



Riga Nordic University



СУМСЬКИЙ ДЕРЖАВНИЙ
ПЕДАГОГІЧНИЙ УНІВЕРСИТЕТ
ІМЕНІ А.С. МАКАРЕНКА

**Sumy State Pedagogical
University named after
A. S. Makarenko**

**NATURAL SCIENCES AND EDUCATION
IN THE CONTEXT
OF SUSTAINABLE DEVELOPMENT:
BIOLOGICAL, GEOGRAPHICAL,
CHEMICAL, AND ENVIRONMENTAL
DIMENSIONS**

Scientific monograph

Volume 1



IZDEVNIECĪBA
BALTĪJA
PUBLISHING

2026

*Recommended for printing and distribution via Internet
by the Academic Council of Baltic Research Institute
of Transformation Economic Area Problems according
to the Minutes № 9 dated 30.09.2026*

EDITORS:

Djakons Romans – Dr.sc.ing., Professor, Academician, Chairman of the Board of Riga Nordic University;

Babenko Olena Mykhailivna – Candidate of Pedagogical Sciences, Associate Professor, Head of the Department of Human Biology, Chemistry, and Methods of Teaching Biology at Sumy State Pedagogical University named after A. S. Makarenko;

Kornus Anatolii Oleksandrovych – Candidate of Geographic Sciences, Associate Professor, Deputy Dean for Research of the Faculty of Natural Sciences and Geography, Associate Professor at the Department of General and Regional Geography at Sumy State Pedagogical University named after A. S. Makarenko;

Lytvynenko Yuliia Ivanivna – Candidate of Biological Sciences, Associate Professor, Head of the Department of Biology and Methods of Teaching Biology at Sumy State Pedagogical University named after A. S. Makarenko.

REVIEWERS:

Danylchenko Olena Serhiivna – Candidate of Geographical Sciences, Associate Professor, Associate Professor at the Department of General and Regional Geography, Sumy State Pedagogical University named after A. S. Makarenko;

Vakal Anatolii Petrovych – Candidate of Biological Sciences, Associate Professor, Associate Professor at the Department of Biology and Biology Teaching Methods, Sumy State Pedagogical University named after A. S. Makarenko;

Vakal Yuliia Serhiivna – Doctor of Philosophy, Associate Professor at the Department of Human Biology, Chemistry and Chemistry Teaching Methods, Sumy State Pedagogical University named after A. S. Makarenko.

Natural Sciences and Education in the Context of Sustainable Development: Biological, Geographical, Chemical, and Environmental Dimensions: Scientific monograph. Riga, Latvia : “Baltija Publishing”, 2026. Volume 1. 504 p.

© Riga Nordic University, 2026

ISBN 978-9934-26-714-7

© Sumy State Pedagogical
University named after A. S. Makarenko, 2026

CONTENTS

FUNDAMENTAL AND APPLIED RESEARCH IN BIOLOGY AND ECOLOGY

Chapter 1

Growth response of near-isogenic bread wheat (<i>Triticum aestivum</i> L.) lines carrying different <i>PPD</i> alleles under <i>in vivo</i> and <i>in vitro</i> conditions (Avksentieva O.)	2
1. Analysis of the growth response of near-isogenic wheat lines carrying <i>PPD</i> alleles during early ontogeny	7
2. Analysis of the growth response of primary calli of near-isogenic wheat lines carrying different <i>PPD</i> alleles	10
3. Comparative analysis of the growth response of wheat NILs carrying <i>PPD</i> genes under <i>in vivo</i> and <i>in vitro</i> conditions	15

Chapter 2

Health assessment of species-rich urban lawn mixtures in sustainable urban green infrastructure (Golosna L., Bobrova O., Afanasieva O., Holubec V.)	22
1. Materials and methods	24
2. Results	30
3. Discussion	38

Chapter 3

Natural antimicrobial compounds of plant origin: mechanisms of action and prospects for application against bacterial infection agents (Derkach T.)	45
1. Main antibacterial mechanisms of action of phytochemicals	46
2. Specific mechanisms of action of individual phytochemical classes	52
3. Pathogen-specific manifestations of antibacterial activity	55
4. Discussion	60

Chapter 4

Decision framework for assessing the environmental safety of renewable energy in agri-food systems (Kryshstop Ye.)	75
1. Conceptual and methodological foundations	76
2. Comparative analysis of assessment methods	83
3. The decision framework and its application	94

Chapter 5

Environmental and technological solutions for the protection and sustainable use of water resources (Litvak O.) 108

1. Environmental and technological solutions for water treatment and pollution reduction in water bodies 110
2. Rational water use within the sustainable development framework . . . 121

Chapter 6

Review of current research on the effects of heavy metals on tissue regeneration processes: ecological and medical-biological aspects (Lukonin O., Smyrnov V.) 137

1. Ecological aspects of heavy metal contamination of aquatic ecosystems and their bioaccumulation 139
2. Molecular and cellular mechanisms of heavy metal effects on tissue regeneration processes 143
3. Clinical consequences of heavy metal bioaccumulation and conceptual research model 147

Chapter 7

Methodological framework for risk-based assessment of recreational waters and its application at selected sites in Mykolaiv Oblast (Magas N.) 156

1. Theoretical and methodological framework and research design 158
2. Overall physicochemical characterization of recreational sites 164
3. Microbiological safety, risk characterization and management priorities . 166
4. Discussion 170

Chapter 8

Current approaches in maintenance gut health in farming animals.

Part 1. The isotonic protein blend inhibits porcine epidemic diarrhea virus excretion and ameliorates intestinal barrier

(Masiuk D., Nedzvetsky V., Kokariiev A.) 177

1. Materials and methods to assess the intestinal disturbances in PEDV infected animals 179
2. Results on PEDV excretion dynamics 182
3. The protective effect of Isotonic Protein Formulation on PEDV proliferation 184

Chapter 9

Current approaches in maintenance gut health in farming animals.

Part 2. Glycerides blend modulates intestinal barrier functioning in piglets (Masiuk D., Nedzvetsky V., Kokariiev A.) 193

1. Methodological approaches to study the bioeffects of fatty acids blend 195
2. The results on bioeffects of fatty acids blend 197
3. The protective effects of fatty acids blend supplementation 199

Chapter 10

Current approaches in maintenance gut health in farming animals.

Part 3. The prospects of organic acids supplementation in poultry feed additives (Masiuk D., Nedzvetsky V., Kokariiev A.) 210

1. Application of SCFAS in poultry farming 212
2. Effect of SCFA on metabolism and immune defence 215
3. Use of medium-chain fatty acids 217
4. Application of organic acid mixtures in poultry farming 219

Chapter 11

Current approaches in maintenance gut health in farming animals.

Part 4. The current progress in monoglycerides supplementation to provide antibiotics restriction in swine husbandry (Masiuk D., Nedzvetsky V., Kokariiev A.) 228

1. General characteristics of monoglycerides 229
2. Effect of monoglycerides on perfomance and feed conversion in pigs 231
3. Effectiveness of monoglycerides in the treatment and prevention of swine diseases 233
4. Effects of monoglycerides on intestinal morphology and microbiome in pigs 237

Chapter 12

Current approaches in maintenance gut health in farming animals.

Part 5. Monoglyceride blend supplementation modulates intestinal molecular markers expression and microbiome status in the duodenum of broiler chickens (Masiuk D., Nedzvetsky V.) 246

1. Methodology of the study of intestinal health assessment 248
2. The results on intestinal health assessment 250
3. The intestinal health maintenance is key task to provide poultry profitability 258

Chapter 13

Biological basis of industrial hemp breeding in the context of sustainable development (Mishchenko S.)	265
1. Genetic and selection control of sexual structure and heterosis	267
2. Genetic and biochemical foundations of breeding for cannabinoid profiles and other biochemical traits	269
3. Anatomical and morphological differentiation of fiber and <i>in vitro</i> technology	272
4. Climate-oriented breeding of industrial hemp	274

Chapter 14

System for ensuring virological purity of veterinary immunobiological drugs in the context of pestivirus contamination (Napnenko O., Solomon V., Midyk S.)	283
1. Research materials and methods	286
2. Analysis of contamination risk groups	288
3. Development of a test system for detecting contamination	289
4. Determination of sensitivity and specificity of the test system	291
5. Comparative study of the effectiveness of the methods	292
6. Research on pestivirus contamination of biological raw materials and products	294
7. Study of pestivirus contamination of cell cultures	295

Chapter 15

Atmospheric air: global trends, monitoring, and climate challenges (Pytuliak M., Pytuliak M.)	302
1. Contemporary sources and chemical mechanisms of technogenic atmospheric air pollution	304
2. Fine particulate matter (PM ₁₀ , PM _{2.5}) and its transboundary transport	316
3. Environmental monitoring of atmospheric air	320
4. Atmospheric pollution and climate change	332

Chapter 16

Consumers' self-extension and experiences of switching to more sustainable food-related products (Svietlichna M., Hjelm J., Wijesinghe P. G. Sachini D.)	350
1. Theoretical framework and literature review. Self-congruity theory	352
2. Health, social influence and barriers to sustainable consumption	354

Chapter 17

Assessment of the cumulative impact of wind stations on seasonal bird complexes within the boundaries of man-made territories of the Northwestern Azov region (Sydorenko A.) 363

1. Migration corridors within the Zaporizhzhia wind farm area 364
2. Seasonal characteristics of migration and population connectivity. Stopover sites within the study region 368
3. Cumulative impacts within the wind farm area 375
4. Barrier effects and cumulative impacts of wind farms on birds. 378

Chapter 18

The impact of military actions on the ecological state of Ukraine's territorial communities and directions for the restoration of natural ecosystems (Sovhira S.) 390

1. The ecological state of territorial communities under armed aggression: scale, categories and consequences of destructive processes. . 391
2. Directions for the restoration of natural ecosystems and strategies for the ecological rehabilitation of territorial communities. 400

Chapter 19

Ecological resilience capacity of urban soil-landscape systems under compound shocks: a methodological framework for robust management decision making (Titenko G.) 413

1. Conceptual foundations of the ecological resilience of urban soil-landscape systems. 415
2. A methodological approach to assessing resilience capacity under compound shocks. 426
3. Developing and justifying robust management strategies for urban areas. 435

Chapter 20

Monitoring and status of introduced populations of species of the genus *Pulsatilla* Mill. in cultivated plant communities (Chekanov M., Kuzemko A., Didenko I.) 452

1. Research methods and materials 453
2. The experience of introducing species of the genus *Pulsatilla* L. *ex situ*. 454
3. The status of introduced populations of *P. patens* and *P. pratensis* *ex situ* at the National Dendrological Park "Sofiyivka" 458

Chapter 21

The diversity of natural oak forest phytocenoses in typical steppe ravines of the Dnipropetrovsk region

(Yakovlieva-Nosar S., Bessonova V.) 469

1. Research objects and methods. 470

2. Characteristics of oak forest phytocenoses in the Right-Bank tracts . . 474

3. Characteristics of oak forest phytocenoses in the Left-Bank tracts . . . 486

01

SECTION

FUNDAMENTAL
AND APPLIED
RESEARCH
IN BIOLOGY
AND ECOLOGY

GROWTH RESPONSE OF NEAR-ISOGENIC BREAD WHEAT (*TRITICUM AESTIVUM* L.) LINES CARRYING DIFFERENT *PPD* ALLELES UNDER *IN VIVO* AND *IN VITRO* CONDITIONS

Avksentieva Olha

DOI <https://doi.org/10.30525/978-9934-26-714-7-1>

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops¹, accounting for more than one-third of global grain production and serving as a major source of plant-derived protein and carbohydrates² for populations worldwide. Rapid population growth, global climate change, and increasingly adverse agricultural conditions have intensified the need to develop new wheat cultivars capable of maintaining stable productivity across a wide range of environmental conditions. Consequently, one of the major objectives of modern wheat breeding is to investigate the genetic systems regulating plant growth, development, and adaptation to environmental factors³.

Among the most stable and evolutionarily conserved environmental signals is the photoperiod, defined as the daily duration of light and darkness at a given geographical location. In bread wheat, photoperiod sensitivity is an evolutionarily conserved and genetically determined trait controlled by the Photoperiod (*PPD*)⁴ gene system. The *PPD* genes play a pivotal role in the regulation of wheat ontogeny and are represented by three homoeologous loci, *PPD-A1*, *PPD-B1*, and *PPD-D1*, located on chromosomes 2A, 2B, and 2D,

¹ Erenstein, O., Jaleta, M., Mottaleb, K. A., Sonder, K., Donovan, J., Braun, H.-J., et al. (2022). Global Trends in Wheat Production, Consumption and Trade. Wheat Improvement. Springer, Cham. C. 47–66. DOI: 10.1007/978-3-030-90673-3_4

² Gul K., Yousuf B., Singh A. K., Singh P., Wani A. A. Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*. 2023. DOI: 10.3389/fnut.2023.1053196

³ Dubrovna O. V., Mykhalska S. I., Komisarenko A. G. Modern advancements in Agrobacterium-mediated wheat transformation technology. *Фізіологія рослин і генетика*. 2025. Т. 57, № 4. С. 279–307. DOI: 10.15407/frg2025.04.279

⁴ Pérez-Gianmarco T. I., Slafer G. A., González F. G. Photoperiod-sensitivity genes shape floret development in wheat. *Journal of Experimental Botany*. 2019. Vol. 70, No. 4. P. 1339–1348. DOI: 10.1093/jxb/ery449

respectively. These genes constitute key components of the molecular pathway underlying photoperiod perception and determine the timing of the transition from vegetative to reproductive development. Dominant *PPD-1a* alleles confer photoperiod insensitivity by accelerating heading regardless of day length, whereas recessive *PPD-1b* alleles maintain long-day photoperiod sensitivity. Therefore, allelic variation at the *PPD* loci is a major determinant of the adaptation of wheat cultivars to diverse agro-climatic environments.

Over the past decade, substantial evidence has accumulated regarding the influence of *PPD* genes on heading time, productivity, and grain yield⁵. However, numerous studies have demonstrated that the functions of these genes extend far beyond the regulation of photoperiodic responses. They exert pleiotropic effects on plant height, growth rate, root system development⁶, biomass accumulation, photosynthetic efficiency, nitrogen use efficiency, productive tillering, and individual yield components⁷. These effects are believed to result from complex interactions between *PPD* genes, components of the circadian clock⁸, hormonal pathways regulating plant growth, and the vernalization (*VRN*)⁹ genes, which together constitute an integrated regulatory network controlling plant development.

Particular attention has recently been directed toward the early stages of plant ontogeny¹⁰, during which the fundamental morphological organization of the plant is established and the balance between shoot and root development is determined. Seedling growth, root system development, and the accumulation

⁵ Alvarez M. A., Slafer G. A., González F. G. et al. Photoperiod-1 regulates the wheat inflorescence transcriptome to influence spikelet architecture and flowering time. *Current Biology*. 2024. Vol. 34, No. 11. P. 2330–2343.e4. DOI: 10.1016/j.cub.2024.04.029

González F. G., Slafer G. A., Miralles D. J. et al. Features of Ppd-B1 expression regulation and their impact on the flowering time of wheat near-isogenic lines. *BMC Plant Biology*. 2017. Vol. 17. Article 107. DOI: 10.1186/s12870-017-1126-z

Royo C, Dreisigacker S, Alfaro C, Ammar K, Villegas D. Effect of Ppd-1 genes on durum wheat flowering time and grain filling duration in a wide range of latitudes. *The Journal of Agricultural Science*. 2016. 154(4) P. 612–631. DOI:10.1017/S0021859615000507

⁶ Makhoul M., Schlichtermann R.-H., Ugwuanyi S. et al. Novel PHOTOPERIOD-1 gene variants associate with yield-related and root-angle traits in European bread wheat. *Theoretical and Applied Genetics*. 2024. Vol. 137. Article 125. DOI: 10.1007/s00122-024-04634-9

⁷ Kitagawa S., Shimada K., Murai K. Effect of Ppd-1 on flowering time in wheat is associated with activation of FT1 and genes related to development of the inflorescence. *Breeding Science*. 2012. Vol. 62, No. 2. P. 184–190. DOI: 10.1270/jsbbs.62.184

⁸ Faure S., Turner A. S., Gruszka D. et al. Mutation at the ELF3 gene disrupts the circadian clock and causes barley photoperiod insensitivity. *Nature Genetics*. 2012. Vol. 44. P. 123–130.

⁹ Alvarez M. A., Tranquilli G., Lewis S., Kippes N., Dubcovsky J. Genetic and physical mapping of the photoperiod-insensitive gene Ppd-A1 and interaction with Vrn-1 alleles in wheat. *Molecular Breeding*. 2016. Vol. 36. Article 57. DOI: 10.1007/s10142-016-0490-3

Diallo A., Kane N, Agharbaoui Z, Badawi M, Sarhan F. Heterologous expression of wheat VERNALIZATION 2 (TaVRN2) gene in Arabidopsis delays flowering and enhances freezing tolerance. *PLoS One*. 2010 Jan 13;5(1):e8690. DOI: 10.1371/journal.pone.0008690

¹⁰ Carrera-Castaño G., Calleja-Cabrera J., Pernas M. et al. An updated overview on the regulation of seed germination. *Plants*. 2020. Vol. 9, No. 6. Article 703. DOI: 10.3390/plants9060703

of fresh and dry biomass largely determine the subsequent adaptation of plants to environmental stresses, including drought, salinity¹¹, nutrient deficiency, and temperature extremes¹². Despite numerous studies addressing the productivity of wheat cultivars carrying different *PPD*¹³ alleles, the influence of these genes on early vegetative growth remains insufficiently understood, and the available data are often inconsistent.

Near-isogenic lines (NILs)¹⁴ represent one of the most informative genetic models for assessing the contribution of individual genes to phenotypic expression. These lines differ only in one or a few target loci while sharing an otherwise nearly identical genetic background. Consequently, the influence of background genetic variation is minimized, allowing the direct evaluation of the role of a particular gene in the expression of a given trait. One of the best-characterized NIL collections was developed using the winter wheat cultivar Myronivska 808 and has been widely employed to investigate the functional roles of *PPD*, *VRN*, and other developmental regulatory genes in wheat.

In parallel with *in vivo* studies, *in vitro* tissue culture techniques have become increasingly important for investigating the expression of genetic characteristics at the cellular level¹⁵. Callus culture, consisting of a population of isolated cells that develop independently of the regulatory influences operating within the intact plant, represents a versatile experimental system for studying cellular dedifferentiation, proliferation, morphogenesis, and plant regeneration¹⁶. It is well established that callus induction, callus growth rate, and morphogenetic competence are strongly genotype-dependent. Consequently, plant tissue

¹¹ Iyem E., Yıldırım M., Kızılgöçü F. Comparative study on germination and seedling growth of wheat cultivars under salt stress regimes. *International Journal of Agriculture, Environment and Food Sciences*. 2020. Vol. 4, No. 4. DOI: 10.31015/jaefs.2020.4.8

¹² Hussain S., Chachar Q., Chachar S. et al. Temperature regimes on seeds germination and growth parameters of wheat (*Triticum aestivum* L.) genotypes at early seedling stage. *Pakistan Journal of Scientific and Industrial Research*. 2020. Vol. 63, No. 3. P. 214–225. DOI: 10.52763/PJSIR.BIOL.SCI.63.3.2020.214.225

¹³ Seki M., Chono M., Matsunaka H. et al. Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars. *Breeding Science*. 2011. Vol. 61, No. 4. P. 405–412.

Song T., Shi C., Wang Y. et al. Molecular characterization of a novel photoperiod-insensitive allele *Ppd-B1a.3* and its effect on heading date in Chinese wheat (*Triticum aestivum*). *Journal of Plant Research*. 2025. DOI: 10.1007/s10265-024-01609-1

¹⁴ Ning S., Li S., Xu K. et al. Development and characterization of near-isogenic lines derived from synthetic wheat revealing the 2 kb insertion in the *PPD-D1* gene responsible for heading delay and grain number improvement. *International Journal of Molecular Sciences*. 2023. Vol. 24, No. 13. Article 10834. DOI: 10.3390/ijms241310834

¹⁵ Kumléhn J. et al. Opportunities and challenges of *in vitro* tissue culture systems in the era of crop genome editing. *International Journal of Molecular Sciences*. 2023. Vol. 24, No. 15. Article 11920. DOI: 10.3390/ijms241511920

¹⁶ Wijerathna-Yapa A., Ramtekey V., Ranawaka B., Basnet B. R. Applications of *in vitro* tissue culture technologies in breeding and genetic improvement of wheat. *Plants*. 2022. Vol. 11, No. 17. Article 2273. DOI: 10.3390/plants11172273

culture has become an indispensable tool not only in plant biotechnology but also in fundamental studies of the genetic mechanisms regulating plant growth and development.

An important characteristic of callus tissue is its cytological organization. Cell density, cell size, the proportion of meristematic and highly vacuolated cells, and the degree of cellular differentiation reflect the physiological status of the culture and largely determine its regenerative capacity. Cytological analyses therefore provide valuable insight into the realization of genetic programs under *in vitro* conditions and facilitate the understanding of the relationships between growth processes at both the organismal and cellular levels.

Although numerous investigations have addressed the functional significance of *PPD* genes, most have focused primarily on heading time, photoperiodic responses, or yield formation. Comparatively little attention has been paid to the integrated evaluation of growth processes during early ontogeny, biomass accumulation, shoot-to-root relationships, and the manifestation of these genetic effects under tissue culture conditions. Moreover, studies integrating analyses of plant growth *in vivo* with callus formation and the cytological characteristics of callus cells *in vitro* using the same genetic material remain scarce. Such an integrated approach provides a deeper understanding of the pleiotropic effects of *PPD* genes and the mechanisms through which they regulate plant growth and development.

Therefore, the present study aimed to investigate the growth responses of near-isogenic lines of the bread wheat cultivar Myronivska 808, differing in the dominant alleles *PPD-A1a*, *PPD-B1a*, and *PPD-D1a*, using a comprehensive assessment of plant morphometric traits under *in vivo* conditions, fresh biomass accumulation, callus induction, and the cytological characteristics of callus cells cultured *in vitro*. The findings of this study will contribute to a better understanding of the pleiotropic functions of photoperiod sensitivity genes and may facilitate the development of highly productive wheat cultivars with improved growth performance, enhanced environmental adaptability, and superior biotechnological potential.

MATERIALS AND METHODS

Plants materials

A set of near-isogenic lines (NILs) of bread wheat (*Triticum aestivum* L.) differing in photoperiod sensitivity (*PPD*) genes and developed in the genetic background of the winter wheat cultivar Myronivska 808 was used as the experimental material. The use of NILs minimized the influence of background genetic variation and enabled the direct assessment of the contribution of individual *PPD* alleles to plant growth responses.

Table 1

**Characteristics of the near-isogenic lines (NILs)
of the wheat cultivar Myronivska 808**

Near-isogenic line (NIL)	Genotype	Growth habit	Developmental rate
Ppd-D1a	<i>Ppd-D1a Ppd-B1b Ppd-A1b</i>	Winter	Rapid
Ppd-B1a	<i>Ppd-D1b Ppd-B1a Ppd-A1b</i>	Winter	Slow
Ppd-A1a	<i>Ppd-D1b Ppd-B1b Ppd-A1a</i>	Winter	Rapid
Myronivska 808	<i>ppd-D1b ppd-B1b ppd-A1b</i>	Winter	Slow

In vivo experiments

Plant growth responses were evaluated under controlled pot-culture conditions. Plants were grown in soil substrate under the following environmental conditions: light intensity of 15 kLux, day/night temperatures of 22/18 °C, a 16 h/8 h light/dark photoperiod, and soil moisture maintained at 70% of field capacity. Plants were cultivated for 21 days, corresponding to the tillering stage. At the end of the experiment, plant height, shoot length, root length, shoot-to-root length ratio, and the fresh biomass of shoots and roots were determined.

In vitro experiments

To investigate growth responses at the cellular level, explants were introduced into *in vitro* culture and primary callus formation was induced using standard plant tissue culture techniques. Mature embryos isolated from surface-sterilized grains of the NILs served as explants. The embryos were cultured in Petri dishes containing Murashige and Skoog (MS) basal medium supplemented with 2 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D). Cultures were incubated in darkness at 26 °C for 21 days. Following cultivation, primary callus induction frequency and the callus growth index (GI) were determined. Callus induction frequency was calculated as the percentage of explants producing callus relative to the total number of cultured explants. The growth index was determined from the increase in callus surface area between the first and third weeks of cultivation using ImageJ software.

The cytological characteristics of callus tissue were evaluated by determining cell density (number of cells per milligram of tissue) and mean cell length. For cell density analysis, calli were macerated in 10% chromic acid for 30 min, after which the cells were counted using a Goryaev counting chamber. Cell length was measured using an ocular micrometer under a LOMO Mikmed-1 light microscope. At least 100–150 cells were analyzed for each experimental variant.

Statistical analysis

All experiments were performed in three biological replicates. In the *in vivo* experiments, 25–30 plants of each NIL were analyzed, whereas 21–28 primary calli were evaluated in the *in vitro* experiments. Statistical analyses were performed using Statistica 5.0 (StatSoft Inc., USA). Differences between mean values were assessed using the least significant difference (LSD_{0.05}) test. Graphs and tables were prepared using Microsoft Excel 2019 (Microsoft Corp., USA). Data are presented as mean $\pm$ standard deviation (mean $\pm$ SD).

1. Analysis of the Growth Response of Near-Isogenic Wheat Lines Carrying PPD Alleles during Early Ontogeny

Plant growth during the early stages of ontogeny is one of the most critical determinants of subsequent development, productivity, and adaptation to adverse environmental conditions. Early ontogeny encompasses seed germination, seedling establishment, root system development, and the formation of the first leaves. During this period, intensive cell division and elongation occur, accompanied by the activation of photosynthesis, water uptake, and mineral nutrition, thereby ensuring the establishment of a vigorous and viable plant.

The genetic regulation of wheat growth is governed by the coordinated action of numerous genes controlling the biosynthesis of enzymes, phytohormones, and regulatory proteins. Of particular importance are genes involved in the biosynthesis and signaling of gibberellins, auxins, and cytokinins, which regulate cell division, elongation, and differentiation. Equally important are the *Reduced height (Rht)* genes, which determine plant sensitivity to gibberellins and influence stem growth from the earliest developmental stages. In addition to genetic factors, environmental conditions—including temperature, soil moisture, light intensity, and nutrient availability—substantially affect gene expression and plant growth.

Thus, wheat growth during early ontogeny is the result of complex interactions between genetic mechanisms and environmental factors. Understanding the genetic regulation of these processes is essential for the development of high-yielding and stress-tolerant wheat cultivars capable of adapting to changing climatic conditions while maintaining stable productivity.

The early stages of wheat ontogeny are particularly important for the establishment of plant adaptive potential, as this period determines root system architecture, biomass accumulation, and the intensity of growth processes. It has been demonstrated that the photoperiod sensitivity genes *PPD-1* (*PPD-A1*, *PPD-B1*, and *PPD-D1*) regulate not only heading time but also influence early

growth rate, leaf area development, root system architecture, and the efficiency of resource utilization by the plant¹⁷.

1.1. Analysis of Linear Growth in Near-Isogenic Lines Carrying Different PPD Alleles

The analysis demonstrated that all near-isogenic lines (NILs) carrying dominant *PPD* alleles exhibited greater total plant length than the recurrent cultivar Myronivska 808 (Table 2).

Table 2

Growth response of near-isogenic wheat lines carrying different PPD alleles based on linear growth parameters at the tillering stage

Near-isogenic line	Plant length (cm)	Shoot length (cm)	Root length (cm)	Shoot/root ratio
<i>PPD D1a</i>	45,41	33,43	12,11	2,76
<i>PPD B1a</i>	46,43	32,72	13,82	3,34
<i>PPD A1a</i>	42,22	32,53	9,87	3,31
Myronivska 808	39,84	33,12	6,83	4,85
<i>LSD_{0.05}</i>	3,02	3,03	0,89	0,25

The greatest plant length was observed in the *PPD-B1a* NIL (46.43 cm), exceeding that of the recurrent cultivar by 6.59 cm (16.5%). A similarly high value was recorded for the *PPD-D1a* genotype (45.41 cm), which was 5.57 cm (14.0%) longer than the control. The *PPD-A1a* NIL also exhibited greater plant length (42.22 cm), exceeding the control by 2.38 cm (6.0%); however, this difference did not exceed the least significant difference ($LSD_{0.05} = 3.02$ cm) and therefore was not statistically significant.

The most pronounced genotypic differences were observed in root development. The longest roots were recorded in the *PPD-B1a* NIL (13.82 cm), representing nearly a twofold increase compared with the recurrent cultivar (6.83 cm). Root length reached 12.11 cm in the *PPD-D1a* line and 9.87 cm in the *PPD-A1a* line. In all NILs, root length significantly exceeded that of Myronivska 808, as the observed differences were greater than the corresponding $LSD_{0.05}$ value (0.89 cm).

In contrast, shoot length varied only slightly among the studied genotypes (32.53–33.43 cm), indicating that the principal effect of the dominant *PPD* alleles during early ontogeny was associated with enhanced root system development rather than shoot elongation.

Significant differences were also observed in the shoot-to-root ratio. The lowest values were recorded for the *PPD-D1a* and *PPD-B1a* NILs,

¹⁷ Hyles, J., Bloomfield, M.T., Hunt, J.R. *et al.* Phenology and related traits for wheat adaptation. *Heredity* 125, 417–430 (2020). <https://doi.org/10.1038/s41437-020-0320-1>

reflecting relatively greater investment in root growth. Conversely, the recurrent cultivar Myronivska 808 exhibited the highest shoot-to-root ratio (4.85), indicating considerably weaker root development relative to the aerial part of the plant.

These findings are consistent with current understanding of *PPD-1* gene function. In addition to regulating photoperiodic responses, *PPD* genes exert pleiotropic effects on plant growth by influencing meristem activity, developmental rate, and vegetative biomass accumulation from the earliest stages of plant development. The observed stimulation of root growth in NILs carrying dominant *PPD* alleles further supports the role of these genes in coordinating developmental processes beyond the regulation of flowering time.

1.2. Analysis of Fresh Biomass Accumulation

The analysis of fresh biomass accumulation demonstrated that the effects of different *PPD* alleles were manifested not only in linear growth but also in the distribution of biomass among individual plant organs (Table 3). The greatest total fresh biomass was recorded in the *PPD-B1a* NIL (4.95 mg), which significantly exceeded that of the *PPD-A1a* line (3.85 mg) and was slightly higher than that of the recurrent cultivar Myronivska 808 (4.63 mg). The *PPD-D1a* NIL also produced greater total biomass than the control, reaching 4.72 mg.

The most pronounced differences among the genotypes were observed in root biomass accumulation. The highest root fresh biomass was recorded in the *PPD-B1a* (1.11 mg) and *PPD-A1a* (1.05 mg) lines, whereas the recurrent cultivar accumulated only 0.54 mg. Thus, root biomass accumulation in the NILs was approximately twofold greater than that of the control genotype.

Table 3

Growth response of near-isogenic wheat lines carrying different *PPD* alleles based on fresh biomass accumulation at the tillering stage

Near-isogenic line	Plant fresh biomass (mg)	Shoot fresh biomass (mg)	Root fresh biomass (mg)	Shoot/root biomass ratio
<i>PPD D1a</i>	4,72	3,92	0,78	5,11
<i>PPD B1a</i>	4,95	3,64	1,11	3,32
<i>PPD A1a</i>	3,85	3,68	1,05	3,51
Myronivska 808	4,63	4,09	0,54	7,64
<i>LSD_{0.05}</i>	0,48	0,21	0,26	1,98

In contrast, the highest shoot fresh biomass was observed in Myronivska 808 (4.09 mg). However, the difference between the recurrent cultivar and the *PPD-D1a* NIL (3.92 mg) was smaller than the corresponding *LSD_{0.05}* value (0.21 mg)

and was therefore not statistically significant. These findings indicate that the principal effect of the dominant *PPD* alleles was associated with stimulation of root development rather than increased shoot biomass accumulation.

The shoot-to-root biomass ratio provided an integrated assessment of biomass partitioning within the plant. The highest ratio was observed in the recurrent cultivar (7.64), indicating preferential allocation of assimilates to shoot growth. In contrast, substantially lower values were recorded in the *PPD-B1a* (3.32) and *PPD-A1a* (3.51) NILs, reflecting a more balanced distribution of assimilates between aboveground and belowground organs and a relatively greater investment in root development.

Recent studies have demonstrated that photoperiod-insensitive *PPD-I* alleles, particularly *PPD-D1a* and *PPD-B1a*, accelerate plant development, modify plant architecture, and influence biomass accumulation well before the transition to the reproductive phase. The present results further support these observations, demonstrating that substitution of individual *PPD-I* alleles in the near-isogenic background of the cultivar Myronivska 808 substantially affects plant growth characteristics during the earliest stages of ontogeny.

Among the investigated genotypes, the *PPD-B1a* NIL exhibited the most pronounced positive effect on root system development, as evidenced by the greatest plant length, maximum root length and root biomass, and the lowest shoot-to-root ratios for both linear growth and biomass accumulation. The *PPD-D1a* NIL also displayed vigorous plant growth and a well-developed root system. Although the influence of the *PPD-A1a* allele was comparatively less pronounced, this genotype nevertheless outperformed the recurrent cultivar for most of the evaluated growth parameters.

Overall, the obtained results indicate that dominant *PPD* alleles determine not only the photoperiodic response of wheat plants but also influence assimilate partitioning among plant organs, promoting the development of a more vigorous root system during early ontogeny. Such modifications may enhance plant adaptation to unfavorable environmental conditions, particularly water deficit, thereby supporting the increasingly recognized pleiotropic role of *PPD* genes in regulating wheat growth, development, and stress adaptation.

2. Analysis of the Growth Response of Primary Calli of Near-Isogenic Wheat Lines Carrying Different *PPD* Alleles

2.1. Callus Induction from Mature Wheat Embryos

In vitro tissue culture is one of the most important tools of modern plant biotechnology and is widely used for cell selection, genetic transformation, production of somaclonal variants, and the rapid propagation of valuable

genotypes. In bread wheat (*Triticum aestivum* L.), mature embryos¹⁸ are among the most commonly used explants because they are available throughout the year, exhibit high viability, and are relatively easy to sterilize. Unlike immature embryos, the use of mature seeds does not require donor plants to be grown at specific developmental stages, thereby considerably simplifying biotechnological research¹⁹.

Callus induction begins with the dedifferentiation of scutellum cells or embryonic meristematic tissues in response to exogenous phytohormones. The most widely used induction medium is Murashige and Skoog (MS) basal medium supplemented with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations ranging from 1 to 3 mg L⁻¹. Auxins initiate intensive cell division, ultimately resulting in the formation of undifferentiated callus tissue.

The efficiency of callus induction is largely genotype-dependent. Numerous studies have demonstrated that wheat cultivars and breeding lines differ substantially in callus induction frequency, callus growth rate, and regeneration capacity. These differences are genetically determined through the regulation of cell division, morphogenesis, and hormonal signaling pathways. Genes involved in phytohormone responses, meristem activity, and photoperiod sensitivity may also influence the proliferative activity of cultured cells under *in vitro* conditions²⁰.

The quality of the induced callus is as important as its quantity. Compact, light-yellow or cream-colored embryogenic callus is considered the most suitable material for plant regeneration because it is characterized by high mitotic activity and consists predominantly of small, isodiametric cells with large nuclei. In contrast, loose, watery, or dark-colored callus generally possesses low morphogenetic potential and rapidly loses its capacity for organogenesis.

Callus induction is also strongly influenced by culture conditions. Optimal induction is generally achieved at temperatures of 24–26°C, under complete darkness during the induction stage, and on media containing elevated concentrations of sucrose as the primary carbon source. Furthermore, the efficiency of callus formation depends on the physiological status of the seeds, storage conditions, and the developmental stage of the embryos. The

¹⁸ Tamimi S. M., Othman H. Callus induction and regeneration from germinating mature embryos of wheat (*Triticum aestivum* L.). *Sains Malaysiana*. 2021. Vol. 50, No. 4. P. 889–896. DOI: 10.17576/jsm-2021-5004-01

¹⁹ Zhou Z., Xu Y., Zhao Y. et al. Overcoming genotypic dependency and bypassing immature embryos in wheat transformation by using morphogenic regulators. *Science China Life Sciences*. 2024. Vol. 67. P. 1535–1538. DOI: 10.1007/s11427-023-2565-9

²⁰ Fehér A. Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Frontiers in Plant Science*. 2019. Vol. 10. Art. 536. DOI: 10.3389/fpls.2019.00536

optimization of culture media and plant growth regulators can substantially increase the frequency of morphogenetically competent callus, even in genotypes exhibiting inherently low regeneration capacity.

Thus, mature wheat embryos represent a convenient and reliable explant source for callus culture. Although their efficiency is generally lower than that of immature embryos, they provide high experimental reproducibility, making them particularly valuable for genetic, breeding, and biotechnological studies. Investigation of callus formation in different genotypes, especially near-isogenic lines, provides an opportunity to elucidate the role of individual genes in the regulation of *in vitro* morphogenesis and contributes to the development of efficient systems for cell selection and plant regeneration.

2.2. Role of the PPD Gene System in the Regulation of Primary Callus Formation

During the experiments, all investigated near-isogenic lines were successfully introduced into *in vitro* culture, resulting in the production of primary calli. Based on their phenotypic characteristics, the calli of different origins did not differ morphologically (Fig. 1A). The primary calli were creamy-white in color, matte, opaque, compact, and homogeneous in structure (Fig. 1B).

Cytological analysis revealed typical callus cells, which were relatively large, elongated or curved, with thin cellulose cell walls, a large vacuole, and a clearly visible nucleus (Fig. 1C).

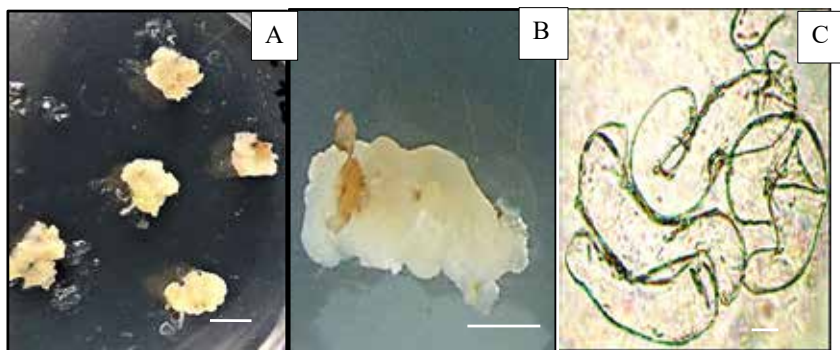


Fig. 1. Primary callus induction from mature wheat embryos. (A) General appearance of callus tissue after 21 days of cultivation; (B) primary callus formed from mature embryos; (C) typical mature callus cells (×400).

Scale bars: 1 cm (A, B) and 1 μ m (C)

Analysis of the experimental results demonstrated that the near-isogenic lines (NILs) of the bread wheat cultivar Myronivska 808, differing in their *PPD*

alleles, exhibited distinct capacities for callus induction and callus growth under *in vitro* culture conditions (Fig. 2). These differences indicate that photoperiod sensitivity genes, in addition to their established role in regulating the transition from vegetative to reproductive development, may indirectly influence cell proliferation, morphogenetic competence, and growth intensity in isolated tissue culture.

The most pronounced growth response was observed in the *PPD-B1a* near-isogenic line, which exhibited a callus induction frequency of 96.1% (Fig. 2A) and a callus growth index of 32.43% (Fig. 2B). Compared with the recurrent cultivar Myronivska 808, these values were approximately 1.6-fold and almost fourfold higher, respectively. These findings demonstrate the considerably greater morphogenetic potential of the *PPD-B1a* genotype and its enhanced capacity for intensive cell proliferation under artificial culture conditions.

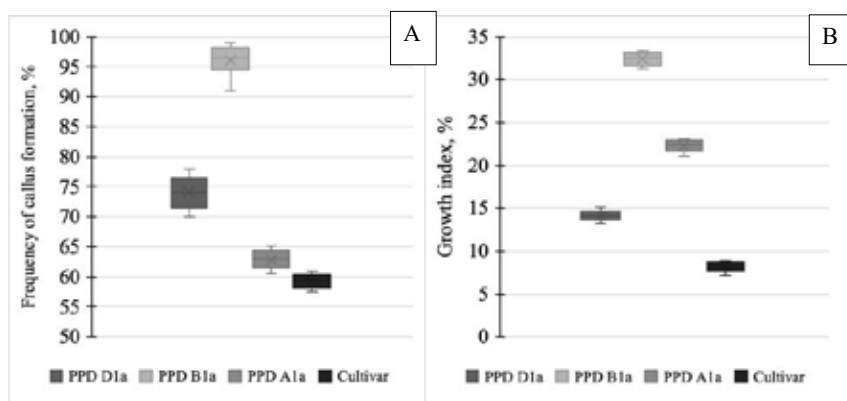


Fig. 2. Growth response of primary calli of near-isogenic wheat lines carrying different *PPD* alleles (A – callus induction frequency, B – callus growth index). Data are expressed as percentages

The high callus induction capacity observed in the *PPD-B1a* line may be associated with more efficient regulation of the cell cycle and enhanced mitotic activity. Callus formation is initiated through the dedifferentiation of somatic cells followed by their transition to intensive cell division. This process is controlled by a complex interaction between genetic factors and the phytohormonal system, particularly auxins and cytokinins. Altered expression of genes involved in photoperiodic regulation may affect the hormonal balance within cultured cells, thereby influencing both the rate of callus induction and the subsequent growth of callus tissue.

2.3. Cytological Analysis of Callus Tissues of Wheat NILs Carrying PPD Genes

The cytological analysis confirmed the results of the morphometric studies (Fig. 3). Among the investigated near-isogenic lines (NILs), the *PPD-B1a* isolate was characterized by the highest cell density in the callus tissue (4.23×10^6 cells mg^{-1}) (Fig. 3A), indicating a high level of mitotic activity. At the same time, the average cell length was lower ($1.05 \times 10^2 \mu\text{m}$) (Fig. 3B) than in the other experimental variants. This pattern is typical of tissues exhibiting intensive proliferation, where cell division predominates over subsequent cell growth and elongation. Smaller cells are physiologically younger and possess higher metabolic activity, thereby ensuring rapid accumulation of callus biomass.

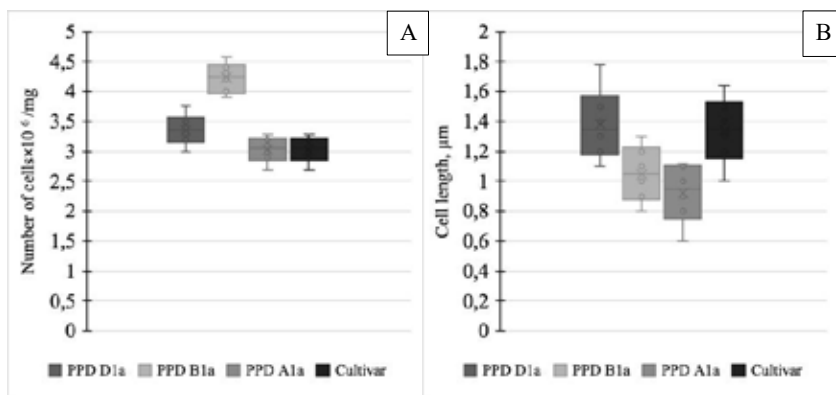


Fig. 3. Cytological characteristics of callus cells of wheat NILs carrying PPD genes in the cultivar Myronivska 808 (A – Cell density; B – Cell length)

In contrast to the *PPD-B1a* isolate, the *PPD-D1a* line exhibited a lower growth index (14.13%), although the number of cells remained relatively high (3.36×10^6 cells mg^{-1}). However, this isolate was characterized by the greatest average cell length (1.38 μm). This finding may indicate the predominance of cell elongation processes over active cell division. Such a growth pattern is commonly observed when mitotic activity declines, and tissue biomass increases primarily through the enlargement of already differentiated cells.

The *PPD-A1a* isolate occupied an intermediate position for most of the investigated parameters. The frequency of callus formation reached 62.9%, while the growth index was 22.3%, exceeding the corresponding values of the original

cultivar but remaining lower than those of the *PPD-B1a* isoline. Nevertheless, the callus tissue contained the lowest number of cells (2.57×10^6 cells mg^{-1}), and their average length was also the smallest (0.92 μm). This combination may indicate relatively low rates of both cell division and subsequent cell expansion, which is likely associated with the functional characteristics of the corresponding *PPD* allele.

The original cultivar Myronivska 808 exhibited the lowest indices of growth activity. The frequency of callus formation was only 59.3%, while the growth index reached 8.15%. These results indicate a considerably lower regenerative capacity compared with the investigated NILs. Therefore, the introduction of individual *PPD* gene alleles into the genetic background of the cultivar can substantially modify the response of plant tissues to *in vitro* culture conditions.

The obtained results are consistent with current concepts regarding the multifunctional role of photoperiod response (*PPD*) genes. Recent studies have demonstrated that *PPD* genes regulate not only heading time but also influence the intensity of growth processes, photosynthetic efficiency, hormonal status, adaptation to abiotic stresses, and meristem activity. Consequently, their effects may also be manifested under *in vitro* culture conditions, where tissue development is determined primarily by intrinsic physiological and biochemical mechanisms.

Thus, the results of the present study demonstrate that different alleles of the *PPD* genes exert distinct effects on the morphogenetic potential of wheat callus tissue. The *PPD-B1a* isoline exhibited the highest biotechnological value, providing the greatest frequency of callus formation, the highest growth index, and the largest population of actively proliferating cells. These characteristics make this genotype a promising candidate for biotechnological applications, including plant regeneration, cell selection, and genetic transformation of wheat.

3. Comparative Analysis of the Growth Response of Wheat NILs Carrying *PPD* Genes under *In Vivo* and *In Vitro* Conditions

A comparative analysis of the experimental results demonstrated that the effects of *PPD* genes are manifested both at the level of the whole plant (*in vivo*) and at the level of isolated cells and tissues (*in vitro*). Despite the substantial differences between these experimental systems, a common pattern was observed in both cases: the dominant alleles of the *PPD* genes promoted growth processes compared with the original cultivar *Myronivska 808*.

Under *in vivo* conditions, the most pronounced effect was observed in the *PPD-B1a* isoline, which exhibited the greatest plant height (46.43 cm), the longest root system (13.82 cm), and the highest fresh plant biomass (4.95 mg). Furthermore, this isoline showed the lowest shoot-to-root ratio, indicating more

intensive root system development. A similar trend was observed for the *PPD-D1a* isoline, although its values were slightly lower. The least pronounced positive effect among the NILs was recorded for *PPD-A1a*, whereas the original cultivar *Myronivska 808* exhibited the poorest root growth and a less efficient distribution of biomass between aboveground and belowground organs.

A similar pattern was also observed under *in vitro* conditions. The *PPD-B1a* isoline exhibited the highest frequency of callus formation (96.1%), the maximum growth index (32.43%), and the greatest cell density in the callus tissue (4.23×10^6 cells mg^{-1}). These results indicate a high proliferative activity of callus cells and a considerable morphogenetic potential of this genotype. In contrast, the original cultivar *Myronivska 808* was characterized by the lowest levels of callus induction and callus growth, consistent with its lower growth performance under natural conditions.

At the same time, certain differences were observed between the *in vivo* and *in vitro* systems. In the intact plant, growth is determined by the integrated interaction of the photosynthetic apparatus, the root system, assimilate transport, hormonal regulation, and environmental factors. In contrast, under *in vitro* culture conditions, cell development occurs in a controlled environment on an artificial nutrient medium, where intrinsic genetic mechanisms, hormonal balance, and the capacity of cells for dedifferentiation and re-entry into the cell cycle play the predominant roles. Consequently, individual parameters do not always show complete correspondence between the two systems. For example, the *PPD-D1a* isoline displayed vigorous plant growth *in vivo*, whereas under *in vitro* conditions its callus exhibited a relatively lower growth index despite possessing the largest average cell size. This observation suggests that cell expansion predominated over intensive cell proliferation.

Particular attention should be given to the *PPD-B1a* isoline, which exhibited the best overall performance in both experimental systems. Plants of this genotype showed intensive root system development and the greatest biomass accumulation, while under *in vitro* conditions they demonstrated the highest frequency of callus formation, the greatest growth index, and the highest cell density in the callus tissue. This correspondence indicates that the *PPD-B1a* allele positively affects not only whole-plant growth but also the fundamental processes of cell division and morphogenesis.

The obtained results support the current understanding of the multifunctional role of *PPD* genes. Although these genes are traditionally regarded as regulators of photoperiod sensitivity and heading time, their function is not limited to reproductive development. The findings suggest that *PPD* genes are also involved in the regulation of meristem activity, plant hormonal status, cell division, and growth processes during the early stages of ontogeny. The

consistency of the observed trends under both natural and tissue culture conditions indicates that the effects of *PPD* alleles are genetically determined and are expressed regardless of the level of biological organization.

In conclusion, the comparative analysis demonstrated that the results obtained under *in vivo* and *in vitro* conditions are complementary. Among the investigated genotypes, the *PPD-B1a* isoline proved to be the most promising, combining vigorous plant growth, a well-developed root system, and the highest morphogenetic potential of callus tissue. These characteristics make this genotype a valuable candidate for breeding programs, cell selection, and biotechnological research aimed at improving wheat adaptability and productivity.

CONCLUSIONS

The present study demonstrated that the substitution of individual dominant *PPD* alleles into the genetic background of the bread wheat cultivar Myronivska 808 significantly affected plant growth and morphogenetic responses under both *in vivo* and *in vitro* conditions. Although *PPD* genes are primarily recognized as regulators of photoperiod sensitivity and heading time, the obtained results provide further evidence of their pleiotropic effects on early plant development, root system architecture, biomass accumulation, and the proliferative activity of cultured cells.

Among the investigated near-isogenic lines, the *PPD-B1a* genotype consistently exhibited the most favorable performance. Under *in vivo* conditions, it showed the greatest plant height, the most developed root system, the highest fresh biomass accumulation, and a more balanced allocation of assimilates between shoots and roots. Under *in vitro* conditions, the same genotype demonstrated the highest frequency of callus induction, the greatest callus growth index, and the highest density of actively proliferating cells, indicating superior morphogenetic competence and regenerative potential.

The comparative analysis revealed a strong correspondence between plant growth under natural conditions and cellular behavior in tissue culture. Although the physiological mechanisms regulating development differ between *in vivo* and *in vitro* systems, the overall effects of the dominant *PPD* alleles remained consistent, suggesting that their influence is genetically determined and expressed across different levels of biological organization. The contrasting response of the *PPD-D1a* isoline, characterized by vigorous plant growth but relatively lower callus proliferation accompanied by larger cell size, further indicates that different *PPD* alleles may differentially regulate the balance between cell division and cell expansion.

Overall, the findings highlight the multifunctional role of *PPD* genes in the regulation of wheat growth and development beyond their established function in flowering-time control. The superior performance of the *PPD-B1a* isoline identifies this genotype as a valuable source of favorable alleles for wheat improvement. Its high growth vigor, enhanced root development, and elevated morphogenetic potential make it particularly suitable for breeding programs, cell selection, plant regeneration, and genetic transformation.

Thus, the results of the present study demonstrate that the dominant *PPD* alleles exhibit pleiotropic effects manifested at both the whole-plant and cellular levels. These findings indicate that the *PPD* gene system can be regarded as one of the key regulators of growth processes in wheat. These results contribute to a better understanding of the biological functions of *PPD* genes and provide a scientific basis for their application in modern wheat breeding and plant biotechnology.

SUMMARY

The study investigated the growth response of near-isogenic lines (NILs) of bread wheat (*Triticum aestivum* L.) cultivar Myronivska 808, differing in the allelic composition of the photoperiod sensitivity (*PPD*) genes, under *in vivo* and *in vitro* conditions. The aim of the study was to determine the effects of the dominant alleles *PPD-A1a*, *PPD-B1a*, and *PPD-D1a* on plant growth, biomass accumulation, root system development, callus formation, and the cytological characteristics of callus tissues. The results demonstrated that all NILs carrying dominant *PPD* alleles exceeded the original cultivar Myronivska 808 in growth performance during the early stages of ontogenesis. The most pronounced positive effect was observed in the *Ppd-B1a* isoline, which exhibited the greatest plant and root lengths, the highest fresh root biomass accumulation, and a more balanced shoot-to-root ratio. Under *in vitro* tissue culture conditions, this isoline also showed the highest callus induction frequency, the greatest growth index, and the highest cell density in callus tissue. These findings indicate that *PPD* genes exert a consistent genetic effect on growth regulation under both *in vivo* and *in vitro* conditions. The obtained results confirm the multifunctional role of *PPD* genes, which regulate not only photoperiodic response and heading time but also influence growth intensity, root system development, biomass accumulation, and cell proliferative activity. The *PPD-B1a* isoline can be recommended as a promising source material for wheat breeding programs and biotechnological research aimed at developing high-yielding and environmentally adaptable wheat cultivars.

Bibliography

1. Alvarez M. A., Slafer G. A., González F. G. et al. Photoperiod-1 regulates the wheat inflorescence transcriptome to influence spikelet architecture and flowering time. *Current Biology*. 2024. Vol. 34, №. 11. P. 2330–2343.e4. DOI: 10.1016/j.cub.2024.04.029
2. Alvarez M. A., Tranquilli G., Lewis S., Kippes N., Dubcovsky J. Genetic and physical mapping of the photoperiod-insensitive gene Ppd-A1 and interaction with Vrn-1 alleles in wheat. *Molecular Breeding*. 2016. Vol.36. Article 57. DOI: 10.1007/s10142-016-0490-3
3. Bentley A. R., Turner A. S., Gosman N. et al. Phenology and related traits for wheat adaptation. *Heredity*. 2020. Vol. 125. P. 417–430. DOI: 10.1038/s41437-020-0320-1
4. Carrera-Castaño G., Calleja-Cabrera J., Pernas M. et al. An updated overview on the regulation of seed germination. *Plants*. 2020. Vol. 9, №. 6. Article 703. DOI: 10.3390/plants9060703
5. Diallo A, Kane N, Agharbaoui Z, Badawi M, Sarhan F. Heterologous expression of wheat VERNALIZATION 2 (TaVRN2) gene in Arabidopsis delays flowering and enhances freezing tolerance. *PLoS One*. 2010 Jan 13;5(1):e8690. DOI: 10.1371/journal.pone.0008690
6. Dubrovna O. V., Mykhalska S. I., Komisarenko A. G. Modern advancements in Agrobacterium-mediated wheat transformation technology. *Фізіологія рослин і генетика*. 2025. Т. 57, № 4. С. 279–307. DOI: 10.15407/frg2025.04.279
7. Erenstein O., Jaleta M., Mottaleb K. A., Sonder K., Donovan J., Braun H.-J. et al. Global trends in wheat production, consumption and trade. *Wheat Improvement*. Cham : Springer, 2022. P. 47–66. DOI: 10.1007/978-3-030-90673-3_4
8. Faure S., Turner A. S., Gruszka D. et al. Mutation at the ELF3 gene disrupts the circadian clock and causes barley photoperiod insensitivity. *Nature Genetics*. 2012. Vol. 44. P. 123–130. DOI: 10.1073/pnas.1120496109
9. Fehér A. Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Frontiers in Plant Science*. 2019. Vol. 10. Art. 536. DOI: 10.3389/fpls.2019.00536
10. González F. G., Slafer G. A., Miralles D. J. et al. Features of Ppd-B1 expression regulation and their impact on the flowering time of wheat near-isogenic lines. *BMC Plant Biology*. 2017. Vol. 17. Article 107. DOI: 10.1186/s12870-017-1126-z
11. Gul K., Yousuf B., Singh A. K., Singh P., Wani A. A. Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types,

classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*. 2023. DOI: 10.3389/fnut.2023.1053196

12. Hussain S., Chachar Q., Chachar S. et al. Temperature regimes on seeds germination and growth parameters of wheat (*Triticum aestivum* L.) genotypes at early seedling stage. *Pakistan Journal of Scientific and Industrial Research*. 2020. Vol. 63, No. 3. P. 214–225. DOI: 10.52763/PJSIR.BIOL.SCI.63.3.2020.214.225

13. Hyles, J., Bloomfield, M.T., Hunt, J.R. et al. Phenology and related traits for wheat adaptation. *Heredity*. 2020. 125, 417–430. doi.org/10.1038/s41437-020-0320-1

14. Iyem E., Yıldırım M., Kızılgeçi F. Comparative study on germination and seedling growth of wheat cultivars under salt stress regimes. *International Journal of Agriculture, Environment and Food Sciences*. 2020. Vol. 4, № 4. DOI: 10.31015/jaefs.2020.4.8

15. Kitagawa S., Shimada K., Murai K. Effect of Ppd-1 on flowering time in wheat is associated with activation of FT1 and genes related to development of the inflorescence. *Breeding Science*. 2012. Vol. 62, №. 2. P. 184–190. DOI: 10.1270/jsbbs.62.184

16. Kumlehn J. et al. Opportunities and challenges of in vitro tissue culture systems in the era of crop genome editing. *International Journal of Molecular Sciences*. 2023. Vol. 24, № 15. Article 11920. DOI: 10.3390/ijms241511920

17. Makhoul M., Schlichtermann R.-H., Ugwuanyi S. et al. Novel PHOTOPERIOD-1 gene variants associate with yield-related and root-angle traits in European bread wheat. *Theoretical and Applied Genetics*. 2024. Vol. 137. Article 125. DOI: 10.1007/s00122-024-04634-9

18. Ning S., Li S., Xu K. et al. Development and characterization of near-isogenic lines derived from synthetic wheat revealing the 2 kb insertion in the PPD-D1 gene responsible for heading delay and grain number improvement. *International Journal of Molecular Sciences*. 2023. Vol. 24, № 13. Article 10834. DOI: 10.3390/ijms241310834

19. Pérez-Gianmarco T. I., Slafer G. A., González F. G. Photoperiod-sensitivity genes shape floret development in wheat. *Journal of Experimental Botany*. 2019. Vol. 70, №. 4. P. 1339–1348. DOI: 10.1093/jxb/ery449

20. Royo C, Dreisigacker S, Alfaro C, Ammar K, Villegas D. Effect of Ppd-1 genes on durum wheat flowering time and grain filling duration in a wide range of latitudes. *The Journal of Agricultural Science*. 2016. 154(4) P. 612–631. DOI:10.1017/S0021859615000507

21. Seki M., Chono M., Matsunaka H. et al. Distribution of photoperiod-insensitive alleles Ppd-B1a and Ppd-D1a and their effect on heading time in Japanese wheat cultivars. *Breeding Science*. 2011. Vol. 61, № 4. P. 405–412.

22. Song T., Shi C., Wang Y. et al. Molecular characterization of a novel photoperiod-insensitive allele Ppd-B1a.3 and its effect on heading date in Chinese wheat (*Triticum aestivum*). *Journal of Plant Research*. 2025. DOI: 10.1007/s10265-024-01609-1
23. Tamimi S. M., Othman H. Callus induction and regeneration from germinating mature embryos of wheat (*Triticum aestivum* L.). *Sains Malaysiana*. 2021. Vol. 50, No. 4. P. 889–896. DOI: 10.17576/jsm-2021-5004-01
24. Wijerathna-Yapa A., Ramtekey V., Ranawaka B., Basnet B. R. Applications of in vitro tissue culture technologies in breeding and genetic improvement of wheat. *Plants*. 2022. Vol. 11, № 17. Article 2273. DOI: 10.3390/plants11172273
25. Zhou Z., Xu Y., Zhao Y. et al. Overcoming genotypic dependency and bypassing immature embryos in wheat transformation by using morphogenic regulators. *Science China Life Sciences*. 2024. Vol. 67. P. 1535–1538. DOI: 10.1007/s11427-023-2565-9

Information about the author:

Avksentieva Olha,

Candidate of Biological Sciences, Associate Professor,
Head of the Department of Plant and Microorganisms Physiology
and Biochemistry
V. N. Karazin Kharkiv National University
4, Svobody Square, Kharkiv, Ukraine

HEALTH ASSESSMENT OF SPECIES-RICH URBAN LAWN MIXTURES IN SUSTAINABLE URBAN GREEN INFRASTRUCTURE

Golosna Lesia, Bobrova Olena, Afanasieva Oksana, Holubec Vojtech
DOI <https://doi.org/10.30525/978-9934-26-714-7-2>

INTRODUCTION

Urban green infrastructure has become an essential component of sustainable city development. As urbanization intensifies worldwide, cities increasingly face challenges associated with climate change, biodiversity loss, air pollution, elevated temperatures, and the deterioration of environmental quality. Green spaces, including parks, roadside vegetation, urban meadows, and lawns, provide a wide range of ecosystem services that mitigate these negative impacts while improving the quality of life for urban residents^{1,2,3}.

Lawns represent one of the most widespread elements of urban green infrastructure. Beyond their aesthetic value, they perform numerous ecological functions, including temperature regulation through evapotranspiration, carbon sequestration, reduction of airborne particulate matter, stormwater infiltration, soil stabilization, and noise attenuation⁴. Well-designed grasslands also contribute to urban biodiversity by providing habitats for insects and other organisms, thereby supporting ecological connectivity within highly fragmented urban landscapes⁵.

In recent years, the concept of urban lawns has undergone considerable transformation. Conventional intensively managed lawns, typically composed of a few highly productive grass species and requiring frequent mowing,

¹ Ignatieva M. et al. Lawn as a cultural and ecological phenomenon: a conceptual framework for transdisciplinary research. *Urban forestry & urban greening*. 2015. T. 14. №. 2. P. 383–387.

² Aronson M. F. J. et al. Biodiversity in the city: key challenges for urban green space management. *Frontiers in Ecology and the Environment*. 2017. T. 15. №. 4. P. 189–196.

³ Ignatieva M. et al. Lawns in cities: from a globalised urban green space phenomenon to sustainable nature-based solutions. *Land* 2020, 9, 73.

⁴ Robbins P. Lawn people: How grasses, weeds, and chemicals make us who we are. Temple University Press, 2012.

⁵ Stewart G. H. et al. URban Biotopes of Aotearoa New Zealand (URBANZ)(I): composition and diversity of temperate urban lawns in Christchurch. *Urban Ecosystems*. 2009. T. 12. №. 3. P. 233–248.

irrigation, and fertilization, are increasingly being replaced by more diverse vegetation communities^{6,7}. Species-rich grass-legume-forb mixtures are considered a sustainable alternative because they require fewer maintenance inputs, exhibit greater resilience to climatic extremes, and provide enhanced ecological functions⁸. Such mixtures are particularly suitable for green infrastructure associated with transport corridors, including tram tracks, railway embankments, and roadside verges, where plants are exposed to multiple environmental stresses.

Among European cities, Prague represents an outstanding example of integrating species-rich vegetation into urban transport infrastructure. During the last decade, numerous tramway corridors have been converted into vegetated tracks that combine ecological, aesthetic, and engineering functions by reducing heat accumulation, retaining rainwater, suppressing noise, and improving the urban environment^{9,10}. Within this concept, perennial mixtures based on native grasses, legumes, and herbaceous flowering species have been developed to tolerate drought, temperature fluctuations, nutrient-poor soils, and reduced maintenance while simultaneously supporting urban biodiversity.

Despite the increasing implementation of species-rich urban lawns, their long-term sustainability depends not only on their ecological adaptability but also on their health status. Urban vegetation is continuously exposed to numerous biotic stress factors, including pathogenic fungi capable of reducing plant vigor, biodiversity, and aesthetic quality. Diseases affecting grasses, legumes, and associated herbaceous species may impair ecosystem functioning, shorten the persistence of plant communities, and increase maintenance requirements^{11,12}. Climatic fluctuations, especially periods of prolonged drought followed by intensive rainfall, may further alter pathogen dynamics and disease severity in urban ecosystems.

⁶ Wiewióra B., Żurek G. Amenity grasses – a short insight into species, their applications and functions. *Agronomy*. 2023. T. 13. №. 4. P. 1164.

⁷ Ignatieva M. et al. Lawns in cities: from a globalised urban green space phenomenon to sustainable nature-based solutions. *Land*, 2020, 9, 73.

⁸ Parra L. et al. Evaluating the impact of natural products to improve the sustainability of urban lawns. *Cities*. 2024. T. 150. P. 105097.

⁹ Silnice železnice (2019). Zelená tramvajová trať = cirkulární ekonomika a přizpůsobení se klimatu = kolejový absorber hluku s funkcí retence vody BRENS STERED. Dostupné na: <http://www.silnice-zeleznice.cz/clanek/zelena-tramvajova-trat-cirkularni-ekonomika-a-prizpusobeni-se-klimatu-kolejovy-absorber-hluku-s-funkci-retence-vody-brens-stered/>

¹⁰ Tramvajová trať Divoká Šárka – Dědinská. URL: <https://mhd86.cz/2015/01/06/tramvajova-trat-divoka-sarka-dedinska/>

¹¹ Vargas J. M. Management of turfgrass Diseases. Boca Raton : CRC Press, 1994. 320 p.

¹² Miller Jr G. L. Turfgrass diseases. *Agrios' Plant Pathology. Academic Press*. 2024. P. 823–828.

Although diseases of conventional turfgrass have been extensively investigated^{13,14}, comparatively little attention has been devoted to phytopathological risks associated with species-rich urban grassland mixtures developed for sustainable landscaping. Similarly, information concerning the diversity of fungi colonizing native plant mixtures established along tramway green corridors remains scarce. Understanding the relationships between plant diversity, fungal communities, and disease incidence is therefore essential for developing resilient urban vegetation capable of maintaining both ecological functions and long-term stability^{15,16}.

The present study forms part of the project Genetic Resources for Cities and Landscape, conducted by the Czech Agrifood Research Center (CARC; formerly the Crop Research Institute), Prague, Czech Republic. The project aims to develop sustainable plant mixtures based on native grasses, legumes, and herbaceous species suitable for urban environments. Within this framework, several experimental grassland mixtures were established along Prague tramway corridors and evaluated under field conditions.

The objectives of this study were to assess the phytosanitary status of experimental urban lawn mixtures established in Prague, to identify fungal pathogens associated with individual plant species using phytopathological and mycological analyses, and to discuss the implications of phytopathological risks for the development of sustainable urban green infrastructure.

1. Materials and Methods

1.1. Study Area

The study was conducted within the framework of the Czech research project Genetic Resources for Cities and Landscape, which aims to develop sustainable plant mixtures suitable for modern urban environments. Field investigations were carried out during the 2022–2023 growing seasons at experimental sites established along tramway corridors in Prague, the capital city of the Czech Republic. Prague (50°05'N, 14°25'E) is located in the temperate climatic zone of Central Europe and is characterized by a humid continental climate with warm summers and cold winters. The city experiences increasing environmental pressures associated with urbanization, including elevated summer temperatures, prolonged drought periods, air pollution, and intensive

¹³ Hsiang T., Matsumoto N., Millett S. M. Biology and management of *Tiphula* snow mold of turfgrass. *Plant Disease*. 1999. V. 3. No 9. P. 788–798.

¹⁴ Miller Jr G. L. Turfgrass diseases. *Agrios' Plant Pathology*. Academic Press, 2024. P. 823–828.

¹⁵ Kiećana I., Cegiełko M., Mielniczuk E. Fungi occurred on turfgrasses in lawn maintenance. *Acta Scientiarum Polonorum. Hortorum Cultus*. 2015. T. 14. №. 3.

¹⁶ Wiewióra B., Żurek G. Amenity grasses – a short insight into species, their applications and functions. *Agronomy*. 2023. T. 13. №. 4. P. 1164.

anthropogenic disturbance, making it an appropriate model for evaluating the performance of species-rich urban vegetation.

Five experimental sites were selected in different parts of Prague to represent a range of urban conditions associated with tramway green corridors (Figure 1). Three sites (Sídliště Řepy, Spořilov, and Bělohorská) contained experimental plots established with low-growing flowering mixtures, tall flowering mixtures, and drought-tolerant mixtures. Two additional sites (Kubánské náměstí and the Tsiolkovského tramway extension between Vlastina and Dědina) were planted with optimized grass–legume–herb mixtures developed during the final phase of the project. The geographical distribution of the study sites enabled evaluation of vegetation performance under varying local environmental conditions, including differences in solar exposure, soil properties, and urban microclimate.

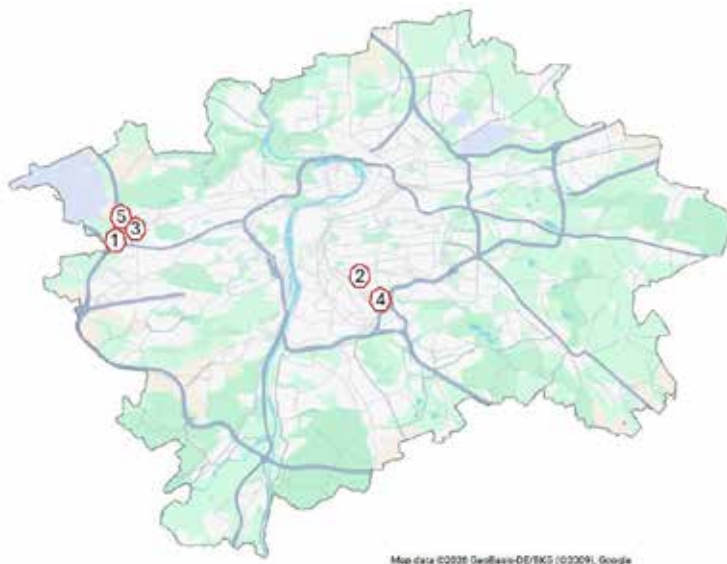


Fig. 1. Location of the experimental sites established along tramway corridors in Prague, Czech Republic. (1) Sídliště Řepy tram terminus; (2) Spořilov tram terminus; (3) Bělohorská tram stop (Prague-Břevnov); (4) Kubánské náměstí tram stop (Prague 10-Vršovice); and (5) Tsiolkovského tram stop between Vlastina and Dědina (Prague 6). Experimental plots included low-growing flowering mixtures, tall flowering mixtures, drought-tolerant mixtures, and optimized grass–legume–herb mixtures established for phytopathological evaluation under urban conditions

Tramway green corridors constitute an important component of Prague's urban green infrastructure. Beyond their aesthetic function, they provide multiple ecosystem services, including biodiversity conservation, mitigation of the urban heat island effect, stormwater infiltration, reduction of dust and noise, and improvement of the urban microclimate. Vegetation established in these corridors is subjected to a combination of abiotic and biotic stress factors, including heat stress, periodic drought, compacted soils, road-derived pollutants, and continuous exposure to naturally occurring plant pathogens. Consequently, tramway corridors represent an ideal environment for evaluating the establishment, persistence, and phytosanitary performance of species-rich lawn mixtures intended for sustainable urban landscaping.

1.2. Experimental Plant Mixtures

Three experimental perennial lawn mixtures were developed within the framework of the project to evaluate alternative approaches to sustainable urban landscaping: (i) a low-growing flowering mixture, (ii) a tall flowering mixture, and (iii) a drought-tolerant mixture. Following the evaluation of their establishment, persistence, and phytosanitary performance under urban conditions, an optimized grass–legume–herb mixture was subsequently developed using the best-performing species.

The mixtures were designed to establish ecologically stable, visually attractive, and low-maintenance vegetation suitable for tramway corridors and other urban green spaces. Species were selected according to their ecological adaptability, ornamental value, drought tolerance, persistence under urban conditions, and compatibility with multispecies plant communities. Particular emphasis was placed on the use of native genetic resources originating from different regions of the Czech Republic. Many of these species naturally occur in habitats characterized by drought, nutrient-poor soils, and other environmental stresses, making them well adapted to the challenging conditions of urban landscapes.

The low-growing flowering mixture consisted of 27 species, including 10 grasses (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Festuca rubra* subsp. *trichophylla*, *Festuca rupicola*, *Helictochloa pratensis*, *Phleum bertolonii*, *Phleum phleoides*, *Poa compressa*, *Poa pratensis*, and *Trisetum flavescens*), three legumes (*Anthyllis vulneraria*, *Trifolium incarnatum*, and *Trifolium pratense*), and 14 perennial herbaceous species, including *Dianthus carthusianorum*, *Dianthus deltoides*, *Echium vulgare*, *Filipendula vulgaris*, *Leontodon autumnalis*, *Leontodon hispidus*, *Linaria vulgaris*, *Matricaria chamomilla*, *Origanum vulgare*, *Plantago lanceolata*, *Plantago media*, *Sanguisorba minor*, *Silene vulgaris*, and *Tragopogon pratensis*.

The tall flowering mixture comprised 22 species, including six grasses (*Brachypodium pinnatum*, *Briza media*, *Bromus erectus*, *Cynosurus cristatus*, *Koeleria pyramidata*, and *Trisetum flavescens*), four legumes (*Astragalus cicer*, *Medicago sativa*, *Onobrychis viciifolia*, and *Securigera varia*), and 12 perennial herbaceous species, including *Achillea ptarmica*, *Anchusa officinalis*, *Artemisia absinthium*, *Betonica officinalis*, *Carum carvi*, *Cichorium intybus*, *Echinops sphaerocephalus*, *Galium album*, *Galium verum*, *Hypericum perforatum*, *Sanguisorba officinalis*, and *Scabiosa ochroleuca*.

The drought-tolerant mixture included 27 species, comprising 12 grasses (*Agrostis capillaris*, *Bothriochloa ischaemum*, *Bromus erectus*, *Festuca filiformis*, *Festuca pallens*, *Festuca rupicola*, *Festuca valesiaca*, *Helictochloa pratensis*, *Melica transsilvanica*, *Phleum bertolonii*, *Poa compressa*, and *Stipa capillata*), five legumes (*Anthyllis vulneraria*, *Lotus corniculatus*, *Medicago lupulina*, *Trifolium incarnatum*, and *Trifolium repens*), and ten perennial herbaceous species (*Achillea millefolium*, *Cichorium intybus*, *Dianthus carthusianorum*, *Plantago lanceolata*, *Plantago media*, *Potentilla argentea*, *Ranunculus bulbosus*, *Sanguisorba minor*, *Thymus pulegioides*, and *Tragopogon pratensis*).

Although the botanical composition differed among the three mixtures, all were designed according to the same ecological principles: the combination of perennial grasses providing structural stability, nitrogen-fixing legumes improving soil fertility, and flowering herbaceous species enhancing biodiversity, ornamental value, and ecological resilience. This multispecies approach was intended to create self-sustaining urban vegetation capable of tolerating drought, low soil fertility, and other environmental stresses while requiring only minimal maintenance.

1.3. Phytopathological Monitoring

Field observations were conducted throughout the 2022–2023 growing seasons, with particular emphasis on periods favorable for the development of fungal diseases. Experimental plots were inspected regularly to evaluate the phytosanitary status of all plant species included in the lawn mixtures. Visual assessments focused on the occurrence of disease symptoms, including leaf spots, chlorosis, necrosis, powdery mildew, anthracnose, stem lesions, wilting, and other abnormalities indicative of pathogen infection.

Plants exhibiting visible disease symptoms were photographed, documented, and collected for subsequent laboratory examination. Representative samples of symptomatic grasses, legumes, and herbaceous species collected during field surveys are shown in Figure 2. The collected material served as the basis for phytopathological diagnosis and mycological isolation of fungal species associated with naturally occurring infections.



Fig. 2. Representative plant samples collected from experimental species-rich lawn mixtures during phytopathological monitoring. Symptomatic grasses, legumes, and herbaceous species were sampled for laboratory diagnosis and mycological analysis

Disease incidence was evaluated for all botanical groups to compare their susceptibility to naturally occurring fungal infections under urban environmental conditions. Particular attention was paid to differences in disease occurrence among grasses, legumes, and herbaceous flowering species, as well as to the distribution and severity of symptoms within the experimental mixtures. No fungicides or other plant protection treatments were applied during the study, allowing the assessment of disease development under natural field conditions.

1.4. Mycological Analysis

Plant samples exhibiting disease symptoms were transported to the laboratory for mycological examination immediately after collection^{17,18}. Leaf, stem, and other infected tissues were processed according to standard phytopathological procedures. Following surface sterilization, tissue fragments were aseptically transferred onto potato dextrose agar (PDA) supplemented with antibiotics to suppress bacterial contamination. Representative plant samples cultured on PDA prior to fungal isolation are shown in Figure 3.

The inoculated Petri dishes were incubated under controlled laboratory conditions until fungal colonies developed. Emerging colonies were examined regularly, and morphologically distinct isolates were subcultured to obtain pure cultures for subsequent identification.

Fungal isolates were identified on the basis of colony morphology and microscopic characteristics using standard taxonomic keys and diagnostic

¹⁷ Watanabe, T. Pictorial Atlas of Soil and Seed Fungi; CRC Press: Boca Raton, FL, USA, 2002. 504 p. [CrossRef]

¹⁸ Seifert, K. A., Morgan-Jones, G. A., Gams, W.; Kendrick, B. The Genera of Hyphomycetes; CBS Biodiversity (Series 10); CBS Fungal Diversity Centre: Utrecht, The Netherlands, 2011. 997 p.

manuals. Identification focused primarily on fungi associated with foliar diseases, anthracnose, powdery mildew, stem lesions, and other diseases affecting grasses, legumes, and herbaceous flowering species. Whenever more than one fungal taxon was isolated from a single host, each isolate was recorded separately to characterize the diversity of the fungal community associated with symptomatic plants.

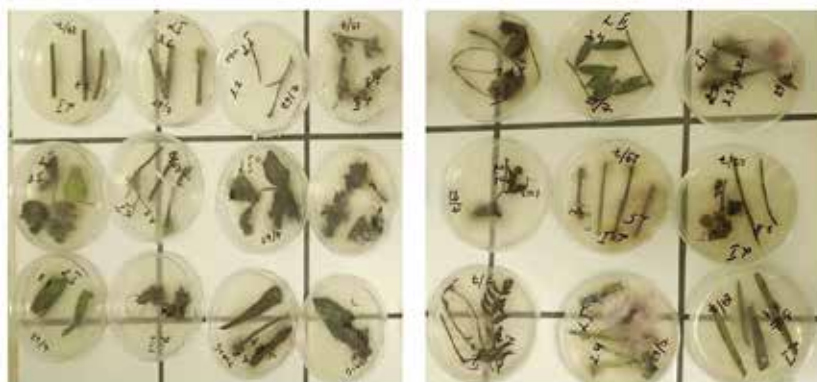


Fig. 3. Representative symptomatic plant samples cultured on potato dextrose agar (PDA) for the isolation of fungi associated with species-rich urban lawn mixtures

1.5. Data Evaluation

The results were evaluated using a descriptive comparative approach, with emphasis on the phytosanitary status of the experimental lawn mixtures, disease incidence among different botanical groups, and the diversity of fungi associated with symptomatic plants. Comparative analyses focused on differences in susceptibility among grasses, legumes, and herbaceous flowering species, as well as on the composition and frequency of fungal taxa isolated from infected plant material.

The identified fungi were grouped according to their taxonomic affiliation and ecological role, enabling comparison of fungal communities associated with the different experimental mixtures. Particular attention was given to the occurrence of the principal phytopathogenic fungi affecting urban lawn vegetation and their distribution among host species.

The results are presented in the following sections using descriptive text supported by photographs and graphical summaries illustrating disease symptoms, fungal diversity, and the comparative phytosanitary performance of the experimental lawn mixtures.

2. Results

2.1. General Health of Experimental Lawns

Phytopathological monitoring conducted during the 2022–2023 growing seasons demonstrated that the experimental species-rich lawn mixtures established at the Prague study sites maintained a generally satisfactory phytosanitary status throughout the observation period. Despite continuous exposure to typical urban environmental stressors, including elevated summer temperatures, periodic soil moisture deficits, atmospheric pollution, and intensive anthropogenic influence, the experimental vegetation established successfully and retained both its ecological functionality and ornamental value.

Most investigated plant species exhibited vigorous growth, good adaptation to local environmental conditions, and no visible signs of serious disease. Throughout the study period, no widespread disease outbreaks or extensive plant mortality were recorded. Disease symptoms, when observed, were generally localized and affected individual plants or species rather than entire plant communities. Consequently, the overall structure, species diversity, and visual quality of the experimental lawns remained stable.

Clear differences in phytosanitary performance were observed among the botanical groups included in the experimental mixtures. Grasses exhibited the highest level of resistance and remained largely free of visible disease symptoms during field observations. In contrast, several leguminous species and, to a lesser extent, some herbaceous flowering plants showed symptoms of fungal diseases, including powdery mildew, anthracnose, and leaf spot diseases. Nevertheless, disease incidence remained low, and infections did not significantly reduce plant vitality, persistence, or competitive ability within the plant communities.

The optimized grass–legume–herb mixture established during the final phase of the project demonstrated particularly good adaptation to urban conditions. The vegetation established rapidly, maintained a balanced species composition, and exhibited only isolated cases of disease throughout the monitoring period. These observations indicate that careful selection of plant species can enhance both the ecological stability and phytosanitary performance of species-rich urban lawns.

Overall, the results demonstrate that perennial mixtures composed of native grasses, legumes, and herbaceous flowering species can maintain good phytosanitary status under challenging urban conditions. The absence of severe disease outbreaks indicates that diverse plant communities are capable of providing stable, resilient, and aesthetically valuable vegetation suitable for sustainable urban green infrastructure. These findings provide the basis for the more detailed evaluation of disease incidence and fungal diversity presented in the following sections.

2.2. Disease Incidence among Plant Groups

The incidence and severity of diseases differed considerably among the botanical groups included in the experimental lawn mixtures, reflecting differences in host susceptibility under urban environmental conditions. Although all mixtures were exposed to similar climatic and anthropogenic stress factors, the occurrence and severity of disease symptoms varied markedly among grasses, legumes, and herbaceous flowering species.

Grasses exhibited the highest level of phytosanitary stability throughout the monitoring period. Most grass species remained free from visible disease symptoms and maintained normal growth and development during both growing seasons. Only isolated cases of minor leaf lesions were observed, and these had no measurable effect on plant vitality or the ornamental quality of the experimental lawns. The generally high resistance of grasses contributed substantially to the long-term stability of the species-rich plant communities.

In contrast, leguminous species proved to be the most susceptible botanical group. Powdery mildew caused by *Erysiphe trifolii* was the most frequently observed disease, particularly on *Trifolium incarnatum* and *Trifolium pratense* during the 2022 growing season. Characteristic white superficial mycelial colonies developed on infected leaves, reducing the photosynthetically active surface and impairing the aesthetic appearance of affected plants. In addition, anthracnose symptoms associated with *Colletotrichum* spp. were recorded on several clover plants, where they appeared as necrotic lesions affecting leaves and petioles. Representative symptoms of both diseases observed during field monitoring are presented in Figure 4.



Fig. 4. Representative symptoms of powdery mildew caused by *Erysiphe trifolii* (upper left and lower left) and anthracnose caused by *Colletotrichum* spp. (right) on clover (*Trifolium* spp.) leaves collected from the experimental lawn mixtures

Besides clover species, symptoms of anthracnose and leaf spot diseases were also observed on several other legumes, including *Medicago sativa*, *Medicago lupulina*, *Onobrychis viciifolia*, and *Securigera varia*. Although symptom expression varied among host species, infections generally remained localized and did not spread extensively within the experimental plots.

Disease symptoms were less frequent among the herbaceous flowering species and were usually restricted to individual plants. Occasional leaf spots, necrotic lesions, and minor tissue discoloration were recorded on *Achillea millefolium*, *Betonica officinalis*, *Cichorium intybus*, and *Silene vulgaris*. Despite these symptoms, the affected plants maintained satisfactory growth and continued to contribute to the structural diversity and ecological value of the lawn mixtures.

Overall, disease incidence remained low across all experimental sites. Differences in susceptibility were associated primarily with plant taxonomic group and host species rather than with the geographical location of the experimental plots. These findings indicate that plant species composition is one of the principal factors influencing the phytosanitary performance of species-rich urban lawns and should therefore be carefully considered when designing resilient vegetation mixtures for sustainable urban green infrastructure.

2.3. Diversity of Fungal Species Associated with Experimental Lawn Mixtures

Laboratory mycological analyses revealed a diverse assemblage of fungi associated with symptomatic plants collected from the experimental lawn mixtures. The recovered isolates comprised phytopathogenic fungi together with saprotrophic and opportunistic species that naturally occur in urban grassland ecosystems. These fungi were associated with a broad spectrum of symptoms, including leaf spots, anthracnose, stem lesions, powdery mildew, and other foliar diseases, reflecting the complexity of fungal communities colonizing species-rich urban vegetation.

Representative symptomatic plant tissues collected during field monitoring and cultured on potato dextrose agar (PDA) are shown in Figure 5. Incubation of infected plant material enabled the isolation of fungal colonies that were subsequently purified and identified using morphological and microscopic characteristics.

Mycological examination identified numerous fungal taxa belonging to both phytopathogenic and saprotrophic groups. The most frequently isolated genera included *Alternaria*, *Fusarium*, *Colletotrichum*, *Cladosporium*, *Stemphylium*, *Nigrospora*, *Epicoccum*, *Volutella*, and *Cymadothea*, together with a range of

less frequently occurring fungi. Powdery mildew caused by *Erysiphe trifolii* represented the most conspicuous disease observed under field conditions, whereas *Colletotrichum* spp. were associated with anthracnose symptoms affecting several leguminous species.



Fig. 5. Isolation of fungi from symptomatic plant tissues collected from experimental species-rich lawn mixtures. Representative samples cultured on potato dextrose agar (PDA) prior to fungal identification

Representative microscopic structures of the principal fungal genera identified during the investigation are presented in Figure 6. The considerable variation in colony morphology, conidial shape, pigmentation, and reproductive structures illustrates the taxonomic diversity of fungi associated with the investigated urban lawn vegetation and confirms the reliability of the morphological identification.

The composition of fungal communities differed among the investigated host plants. Leguminous species supported the richest assemblage of fungi, whereas grasses were colonized predominantly by fungi exhibiting relatively low pathogenic activity. Herbaceous flowering species showed an intermediate level of fungal occurrence. Despite the wide taxonomic spectrum of recovered fungi, infections generally remained localized and were not associated with substantial deterioration of plant vitality or the ornamental quality of the lawn communities.

The frequency of occurrence of individual fungal genera isolated during the two-year investigation is summarized in Figure 7. *Alternaria*, *Fusarium*, and *Cladosporium* were among the most frequently detected genera in both growing seasons, while *Epicoccum*, *Stemphylium*, *Nigrospora*, and several other fungi occurred regularly but at lower frequencies. In contrast, genera such as

Volutella, *Sordaria*, and *Trichotecium* were detected only occasionally. Besides phytopathogenic fungi, isolates belonging to saprotrophic and potentially antagonistic genera, including *Trichoderma*, *Chaetomium*, and *Gliocladium*, were also recovered. The coexistence of fungi with different ecological functions reflects the complexity of the microbial communities inhabiting species-rich urban lawns.

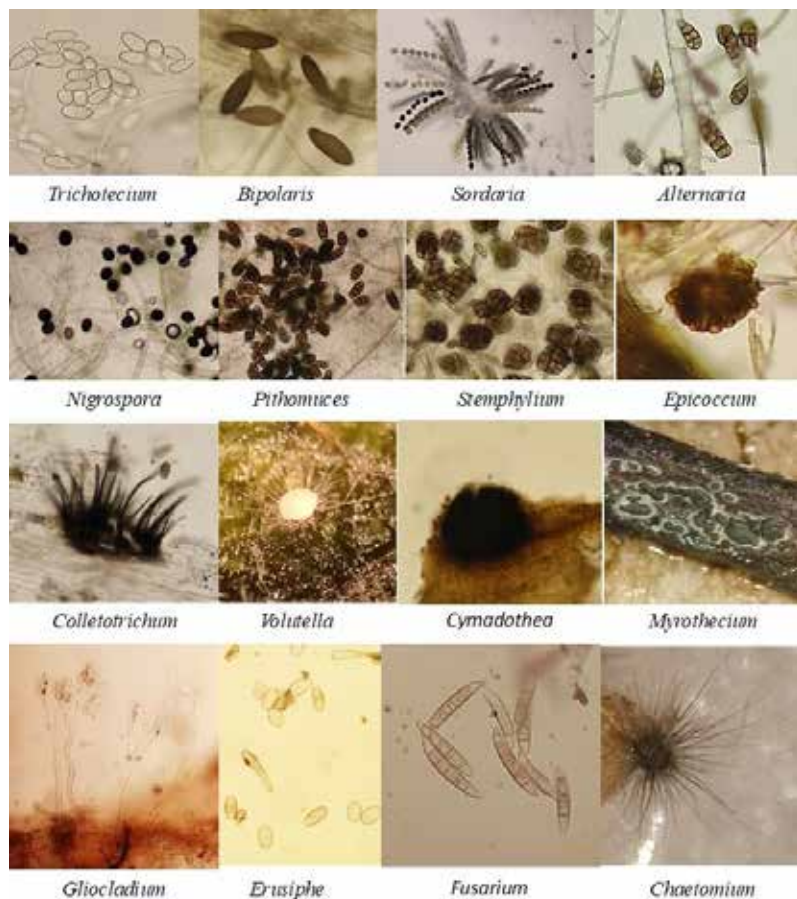


Fig. 6. Representative microscopic structures of selected fungal genera isolated from symptomatic plant tissues collected from experimental lawn mixtures in Prague during the 2022–2023 growing seasons

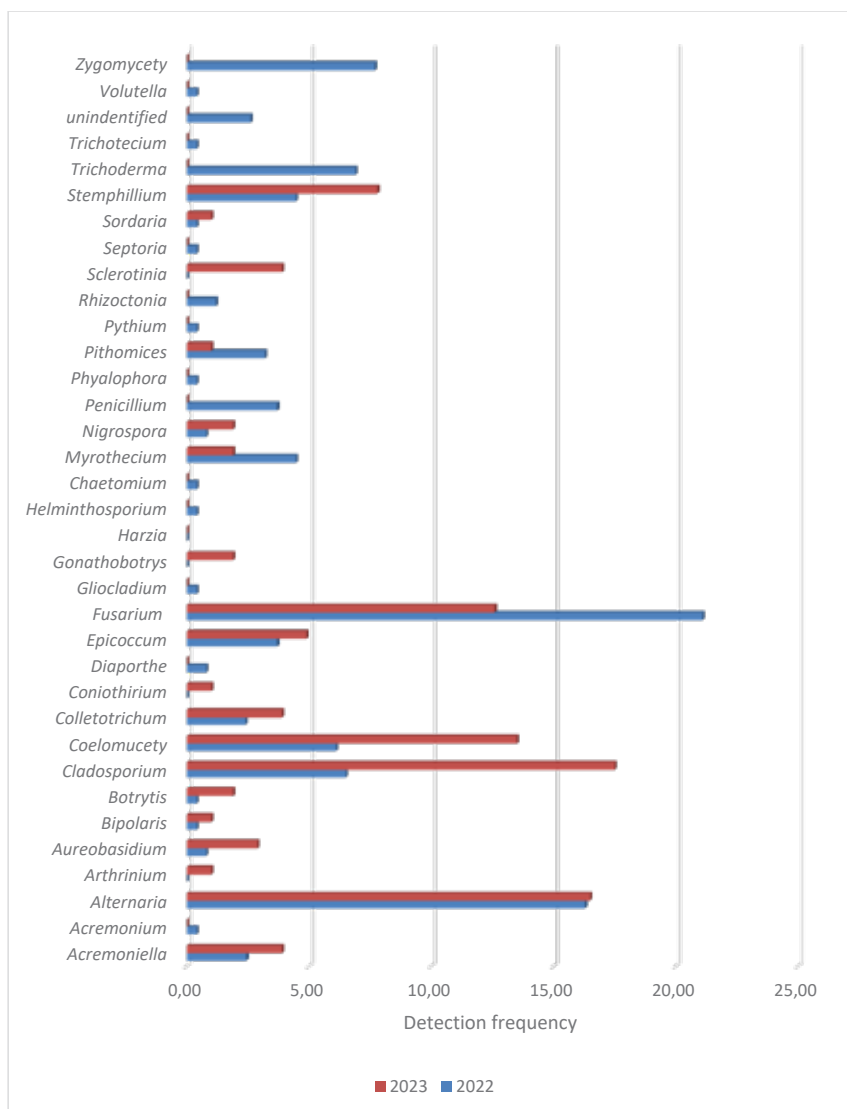


Fig. 7. Frequency of occurrence of fungal genera isolated from experimental species-rich lawn mixtures in Prague during the 2022–2023 growing seasons

Overall, the recovered fungal community represents the natural mycobiota associated with species-rich urban lawns under Central European conditions. Although fungal diversity was relatively high, disease severity remained low throughout the study, indicating that diverse perennial plant communities can maintain satisfactory phytosanitary stability despite the presence of numerous fungal taxa. These findings suggest that increasing plant diversity does not necessarily increase disease risk and further support the use of species-rich lawn mixtures as resilient components of sustainable urban green infrastructure.

2.4. Comparison among Experimental Mixtures

Comparative evaluation of the experimental lawn mixtures demonstrated clear differences in phytosanitary performance, reflecting the influence of botanical composition on disease susceptibility and overall vegetation stability. Although all mixtures were established under comparable environmental conditions and exposed to similar climatic and anthropogenic stress factors, their response to naturally occurring fungal infections differed according to the proportion of grasses, legumes, and herbaceous flowering species.

The low-growing flowering mixture exhibited the highest incidence of disease symptoms, primarily due to the greater susceptibility of clover species to powdery mildew caused by *Erysiphe trifolii*. Although infected plants developed characteristic white mycelial colonies on leaves during periods favorable for disease development, infections remained localized and did not spread throughout the plant community. Consequently, neither the ecological integrity nor the visual quality of the mixture was substantially affected.

The tall flowering mixture was characterized mainly by localized leaf spot diseases and anthracnose affecting several leguminous species. Fungal infections associated with *Colletotrichum*, *Alternaria*, and *Ascochyta* spp. occurred more frequently than in the other mixtures; however, disease severity remained low and had little influence on plant growth, species composition, or community stability.

Among the investigated mixtures, the drought-tolerant mixture demonstrated the highest level of phytosanitary stability. Disease symptoms occurred only sporadically despite prolonged exposure to elevated temperatures, limited soil moisture, and periodic drought. The predominance of drought-adapted grasses and perennial herbaceous species contributed to the resilience of this vegetation type and reduced the overall incidence of fungal diseases.

During the final phase of the project, an optimized grass–legume–herb mixture was developed using the best-performing species identified during field evaluation. Information panels installed at the experimental sites informed the public about the objectives and composition of the three experimental mixtures,

illustrating the practical application of the research within Prague's urban green infrastructure (Figure 8).



Fig. 8. Information panels describing the experimental low-growing flowering, tall flowering, and drought-tolerant lawn mixtures established along Prague tramway corridors as part of the project Genetic Resources for Cities and Landscape

The optimized mixture showed excellent adaptation to urban conditions, combining rapid establishment, balanced species composition, and consistently satisfactory phytosanitary status throughout the monitoring period. Only isolated disease symptoms were observed, indicating that careful selection of compatible species can substantially improve both ecological stability and resistance to naturally occurring fungal infections.

The long-term success of the optimized vegetation is illustrated in Figure 9, which shows a mature species-rich lawn established along the Tšiolkovského tramway extension in Prague. Several years after sowing, the vegetation had developed into a dense, visually attractive, and stable perennial community with a satisfactory phytosanitary condition despite continuous exposure to urban environmental stress.

The comparative evaluation indicates that species composition is one of the principal factors influencing disease development in species-rich urban lawns. Mixtures containing a higher proportion of susceptible leguminous species tended to support greater fungal diversity and higher disease incidence, whereas mixtures dominated by well-adapted grasses and drought-tolerant perennial species exhibited greater resistance to fungal infections. Nevertheless, none of the experimental mixtures experienced disease outbreaks severe enough to impair vegetation establishment, reduce biodiversity, or compromise long-term persistence under urban conditions.

Overall, all investigated mixtures fulfilled the phytosanitary requirements for sustainable urban landscaping, although their performance differed according to botanical composition. The drought-tolerant and optimized mixtures demonstrated the greatest resilience, whereas the low-growing flowering mixture required more careful consideration of susceptible leguminous species

during mixture design. These findings demonstrate that appropriately selected species-rich lawn mixtures can provide durable, biodiverse, and phytosanitarily stable vegetation suitable for modern urban green infrastructure and form the basis for the broader ecological interpretation presented in the following Discussion.

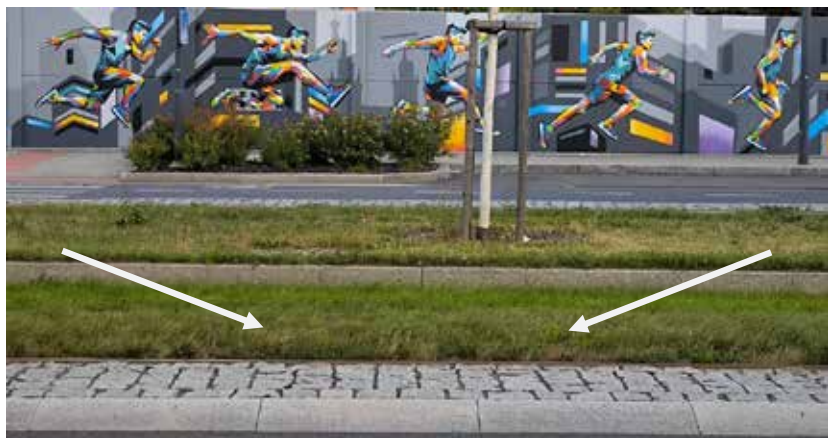


Fig. 9. Established species-rich lawn along the Tsiolkovského tramway extension, Prague, demonstrating successful long-term adaptation and satisfactory phytosanitary performance under urban conditions (summer 2026)

3. Discussion

The present study demonstrates that species-rich urban lawn mixtures composed of native grasses, legumes, and herbaceous flowering plants can maintain satisfactory phytosanitary status under the challenging environmental conditions of a large metropolitan area. Despite continuous exposure to urban stressors, including elevated summer temperatures, periodic soil moisture deficits, atmospheric pollution, and intensive anthropogenic disturbance, the experimental vegetation remained healthy throughout the monitoring period. No severe disease outbreaks or extensive plant mortality were recorded, indicating that species-rich urban lawns can combine ecological functionality with long-term phytosanitary stability^{19,20}.

¹⁹ Ignatieva M. et al. Lawns in cities: from a globalised urban green space phenomenon to sustainable nature-based solutions. *Land* 2020, 9, 73.

²⁰ Wiewióra B., Żurek G. Amenity grasses—a short insight into species, their applications and functions. *Agronomy*. 2023. T. 13. №. 4. P. 1164.

These findings support the growing recognition that increasing plant diversity enhances the resilience of urban green infrastructure²¹. Compared with conventional species-poor turfgrass, diverse perennial plant communities provide greater ecological stability by reducing the dominance of susceptible host species, improving resource utilization, and increasing resistance to both abiotic and biotic stress²². Similar relationships between plant diversity and ecosystem resilience have been reported for natural and semi-natural grasslands, where greater species richness is associated with improved resistance to pests, diseases, and environmental disturbances.

One of the most consistent findings of this study was the difference in disease susceptibility among botanical groups. Grasses remained largely free from visible disease symptoms throughout the monitoring period, whereas leguminous species exhibited greater susceptibility to powdery mildew, anthracnose, and leaf spot diseases^{23,24}. These observations agree with previous studies demonstrating that legumes generally support a more diverse community of fungal pathogens than grasses because of differences in host physiology, canopy structure, and species-specific host–pathogen interactions²⁵.

Although laboratory analyses revealed a diverse assemblage of fungi associated with symptomatic plants, most taxa occurred at relatively low frequencies and caused only localized infections. Importantly, the recovered fungal community comprised not only phytopathogenic fungi but also saprotrophic and potentially antagonistic species^{26,27}. The coexistence of these ecological groups indicates that species-rich urban lawns support complex microbial communities that may contribute to the natural regulation of plant diseases through competition, niche occupation, and other ecological interactions. Consequently, high fungal diversity should not necessarily be interpreted as an increased phytopathological risk.

A particularly important practical outcome of the study was the successful long-term establishment of the experimental lawn mixtures along Prague

²¹ Aronson M. F. J. et al. Biodiversity in the city: key challenges for urban green space management. *Frontiers in Ecology and the Environment*. 2017. T. 15. №. 4. P. 189–196.

²² Stewart G. H. et al. Urban Biotopes of Aotearoa New Zealand (URBANZ)(I): composition and diversity of temperate urban lawns in Christchurch. *Urban Ecosystems*. 2009. T. 12. №. 3. P. 233–248.

²³ Kobes M. et al. The occurrence of *Erysiphe trifolii* on *Lathyrus pratensis* in a foothill area of South Bohemia. *Journal of Agrobiology*. 2011. T. 28. №. 1. P. 25.

²⁴ Kiecana I., Cegiello M., Mielniczuk E. Fungi occurred on turfgrasses in lawn maintenance. *Acta Scientiarum Polonorum. Hortorum Cultus*. 2015. T. 14. №. 3.

²⁵ Simon U. K., Groenewald J. Z., Crous P. W. *Cymadothea trifolii*, an obligate biotrophic leaf parasite of *Trifolium*, belongs to *Mycosphaerellaceae* as shown by nuclear ribosomal DNA analyses. *Persoonia-Molecular Phylogeny and Evolution of Fungi*. 2009. T. 22. №. 1. P. 49–55.

²⁶ Watanabe, T. *Pictorial Atlas of Soil and Seed Fungi*; CRC Press: Boca Raton, FL, USA, 2002. 504 p.

²⁷ Seifert, K. A., Morgan-Jones, G. A., Gams, W., Kendrick, B. *The Genera of Hyphomycetes*; CBS Biodiversity (Series 10); CBS Fungal Diversity Centre: Utrecht, The Netherlands, 2011. 997 p.

tramway corridors. The comparison between newly established vegetation and mature perennial stands demonstrated that carefully designed species-rich mixtures can develop into stable, visually attractive, and phytosanitarily healthy plant communities under intensive urban conditions. These findings confirm that tramway corridors can function not only as transportation infrastructure but also as valuable components of urban green infrastructure, contributing to biodiversity conservation, climate-change adaptation, and the improvement of urban environmental quality²⁸.

From a practical perspective, the results indicate that careful selection of compatible native grasses, legumes, and perennial herbaceous species is essential for developing resilient urban lawns. Regular phytopathological monitoring remains an important management tool for the early detection of emerging diseases; however, the low disease incidence observed throughout the study suggests that well-designed species-rich lawns can be maintained with minimal reliance on fungicides. This approach is consistent with current principles of sustainable urban landscaping and integrated plant protection.

Although the present investigation was limited to experimental sites within Prague and covered two consecutive growing seasons, the results provide valuable evidence supporting the wider application of species-rich lawn mixtures in urban environments. Further long-term studies conducted under different climatic conditions would improve our understanding of the interactions among vegetation diversity, fungal communities, and environmental stress in urban ecosystems.

Overall, the findings demonstrate that species-rich lawn mixtures based on native plant species represent a sustainable alternative to conventional urban turfgrass. Their ability to maintain biodiversity, satisfactory phytosanitary status, and long-term ecological stability supports their wider use in tramway corridors, public parks, residential areas, and other multifunctional urban green spaces. The integrated approach adopted in this study, combining field phytopathological monitoring with laboratory mycological diagnostics, provides a practical framework for evaluating the health and resilience of urban grassland ecosystems.

CONCLUSIONS

The present study demonstrated that species-rich urban lawn mixtures based on native grasses, legumes, and herbaceous flowering plants can maintain satisfactory phytosanitary status under the environmental conditions of a large

²⁸ Winkler J. et al. Green space in an extremely exposed part of the city center «Aorta of Warsaw» – Case study of the urban lawn. *Urban Ecosystems*. 2023. T. 26. №. 5. P. 1225–1238.

metropolitan area. Despite continuous exposure to multiple urban stress factors, disease incidence remained generally low, confirming the suitability of these mixtures for sustainable urban landscaping.

The investigation revealed clear differences in disease susceptibility among botanical groups. Grasses exhibited the highest phytosanitary stability, whereas leguminous species were more frequently affected by fungal diseases, particularly powdery mildew and anthracnose. Laboratory mycological analyses demonstrated that the experimental lawns supported a diverse assemblage of fungi; however, most infections remained localized and did not compromise the ecological stability or visual quality of the vegetation.

Overall, the results confirm that appropriately designed species-rich lawn mixtures represent a resilient and environmentally sustainable alternative to conventional urban turfgrass. The integration of phytopathological monitoring with mycological diagnostics provides valuable information for the management of urban green infrastructure and supports the long-term development of biodiverse, low-maintenance, and climate-resilient urban landscapes.

SUMMARY

Urban green infrastructure plays an increasingly important role in sustainable city development by improving environmental quality, mitigating climate change impacts, supporting biodiversity, and enhancing human well-being. Species-rich urban lawns composed of grasses, legumes, and herbaceous flowering plants are increasingly used as sustainable alternatives to conventional turfgrass because they require lower maintenance while providing a wide range of ecosystem services. However, the long-term sustainability of these vegetation communities depends not only on appropriate species selection and adaptation to urban environments but also on their phytosanitary condition and resistance to plant pathogens. This chapter presents the results of a comprehensive phytopathological assessment of experimental species-rich lawn mixtures established along tramway corridors in Prague, Czech Republic, within the framework of the project *Genetic Resources for Cities and Landscape*. Field phytopathological monitoring and laboratory mycological analyses were conducted to evaluate disease incidence, compare the health status of grasses, legumes, and herbaceous species, and identify fungal taxa associated with naturally occurring infections under urban conditions. The experimental lawns maintained a generally satisfactory phytosanitary status throughout the monitoring period despite exposure to multiple abiotic and biotic stress factors. Disease symptoms occurred predominantly on leguminous species, whereas grasses exhibited substantially greater resistance to fungal infections. Mycological analyses revealed a diverse assemblage of fungi,

including representatives of the genera *Alternaria*, *Fusarium*, *Colletotrichum*, *Ascochyta*, *Stemphylium*, *Nigrospora*, and several others. Although numerous fungal taxa were identified, most infections remained localized and did not compromise the stability, biodiversity, or aesthetic quality of the experimental vegetation. The results demonstrate that appropriately designed species-rich lawn mixtures based on native plant species combine high ecological value with satisfactory phytosanitary performance and are well suited for sustainable urban landscaping. The integration of field phytopathological monitoring with laboratory mycological diagnostics provides a reliable framework for evaluating the health and long-term resilience of species-rich urban lawns and supports evidence-based management of multifunctional urban green infrastructure under changing environmental conditions.

Acknowledgements

This work was supported by the Technology Agency of the Czech Republic (TACR) through Project No. SS01020023, Germplasm for Cities and Landscape. The authors gratefully acknowledge the financial support provided by this project.

Bibliography

1. Aronson M. F. J. et al. Biodiversity in the city: key challenges for urban green space management. *Frontiers in Ecology and the Environment*. 2017. Vol. 15, no. 4. P. 189–196. DOI: 10.1002/fee.1480
2. Hsiang T., Matsumoto N., Millett S. M. Biology and management of *Tiphula* snow mold of turfgrass. *Plant Disease*. 1999. Vol. 3, no. 9. P. 788–798.
3. Ignatieva M. et al. Lawn as a cultural and ecological phenomenon: a conceptual framework for transdisciplinary research. *Urban Forestry & Urban Greening*. 2015. Vol. 14, no. 2. P. 383–387. DOI: 10.1016/j.ufug.2015.04.003
4. Ignatieva M. et al. Lawns in cities: from a globalised urban green space phenomenon to sustainable nature-based solutions. *Land*. 2020. Vol. 9. Art. 73. DOI: <https://doi.org/10.3390/land9030073>
5. Kiecana I., Cegiello M., Mielniczuk E. Fungi occurred on turfgrasses in lawn maintenance. *Acta Scientiarum Polonorum. Hortorum Cultus*. 2015. Vol. 14, no. 3.
6. Kobes M. et al. The occurrence of *Erysiphe trifolii* on *Lathyrus pratensis* in a foothill area of South Bohemia. *Journal of Agrobiology*. 2011. Vol. 28, no. 1. P. 25.
7. Miller G. L. Jr. Turfgrass diseases. *Agrios' Plant Pathology*. Academic Press, 2024. P. 823–828. DOI: 10.1016/B978-0-12-822429-8.00041-8

8. Parra L. et al. Evaluating the impact of natural products to improve the sustainability of urban lawns. *Cities*. 2024. Vol. 150. Art. 105097. DOI: 10.1016/j.cities.2024.105097
9. Robbins P. *Lawn People: How Grasses, Weeds, and Chemicals Make Us Who We Are*. Temple University Press, 2012.
10. Zelená tramvajová trať = cirkulární ekonomika a přizpůsobení se klimatu = kolejový absorbér hluku s funkcí retence vody BRENS STERED. *Silnice železnice*. 2019. URL: <https://silnice-zeleznice.cz/zeleznice/zelena-tramvajova-trat-cirkularni-ekonomika-a-prizpusobeni-se-klimatu-kolejovy-absorber-hluku-s-funkci-retence-vody-brens-stered-130> (дата звернення: 18.06.2026).
11. Simon U. K., Groenewald J. Z., Crous P. W. *Cymadothea trifolii*, an obligate biotrophic leaf parasite of *Trifolium*, belongs to Mycosphaerellaceae as shown by nuclear ribosomal DNA analyses. *Persoonia: Molecular Phylogeny and Evolution of Fungi*. 2009. Vol. 22, no. 1. P. 49–55. DOI: 10.3767/003158509X425350
12. Stewart G. H. et al. Urban Biotopes of Aotearoa New Zealand (URBANZ) (I): composition and diversity of temperate urban lawns in Christchurch. *Urban Ecosystems*. 2009. Vol. 12, no. 3. P. 233–248. DOI: 10.1007/s11252-009-0098-7
13. Vargas J. M. *Management of Turfgrass Diseases*. Boca Raton: CRC Press, 1994. 320 p.
14. Winkler J. et al. Green space in an extremely exposed part of the city center «Aorta of Warsaw»: case study of the urban lawn. *Urban Ecosystems*. 2023. Vol. 26, no. 5. P. 1225–1238. DOI: 10.1007/s11252-023-01380-6
15. Wiewióra B., Żurek G. Amenity grasses: a short insight into species, their applications and functions. *Agronomy*. 2023. Vol. 13, no. 4. Art. 1164. DOI: <https://doi.org/10.3390/agronomy13041164>
16. Watanabe T. *Pictorial Atlas of Soil and Seed Fungi*. Boca Raton: CRC Press, 2002. 504 p. DOI: <https://doi.org/10.1201/9781420040821>
17. Seifert K. A., Morgan-Jones G. A., Gams W., Kendrick B. *The Genera of Hyphomycetes*. Utrecht: CBS Fungal Diversity Centre, 2011. 997 p. (CBS Biodiversity Series; 10). DOI: 10.3767/003158511X617435
18. Tramvajová trať Divoká Šárka – Dědinská. URL: <https://mhd86.cz/2015/01/06/tramvajova-trat-divoka-sarka-dedinska/> (дата звернення: 15.06.2026).

Information about the authors:

Golosna Lesia,

Candidate of Agricultural Sciences
Czech Agrifood Research Center
507/73, Drnovská Street, Praha, Czech Republic;
Institute of Plant Protection of the National Academy
of Agrarian Sciences of Ukraine
33, Vasilkivska Street, Kyiv, Ukraine

Bobrova Olena,

Candidate of Biological Sciences
Czech Agrifood Research Center
507/73, Drnovská Street, Praha, Czech Republic;
Institute for Problems of Cryobiology and Cryomedicine
of the National Academy of Sciences of Ukraine
23, Pereyaslavskaya Street, Kharkiv, Ukraine

Afanasieva Oksana,

Candidate of Agricultural Sciences
Institute of Plant Protection of the National Academy
of Agrarian Sciences of Ukraine
33, Vasilkivska Street, Kyiv, Ukraine

Holubec Vojtech,

Candidate of Sciences
Czech Agrifood Research Center
507/73, Drnovská Street, Praha, Czech Republic

NATURAL ANTIMICROBIAL COMPOUNDS OF PLANT ORIGIN: MECHANISMS OF ACTION AND PROSPECTS FOR APPLICATION AGAINST BACTERIAL INFECTION AGENTS

Derkach Tetiana

DOI <https://doi.org/10.30525/978-9934-26-714-7-3>

INTRODUCTION

The increasing prevalence of antimicrobial resistance among bacterial pathogens has become one of the most urgent challenges in modern medicine. The excessive and inappropriate use of synthetic antibiotics has accelerated the emergence of multidrug-resistant strains, creating a need to develop new antimicrobial agents. Natural plant-derived products have a long history of use in traditional medical systems worldwide, and modern research increasingly confirms their pharmacological efficacy.¹ From a fundamental biological perspective, plant secondary metabolites evolved primarily as chemical defences against competing microorganisms, making the biodiversity of medicinal flora a valuable bioresource studied at the intersection of applied biology and ecology. Investigating their biological functions advances our understanding of plant–microbe interactions in the environment. It aligns with the «One Health» concept, which recognises the interconnection between human, animal, and environmental health. Such an ecologically grounded approach reinforces the relevance of this research to fundamental and applied studies in biology and ecology. So, phytochemicals are attracting considerable attention as potential antimicrobial agents.

Secondary metabolites of medicinal plants represent a vast library of chemically diverse compounds with proven antimicrobial, anti-inflammatory, and antioxidant properties. Among them, phenolic compounds, flavonoids, terpenoids, and alkaloids constitute the predominant classes of biologically active substances. Phenols and their derivatives, including flavonoids and phenolic acids, form one of the largest groups of plant secondary metabolites and are

¹ Newman D. J., Cragg G. M. Natural products as sources of new drugs from 1981 to 2019. *Journal of Natural Products*. 2020. Vol. 83, No 3. P. 770–803. DOI: 10.1021/acs.jnatprod.9b01285

present in virtually all species of medicinal plants.² Flavonoids, a subclass of phenolic compounds, exhibit diverse structural variations and possess multiple mechanisms of antimicrobial action.³ Terpenoids and their derivatives are found predominantly in essential oils⁴ and have well-documented antibacterial properties. Alkaloids, nitrogen-containing compounds with complex structures, represent another class of secondary metabolites with antimicrobial potential.⁵ The chemical diversity and multiplicity of mechanisms of action of all these compounds make them attractive candidates for combating antibiotic-resistant bacteria.

However, comparative studies of mechanisms across several classes of compounds and bacterial species remain limited. Understanding the mechanisms by which these plant compounds exert their antimicrobial effects is essential for rational drug development and the optimisation of therapeutic applications. Unlike single-target antibiotics, plant metabolites demonstrate polypharmacological activity, which may reduce the likelihood of resistance development. In addition, the use of plant compounds in topical applications, such as dermatology and wound treatment, offers clear advantages, including reduced systemic toxicity and improved tolerability.⁶

This study aims to investigate and compare the mechanisms of antimicrobial action of flavonoids, terpenoids, phenols, and alkaloids derived from medicinal plants against three clinically significant bacterial species: *S. aureus*, *E. coli*, and *C. acnes*. This study seeks to elucidate patterns of antimicrobial activity specific to individual compounds and mechanisms, characterise differential susceptibility among bacterial species, and provide scientific evidence supporting the therapeutic potential of these plant compounds as alternatives or adjuncts to conventional antimicrobial agents.

1. Main Antibacterial Mechanisms of Action of Phytochemicals

1.1. Studied pathogenic bacteria and biologically active compounds

Staphylococcus aureus is a facultatively anaerobic Gram-positive coccid bacterium and one of the most important bacterial pathogens in humans. It

² Taguri T., Tanaka T., Kouno I. Antimicrobial activity of 10 different plant polyphenolic compounds against bacteria associated with periodontal disease in vitro. *Journal of Periodontal Research*. 2024. Vol. 59, No 3. P. 457–469. DOI: 10.1248/bpb.27.1965

³ Cushnie T. P., Lamb A. B. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*. 2021. Vol. 26, No 5. P. 343–356. DOI: 10.1016/j.ijantimicag.2005.09.002

⁴ Tian F., Woo S. Y., Lee S. Y., Park S. B., Zheng Y., Chun H. S. Antifungal activity of essential oil and plant-derived natural compounds against *Aspergillus flavus*. *Antibiotics*. 2022. Vol. 11, No 12. Art. 1727. DOI: 10.3390/antibiotics11121727

⁵ Zhang X. Z. et al. Natural alkaloids as antimicrobial agents: mechanisms, potentials and challenges. *Molecules*. 2026. Vol. 31, No 7. Art. 1204. DOI: 10.3390/molecules31071204

⁶ Fais A., Era B. Phytochemical composition and biological activity. *Plants*. 2024. Vol. 13, No 3. Art. 331. DOI: 10.3390/plants13030331

colonises the skin and the anterior nasal cavity in many healthy individuals. However, when the body's barrier functions are impaired, this can lead to a wide range of diseases, including impetigo, abscesses, cellulitis, osteomyelitis, endocarditis, and pneumonia.⁷

The clinical significance of *S. aureus* is greatly increased by its ability to acquire antibiotic resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to most β -lactam antibiotics and are associated with various infections.⁸ *S. aureus* can also form biofilms on the surfaces of wounds, catheters, and other medical devices. Bacteria within biofilms exhibit reduced susceptibility to antibiotics and immune defence mechanisms, which complicate the eradication of infection.⁹ Plant phytochemical compounds, such as flavonoids, terpenoids, phenolic compounds, and alkaloids,⁵ are actively being investigated as potential agents against *S. aureus*.

Escherichia coli is a Gram-negative, facultatively anaerobic bacterium that normally inhabits the intestines. Many strains are harmless commensals, but pathogenic variants can cause urinary tract infections, gastrointestinal infections, and sepsis, with an increasing prevalence of strains producing extended-spectrum β -lactamases and carbapenem-resistant strains. Important pathogenic groups include enterotoxigenic, enterohemorrhagic, enteropathogenic, enteroaggregative, uropathogenic, and enteroinvasive *E. coli*.¹⁰ Unlike Gram-positive bacteria, *E. coli* has an outer membrane with lipopolysaccharide, limiting the penetration of hydrophobic and large molecules. The periplasmic space contains enzymes such as β -lactamases, while the inner membrane harbours efflux systems that export toxic compounds.¹⁰ These features often make *E. coli* less susceptible to many plant extracts than *S. aureus*.

Cutibacterium acnes – formerly known as *Propionibacterium acnes*, although both names are found in the literature – is a slow-growing, anaerobic Gram-positive bacterium of the normal microbiota of sebaceous areas of the skin.¹¹ *C. acnes* is primarily associated with acne vulgaris, a highly prevalent dermatological disease affecting millions of people worldwide. Some strains are more strongly associated with acne than others, indicating that pathogenicity

⁷ Sutton J. A. F. et al. *Staphylococcus aureus* cell wall structure and dynamics during host-pathogen interaction. *PLoS Pathogens*. 2021. Vol. 17, No 3. Art. e1009468. DOI: 10.1371/journal.ppat.1009468

⁸ Chalmers S. J., Wylam M. E. Methicillin-resistant *Staphylococcus aureus* infection and treatment options. *Methods in Molecular Biology*. 2020. Vol. 2069. P. 229–251. DOI: 10.1007/978-1-4939-9849-4_16

⁹ Peng Q. et al. A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. *Antibiotics*. 2023. Vol. 12, No 1. Art. 12. DOI: 10.3390/antibiotics12010012

¹⁰ Naidoo N., Zishiri O.T. Presence, Pathogenicity, Antibiotic Resistance, and Virulence Factors of *Escherichia coli*: A Review. *Bacteria*. 2025. Vol. 4, No 1. Art. 16. DOI: 10.3390/bacteria4010016

¹¹ Cogen A. L., Nizet V., Gallo R. L. Skin microbiota: A source of disease or defence? *British Journal of Dermatology*. 2023. Vol. 189, No 3. P. 299–309. DOI: 10.1111/j.1365-2133.2008.08437.x

depends on strain-level differences rather than the mere presence or absence of the microorganism.¹² *C. acnes* also forms biofilms, contributing to follicular persistence and reducing susceptibility to antimicrobial agents. The potential effect of medicinal compounds in acne may be twofold. First, they may directly inhibit the growth of *C. acnes*. Second, many phytochemicals possess anti-inflammatory, antioxidant, or skin-modulating properties.

Structural differences between Gram-positive and Gram-negative bacteria, associated with differences in cell wall architecture and outer membrane composition, create distinct barriers to the penetration of antimicrobial agents, making these bacteria suitable models for evaluating compound efficacy. In addition, *C. acnes* presents unique challenges related to biofilm formation and anaerobic metabolism, which are increasingly recognised as important virulence factors.

The paper considers antibacterial effects for 4 classes of phytochemicals.

Flavonoids are distributed in fruits, vegetables, and other plant materials. They usually contain a C6-C3-C6 carbon skeleton consisting of two aromatic rings connected by a three-carbon bridge. Flavonoids are divided into several structural subclasses, such as flavonols, flavones, flavanones, isoflavonoids, catechins, anthocyanins, etc.^{13,14} Examples of antibacterial flavonoids include quercetin, kaempferol, apigenin, luteolin, baicalin, naringenin, EGCG, catechin, and rutin.

Terpenoids, also known as isoprenoids, are one of the largest groups of plant secondary metabolites. They are derived from isoprene units and include monoterpenes, sesquiterpenes, diterpenes, triterpenes, and related compounds. Many terpenoids are major constituents of essential oils obtained from aromatic medicinal herbs.¹⁵ Examples of antibacterial terpenoids include thymol, carvacrol, menthol, linalool, terpinen-4-ol, α -pinene, 1,8-cineole, borneol, geraniol, and citral.

Phenols and polyphenols. The term «phenolic compounds» refers to plant metabolites containing one or more hydroxyl groups attached to aromatic rings. This broad category partially overlaps with flavonoids but also includes simple phenols, phenolic acids, tannins, stilbenes, lignans, and other polyphenols.

¹² Mayslich C., Grange P. A., Dupin N. *Cutibacterium acnes* as an opportunistic pathogen: an update of its virulence-associated factors. *Microorganisms*. 2021. Vol. 9, No 2. Art. 303. DOI: 10.3390/microorganisms9020303

¹³ Farhadi F. et al. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. *Phytotherapy Research*. 2019. Vol. 33, No 1. P. 13–40. DOI: 10.1002/ptr.6208

¹⁴ Górniak I., Bartoszewski R., Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*. 2019. Vol. 18, No 1. P. 241–272. DOI: 10.1007/s11101-018-9591-z

¹⁵ Yamaguchi T. Antibacterial effect of the combination of terpenoids. *Archives of Microbiology*. 2022. Vol. 204. Art. 520. DOI: 10.1007/s00203-022-03142-y

In the context of medicinal herbs, phenols and polyphenols contribute to antimicrobial, antioxidant, anti-inflammatory, and astringent effects.^{16,17} Examples of antibacterial phenolic compounds include eugenol, gallic acid, caffeic acid, rosmarinic acid, ferulic acid, chlorogenic acid, curcumin, tannic acid, ellagic acid, and resveratrol.

Alkaloids are nitrogen-containing secondary metabolites that exhibit considerable structural diversity. Many alkaloids exhibit strong biological activity, and some are used in drug development.¹⁸ Examples of antibacterial alkaloids include berberine, palmatine, sanguinarine, chelerythrine, piperine, harmine, tetrandrine, etc.

Plant-derived antibacterial compounds usually act simultaneously on multiple cellular targets, distinguishing them from synthetic antibiotics, which have a single-target mechanism of action. This polypharmacological activity makes phytochemicals promising sources of new antibacterial agents, antibiotic adjuvants, and antivirulence compounds.¹⁹ Ten major mechanisms of antibacterial action of phytochemicals were grouped into four functional categories (Table 1). They all affect *S. aureus*, *E. coli*, and *C. acnes*, but their effectiveness varies depending on the bacterial cell envelope structure, metabolic state, growth conditions, and biofilm-forming capacity.

Table 1

Classification of antibacterial mechanisms of phytochemical compounds

Group	Mechanism	Main target
I. Structural	Membrane damage; disruption of the cell envelope	Cell integrity
II. Metabolic	Inhibition of DNA/RNA synthesis; inhibition of protein synthesis; energy dysfunction; oxidative stress	Intracellular processes
III. Regulatory	Efflux inhibition; suppression of quorum sensing; antibiofilm activity	Adaptation and virulence
IV. Synergistic	Potentialiation of antibiotics	Enhancement of therapy

¹⁶ De Rossi L., Rocchetti G., Lucini L., Rebecchi A. Antimicrobial potential of polyphenols: mechanisms of action and microbial responses – a narrative review. *Antioxidants*. 2025. Vol. 14, No 2. Art. 200. DOI: 10.3390/antiox14020200

¹⁷ De Rossi L., Rocchetti G., Lucini L., Rebecchi A. Antimicrobial potential of polyphenols: mechanisms of action and microbial responses – a narrative review. *Antioxidants*. 2025. Vol. 14, No 2. Art. 200. DOI: 10.3390/antiox14020200

¹⁸ Faisal S. et al. Alkaloids as potential antivirals: a comprehensive review. *Natural Products and Bioprospecting*. 2023. Vol. 13. Art. 4. DOI: 10.1007/s13659-022-00366-9

¹⁹ Ghosh S. et al. A comprehensive review of antimicrobial agents against clinically important bacterial pathogens: prospects for phytochemicals. *Phytotherapy Research*. 2025. Vol. 39. P. 138–161. DOI: 10.1002/ptr.8365

1.2. Structural Group: Two Active Mechanisms

Damage to membrane integrity is the most common mechanism of action for terpenoids and phenols. Hydrophobic phytochemical compounds integrate into the lipid bilayer, disrupt lipid organisation, and cause leakage of potassium ions, ATP, and nucleic acids.²⁰ The Gram-positive bacteria *S. aureus* and *C. acnes* are more susceptible to this mechanism because they lack an outer membrane. *E. coli* is protected by a lipopolysaccharide barrier, which limits the penetration of hydrophobic compounds.²¹

Disruption of the cell envelope is an effect exhibited by certain flavonoids (e.g. baicalein and apigenin) and alkaloids (berberine), which affect peptidoglycan biosynthesis.^{22,23} Curcumin inhibits the polymerisation of the FtsZ protein, thereby disrupting bacterial cytokinesis.²⁴ Compounds that weaken the cell wall can increase susceptibility to β -lactam antibiotics.²⁵

1.3. Metabolic Group: Four Active Mechanisms

Inhibition of nucleic acid synthesis. Flavonoids and alkaloids can inhibit DNA gyrase, topoisomerase IV, and replication.²⁶ The planar aromatic structure of berberine, palmatine, and sanguinarine promotes DNA intercalation. Curcumin may affect the organisation of the bacterial nucleoid.²⁷ In Gram-negative bacteria, the effectiveness of this mechanism is limited by efflux pumps.

Inhibition of enzymes and protein synthesis. Phytochemical compounds can inhibit enzymes involved in energy metabolism, fatty acid synthesis, and

²⁰ Kumar G., Engle K. Natural products acting against *S. aureus* through membrane and cell wall disruption. *Natural Product Reports*. 2023. Vol. 40, No 10. P. 1608–1646. DOI: 10.1039/D2NP00084A

²¹ Lou Z. et al. The antibacterial mechanism and cell damage mechanism of natural antibacterial agents from different sources and their applications in food. *Journal of Food Measurement and Characterization*. 2026. Vol. 20. P. 1362–1386. DOI: 10.1007/s11694-025-03794-6

²² Mu L. et al. The antibacterial activity, action mechanisms and prospects of baicalein as an antibacterial adjuvant. *World Journal of Microbiology and Biotechnology*. 2025. Vol. 41. Art. 304. DOI: 10.1007/s11274-025-04508-2

²³ Boberek J. M., Stach J., Good L. Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. *PLoS One*. 2010. Vol. 5, No 10. Art. e13745. DOI: 10.1371/journal.pone.0013745

²⁴ Rai D. et al. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochemical Journal*. 2008. Vol. 410, No 1. P. 147–155. DOI: 10.1042/BJ20070891

²⁵ Xu S. et al. Plant flavonoids with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *ACS Infectious Diseases*. 2024. Vol. 10, No 9. P. 3086–3097. DOI: 10.1021/acsinfecdis.4c00292

²⁶ Allison D. G., Lambert P. A. Modes of action of antibacterial agents. *Molecular Medical Microbiology* / ed. by Y. W. Tang et al. 3rd ed. Academic Press, 2024. P. 597–614. DOI: 10.1016/B978-0-12-818619-0.00133-7

²⁷ Davidova S., Galabov A. S., Satchanska G. Antibacterial, antifungal, antiviral activity, and mechanisms of action of plant polyphenols. *Microorganisms*. 2024. Vol. 12, No 12. Art. 2502. DOI: 10.3390/microorganisms12122502

virulence factor production.²⁸ Flavonoids interact with enzyme active sites through hydrogen bonds and hydrophobic interactions.²⁹ Tannins precipitate surface proteins, while eugenol and phenolic acids denature proteins and affect redox balance.³⁰ For *C. acnes*, inhibition of lipases is particularly important, since these enzymes hydrolyse sebum triglycerides into pro-inflammatory free fatty acids.

Disruption of energy metabolism. Membrane-active terpenoids, such as carvacrol and thymol, collapse the proton gradient, reduce ATP synthesis, and impair nutrient transport.¹⁶ Flavonoids can inhibit respiratory-chain enzymes and ATP synthase. This mechanism is relevant to all three pathogens.

Induction of oxidative stress. Under certain conditions, phenolic compounds can generate reactive oxygen species (ROS) or disrupt bacterial antioxidant systems.^{31,32} Flavonoids and phenolic acids chelate metal ions and, in the presence of transition metals, promote ROS formation. This mechanism may enhance the effectiveness of antibiotics and immune defence mechanisms.

1.4. Regulatory Group: Three Active Mechanisms

Inhibition of efflux pumps. Efflux pumps, such as NorA in *S. aureus* and AcrAB–TolC in *E. coli*, export antibiotics and toxic compounds from the cell. Some plant compounds inhibit their activity, increasing the intracellular concentration of antimicrobial agents.^{33,34} It is especially important for MRSA and biofilm populations.

Suppression of quorum sensing. Quorum sensing coordinates the expression of virulence factors, motility, and biofilm formation. Phenolic

²⁸ de Souza Nascimento A. M. et al. Antibacterial action mechanisms and mode of trypsin inhibitors: a systematic review. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2022. Vol. 37, No 1. P. 749–759. DOI: 10.1080/14756366.2022.2039918

²⁹ Liu Y. et al. Flavonoids as promising natural compounds for combating bacterial infections. *International Journal of Molecular Sciences*. 2025. Vol. 26, No 6. Art. 2455. DOI: 10.3390/ijms26062455

³⁰ Bae J. Y., Seo Y. H., Oh S. W. Antibacterial activities of polyphenols against foodborne pathogens and their application as antibacterial agents. *Food Science and Biotechnology*. 2022. Vol. 31, No 8. P. 985–997. DOI: 10.1007/s10068-022-01058-3

³¹ Bellanti F. et al. Redox imbalance in inflammation: the interplay of oxidative and reductive stress. *Antioxidants*. 2025. Vol. 14, No 6. Art. 656. DOI: 10.3390/antiox14060656

³² Bhattacharya S. et al. Mitigating ROS signalling pathway-mediated defence mechanism: a novel approach to counteract bacterial resistance using natural antioxidant-based antibiotics. *Phytochemistry Reviews*. 2025. DOI: 10.1007/s11101-025-10179-6

³³ Ramalingam S. et al. Plant-based natural products as inhibitors for efflux pumps to reverse multidrug resistance in *Staphylococcus aureus*: a mini review. *Mini-Reviews in Medicinal Chemistry*. 2024. Vol. 24, No 3. P. 272–288. DOI: 10.2174/1389557523666230406092128

³⁴ Zhang L. et al. Bacterial efflux pump inhibitors reduce antibiotic resistance. *Pharmaceutics*. 2024. Vol. 16, No 2. Art. 170. DOI: 10.3390/pharmaceutics16020170

compounds, flavonoids, and terpenoids can inhibit the synthesis of signalling molecules, block their receptors, or alter gene expression.³⁵ In *S. aureus*, the *agr* system – the accessory gene regulator – is particularly important, as it controls toxin synthesis. In *E. coli*, quorum sensing affects biofilms and motility. In *C. acnes*, these mechanisms have been studied less extensively, but antibiofilm activity remains clinically important.

Antibiofilm activity. Biofilms confer increased tolerance to antibiotics and enhanced immune defence mechanisms. Phytochemicals prevent bacterial adhesion, inhibit matrix synthesis, disrupt quorum sensing, and improve antibiotic penetration.^{36,37} Antibiofilm activity is essential in chronic wounds caused by *S. aureus*, catheter-associated infections with *E. coli*, and follicular persistence of *C. acnes*.

1.5. Synergistic Group: One Active Mechanism

Synergy with antibiotics. Phytochemical compounds can restore the susceptibility of resistant bacteria or reduce the required antibiotic doses by increasing membrane permeability, inhibiting efflux pumps, and suppressing resistance enzymes.^{38,39} Examples include the enhancement of β -lactam and fluoroquinolone activity against *S. aureus* by flavonoids, as well as the increased effectiveness of berberine in combination with efflux pump inhibitors.

2. Specific Mechanisms of Action of Individual Phytochemical Classes

2.1. Flavonoids: Mechanisms and Antibacterial Significance

Flavonoids exert antibacterial effects through several mechanisms. These include disruption of bacterial membranes, inhibition of DNA gyrase and topoisomerases, inhibition of energy metabolism, interference with cell envelope synthesis, suppression of virulence factors, and prevention of

³⁵ Bouyahya A. et al. Mechanisms, anti-quorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria: a comprehensive review. *Molecules*. 2022. Vol. 27, No 5. Art. 1484. DOI: 10.3390/molecules27051484

³⁶ Zhang M. et al. Recent advances on the regulation of bacterial biofilm formation by herbal medicines. *Frontiers in Microbiology*. 2022. Vol. 13. Art. 1039297. DOI: 10.3389/fmicb.2022.1039297

³⁷ Liu Y. et al. Eradicating biofilms: decoding persistent mechanisms and combating therapeutic strategies. *Advanced Healthcare Materials*. 2026. Vol. 15, No 6. Art. e02707. DOI: 10.1002/adhm.202502707

³⁸ Alam M. et al. Synergistic role of plant extracts and essential oils against multidrug resistance and gram-negative bacterial strains producing extended-spectrum β -lactamases. *Antibiotics*. 2022. Vol. 11, No 7. Art. 855. DOI: 10.3390/antibiotics11070855

³⁹ Dai C., Liu Y., Lv F., Cheng P., Qu S. An alternative approach to combat multidrug-resistant bacteria: new insights into traditional Chinese medicine monomers combined with antibiotics. *Advanced Biotechnology*. 2025. Vol. 3. Art. 6. DOI: 10.1007/s44307-025-00059-7

biofilm formation.^{40,41} Some flavonoids also inhibit bacterial efflux pumps, thereby increasing the intracellular accumulation of antibiotics or other toxic compounds.

The antibacterial activity of flavonoids depends strongly on their chemical structure. Aglycones are generally more active than glycosides because of their higher lipophilicity. Hydroxyl groups enhance binding to molecular targets, while structural planarity facilitates DNA intercalation. Galloylation, as seen in EGCG, increases membrane activity. In *S. aureus*, inhibition of DNA gyrase may contribute to growth suppression. In *E. coli*, the penetration of flavonoids is limited by the outer membrane; however, once inside the cell, DNA gyrase remains a potential intracellular target. In *C. acnes*, gyrase inhibition is also relevant, although additional organism-specific studies are needed. Flavonoids can also affect bacterial energy metabolism by altering membrane potential, electron transport, and ATP synthesis. Some flavonoids inhibit ATP synthase or enzymes involved in respiratory metabolism. It may reduce bacterial growth, motility, and active transport.

Efflux pumps are membrane transport proteins that export various substances from the bacterial cell. Inhibition of efflux pumps is one of the main resistance-modifying mechanisms of flavonoids. By suppressing efflux activity, flavonoids increase the intracellular concentration of antibiotics and restore bacterial susceptibility. Flavonoids may reduce bacterial virulence without necessarily producing strong bactericidal activity. They can inhibit adhesion, toxin production, motility, quorum sensing, and biofilm development. Therefore, flavonoids are particularly important not only as direct antibacterial compounds but also as antivirulence and antibiotic-adjuvant agents.

2.2. Terpenoids: Mechanisms and antibacterial significance

The antibacterial effects of terpenoids are closely associated with their hydrophobicity. Many terpenoids are volatile, hydrophobic, and membrane-active compounds. The dominant antibacterial mechanism of terpenoids is the disruption of membrane permeability.

Due to their hydrophobic nature, terpenoids partition into lipid bilayers and disturb membrane structure. They penetrate bacterial membranes, disrupt lipid packing, increase membrane fluidity and permeability, and cause leakage of ions, ATP, and other intracellular components.¹⁵ They may also collapse the proton motive force, reduce ATP synthesis, and interfere with membrane-associated enzymes.

⁴⁰ Ullah S. et al. Important flavonoids and their role as a therapeutic agent. *Molecules*. 2020. Vol. 25, No 22. Art. 5243. DOI: 10.3390/molecules25225243

⁴¹ Dias M. C., Pinto D. C. G. A., Silva A. M. S. Plant flavonoids: chemical characteristics and biological activity. *Molecules*. 2021. Vol. 26, No 17. Art. 5377. DOI: 10.3390/molecules26175377

Terpenoids are usually more active against Gram-positive bacteria than Gram-negative bacteria because the outer membrane of Gram-negative bacteria restricts access to the cytoplasmic membrane. Therefore, *S. aureus* and *C. acnes* are generally more susceptible to terpenoid-mediated membrane damage than *E. coli*. However, small hydrophobic terpenoids may still affect Gram-negative bacteria, especially when combined with other agents that destabilise the outer membrane.

Examples of membrane-active terpenoids include thymol, carvacrol, menthol, linalool, terpinen-4-ol, α -pinene, borneol, etc. Thymol and carvacrol are particularly well known for their ability to disrupt membrane integrity and energy metabolism. Their rapid membrane-targeting activity makes them promising candidates for topical antimicrobial formulations and for combination therapy with conventional antibiotics.

2.3. Phenols and Polyphenols: Mechanisms and antibacterial significance

Phenols and polyphenols are numerous and chemically diverse compounds. Important antibacterial phenolic compounds include eugenol, gallic acid, caffeic acid, ferulic acid, rosmarinic acid, chlorogenic acid, curcumin, tannic acid, ellagic acid, and resveratrol. Flavonoids are technically polyphenols, but they are discussed separately here because they represent a distinct structural class.

Phenolic compounds are relevant to all three target bacteria and may act through several mechanisms. They disrupt bacterial membranes, inhibit extracellular microbial enzymes, bind metal ions required for bacterial metabolism, damage cell walls, interfere with nucleic acid synthesis, modulate oxidative stress, and inhibit biofilm formation.^{2,27} Compounds that combine antibacterial and anti-inflammatory effects are particularly useful, especially if inflammation plays a central role. More hydrophobic phenolic acids generally enter bacterial membranes more easily.

Phenolic compounds can interact with proteins through hydrogen bonding, hydrophobic interactions, and covalent reactions after oxidation to quinones. Tannins are especially known for their ability to precipitate proteins. It inhibits microbial adhesins, enzymes, transport proteins, and cell envelope proteins. Many phenols can also damage bacterial membranes, particularly when they are sufficiently lipophilic.

Eugenol, for example, can disrupt membrane integrity and denature proteins. Curcumin may interfere with bacterial cell division by inhibiting FtsZ polymerisation and by affecting nucleoid organisation. Resveratrol and phenolic acids can influence oxidative balance, enzyme activity, and biofilm formation. In *C. acnes*, phenolic compounds are of special interest because they may combine direct antibacterial activity with anti-inflammatory and antioxidant effects relevant to acne pathogenesis.

2.4. Alkaloids: Mechanisms and antibacterial significance

Alkaloids are nitrogen-containing compounds with diverse structures and biological activities. Some alkaloids possess strong antimicrobial properties, although toxicity may limit their application. Alkaloids can act through several mechanisms. Some intercalate into DNA or inhibit nucleic acid synthesis. Others interfere with cell division, inhibit enzymes, disrupt membranes, or suppress efflux pumps.⁴²

Many alkaloids have planar aromatic structures and positive charges, allowing them to interact with DNA and potentially interfere with replication and transcription. Berberine, palmatine, and sanguinarine are examples of alkaloids that interact with nucleic acids. Berberine is also known for its ability to affect bacterial membranes, inhibit efflux systems, and enhance the activity of other antimicrobial agents.

Regarding the target microorganisms, alkaloids are particularly interesting for *S. aureus*, as they exhibit activity against Gram-positive bacteria and may modify resistance mechanisms. For *E. coli*, the effectiveness of alkaloids is often limited by the outer membrane and efflux systems, but combination strategies may help overcome these barriers. For *C. acnes*, alkaloid-rich extracts require further research; compounds that combine antibacterial and anti-inflammatory properties appear promising.

Some medicinal plants contain combinations of antibacterial compounds and agents that modify resistance. Thus, alkaloids may be more effective in combination therapy than as isolated single agents. Their ability to interact with DNA, inhibit efflux pumps, and potentiate antibiotics makes them valuable candidates for further research, especially as adjuvants in the treatment of resistant bacterial infections.

3. Pathogen-Specific Manifestations of Antibacterial Activity

Although flavonoids, terpenoids, phenols, and alkaloids exhibit broad-spectrum antibacterial activity, their activity differs substantially against *S. aureus*, *E. coli*, and *C. acnes*. Variations in cell envelope architecture, oxygen tolerance, metabolism, virulence systems, and biofilm ecology explain these differences. In general, phytochemicals that disrupt membranes, inhibit efflux pumps, or suppress virulence tend to show stronger activity against Gram-positive organisms such as *S. aureus* and *C. acnes* than against Gram-negative *E. coli*, whose outer membrane limits the penetration of many hydrophobic compounds.⁴³

⁴² Rajput A., Sharma R., Bharti R. Pharmacological activities and toxicities of alkaloids on human health. *Materials Today: Proceedings*. 2022. Vol. 48, pt. 5. P. 1407–1415. DOI: 10.1016/j.matpr.2021.09.189

⁴³ Villanueva X. et al. Effect of chemical modifications of tannins on their antimicrobial and antibiofilm effect against Gram-negative and Gram-positive bacteria. *Frontiers in Microbiology*. 2023. Vol. 13. Art. 987164. DOI: 10.3389/fmicb.2022.987164

3.1. Activity against *Staphylococcus aureus*

Staphylococcus aureus is the causative agent of a wide range of infections. Its cell envelope consists of a thick peptidoglycan layer containing teichoic and lipoteichoic acids, but it lacks the outer membrane characteristic of Gram-negative bacteria. It makes *S. aureus* relatively accessible to hydrophobic phytochemicals, such as terpenoids and phenolic compounds in essential oils.

Methicillin-resistant *S. aureus* strains are of particular concern. Their resistance is mainly associated with the altered penicillin-binding protein PBP2a, encoded by the *mecA* gene. This protein allows cell wall synthesis to continue even in the presence of β -lactam antibiotics. In addition, *S. aureus* possesses efflux pumps, oxidative stress defence systems, virulence factors, adhesins, and a strong biofilm-forming ability. Promising plant-derived compounds should act not only by directly inhibiting bacterial growth or killing cells, but also by weakening virulence factors, suppressing biofilm formation and maturation, and modifying mechanisms of antibiotic resistance.

Flavonoids have been studied for their antistaphylococcal activity.^{13,14} Their mechanisms include membrane disruption, inhibition of nucleic acid-related enzymes, suppression of efflux pumps, interference with cell wall synthesis, and reduction of toxin production. Some flavonoids may attenuate bacterial adhesion and biofilm development, which is especially important in persistent *S. aureus* infections.

Terpenoids are particularly active against *S. aureus* because the Gram-positive envelope provides less protection against hydrophobic molecules. Their main action is to disrupt the cytoplasmic membrane, increasing permeability and causing the leakage of potassium ions, ATP, and other intracellular components. The collapse of the proton motive force reduces ATP synthesis and impairs nutrient transport. Terpenoids may also decrease biofilm formation through several overlapping effects: reduced cell viability, impaired surface adhesion, altered bacterial surface hydrophobicity, and interference with quorum-sensing pathways. When terpenoids are in mixtures, additive or synergistic interactions are possible. Minor components may influence solubility, membrane partitioning, volatility, and overall antimicrobial potency.

Phenolic compounds are also important against *S. aureus*,²² as they can damage membranes, bind proteins, inhibit enzymes, and affect virulence factors. For example, phenolic compounds may reduce toxin secretion, interfere with adhesins, and impair the integrity of the bacterial envelope. Tannins can precipitate proteins and inhibit enzymes required for colonisation and persistence.

Alkaloids have demonstrated activity against *S. aureus*,⁴⁴ especially when combined with efflux pump inhibitors or antibiotics. Compounds such as berberine and sanguinarine may interfere with DNA, inhibit efflux systems, and enhance the intracellular accumulation of antibacterial agents.

Biofilm formation is a key factor in the persistence of *S. aureus* infections. Biofilms protect bacteria from antibiotics, host immune responses, and adverse environmental conditions. Many phytochemicals inhibit the formation and development of *S. aureus* biofilms by preventing initial bacterial adhesion, suppressing extracellular polysaccharide synthesis, disrupting mature biofilm architecture, inhibiting quorum-sensing systems, and improving antibiotic penetration into biofilm.

The *agr* quorum-sensing system is especially important for *S. aureus* virulence because it regulates toxin production, enzyme secretion, and biofilm dispersal. Some phytochemicals can suppress *agr*-mediated expression of virulence factors, including α -hemolysin and other secreted proteins. Such antivirulence activity reduces bacterial pathogenicity without necessarily requiring complete bacterial eradication.

3.2. Activity against *Escherichia coli*

E. coli includes many harmless commensal strains, but pathogenic lineages can cause urinary tract infections, sepsis, neonatal meningitis, gastroenteritis, and intra-abdominal infections. The antibacterial response of *E. coli* differs substantially from that of *S. aureus* because *E. coli* possesses a complex Gram-negative outer membrane.

This outer membrane contains LPS, porins that restrict the entry of compounds according to size and polarity, a periplasmic space containing enzymes such as β -lactamases, a thin peptidoglycan layer, an inner cytoplasmic membrane, and multiple efflux systems, especially AcrAB–TolC. The outer membrane limits the penetration of many hydrophobic terpenoids and phenols. Thus, *E. coli* is often less susceptible to essential oils and lipophilic phytochemicals than Gram-positive organisms.⁴⁴

Flavonoids inhibit *E. coli*, but their potency depends on structure, polarity, and ability to cross the outer membrane, which limits intracellular accumulation.¹³ More hydrophilic flavonoid glycosides pass through porins more easily than hydrophobic aglycones, but glycosides bind less strongly to intracellular targets. In contrast, hydrophobic aglycones interact more effectively with bacterial membranes and molecular targets, but they enter Gram-negative

⁴⁴ Huang P. Y., Hsu C. K., Tang H. J., Lai C. C. Eravacycline: a comprehensive review of in vitro activity, clinical efficacy, and real-world applications. *Expert Review of Anti-infective Therapy*. 2024. Vol. 22, No 6. P. 387–398. DOI: 10.1080/14787210.2024.2351552

cells less efficiently. So, antibacterial activity depends on the balance between permeability and intrinsic target affinity.

Terpenoids may act against *E. coli* by damaging surface-associated cells and disturbing membrane integrity. However, the outer membrane is the major barrier to their activity. Their effects are often enhanced when other compounds or antibiotics destabilise the outer membrane. It makes terpenoids especially relevant in combination strategies rather than as isolated agents against Gram-negative bacteria.

Phenolic compounds show mixed activity against *E. coli*. Small phenols with sufficient hydrophobicity can damage membranes, while more polar phenolic acids may act through enzyme inhibition, metal chelation, oxidative stress modulation, or interference with biofilm formation. However, their effectiveness is still limited by permeability barriers and efflux mechanisms.

The contribution of alkaloids against *E. coli* is often considered more relevant as a bioavailability enhancer and a resistance-modifying mechanism than as a strong direct antibacterial agent. Alkaloids inhibit efflux systems, increasing susceptibility to antibiotics and other phytochemicals. It is important for multidrug-resistant strains, including extended-spectrum β -lactamase-producing and carbapenem-resistant *E. coli*.

Biofilms of *E. coli* play an important role in urinary tract infections, catheter-associated infections, intestinal colonisation, and long-term bacterial persistence in the environment. Inhibition of *E. coli* biofilm formation may occur through suppression of initial adhesion, reduction of cell motility, disruption of quorum-sensing systems, inhibition of extracellular matrix synthesis, and enhancement of oxidative or membrane stress. At the same time, eradicating mature *E. coli* biofilms remains difficult. Most phytochemicals are much more effective at preventing biofilm formation than at destroying already established biofilm structures. It is due to the protective function of the extracellular matrix and the activation of adaptive mechanisms that increase bacterial tolerance to antimicrobial agents.

3.3. Activity against *Cutibacterium acnes*

Cutibacterium acnes contributes to the development of acne vulgaris through follicular colonisation, biofilm formation, lipase production, inflammation, and activation of innate immune pathways. Unlike *S. aureus*, *C. acnes* grows slowly and is adapted to lipid-rich, low-oxygen environments. It produces lipases that hydrolyse sebum triglycerides, releasing free fatty acids that contribute to follicular irritation and inflammation. It also stimulates keratinocytes and immune cells to produce inflammatory mediators, including IL-1 β , IL-8, TNF- α , and other cytokines.

Phytochemicals from medicinal plants may exert a dual action against *C. acnes*, namely (i) direct antibacterial inhibition of follicular *C. acnes*, and (ii) reduction of inflammation, oxidative stress, and biofilm-associated bacterial persistence.

Because acne is an inflammatory disease rather than a simple infection, compounds with both antibacterial and anti-inflammatory activity are especially attractive.⁴⁵ Disease severity is determined not only by bacterial load but also by the intensity of the host inflammatory response. Therefore, phytochemicals may be useful in acne through direct inhibition of *C. acnes* growth, reduction of bacterial lipase activity, suppression of biofilm formation, decreased sebum oxidation, inhibition of NF- κ B and MAPK signalling pathways, antioxidant protection, reduced neutrophil recruitment, and decreased production of pro-inflammatory cytokines.

Flavonoids may help control *C. acnes* through antibacterial, anti-inflammatory, and antioxidant effects. They can inhibit bacterial growth, reduce oxidative stress, and modulate inflammatory signalling pathways involved in acne pathogenesis. Their ability to suppress cytokine production and protect against oxidative damage makes them particularly relevant for inflammatory skin conditions.

Terpenoids are highly relevant to *C. acnes* because many of them are lipophilic and may accumulate in the sebaceous environment. Essential oils used in dermatology often owe their activity to monoterpenes and related compounds. Since *C. acnes* is Gram-positive and lacks an outer membrane, it is relatively vulnerable to membrane-active terpenoids. These compounds may disrupt bacterial membranes, reduce metabolic activity, and interfere with biofilm formation within follicles.

Phenolic compounds may directly affect *C. acnes* while simultaneously reducing inflammatory responses. Eugenol, curcumin, resveratrol, gallic acid, and others may combine antibacterial, antioxidant, and anti-inflammatory properties. This dual activity is valuable in acne, where inflammation and bacterial persistence are closely linked.

Alkaloids or their extracts require further research against *C. acnes*. Compounds that combine antibacterial activity with anti-inflammatory or resistance-modifying effects are potentially useful. However, their safety profile is especially important for dermatological use, since topical irritation and cytotoxicity may limit application.

The main limitations of phytochemicals against *C. acnes* include potential skin irritation, poor solubility, chemical instability, and limited penetration into

⁴⁵ Pannakal S. T. et al. Acnocure, a synergistic antimicrobial and anti-inflammatory combination of thymol and Curcuma turmerones, formulation and time-kill studies against *C. acnes*. *Cosmetics*. 2025. Vol. 12, No 2. Art. 37. DOI: 10.3390/cosmetics12020037

pilosebaceous follicles. These challenges may be addressed through topical delivery systems such as gels, nanoparticles, liposomes, nanoemulsions, or other formulations designed to improve stability, follicular penetration, and local bioavailability.

4. Discussion

The antibacterial properties of medicinal plants depend on several factors. First, results obtained *in vitro* do not always correlate with clinical efficacy.⁴⁶ Many compounds exert antibacterial effects at concentrations that are difficult to achieve safely in humans, especially in systemic infections. Topical applications, such as anti-acne preparations, may achieve higher local concentrations. Second, the composition of herbal extracts varies depending on plant species, cultivar, growth and storage conditions, harvesting time, and extraction method.⁴⁷ Third, antibacterial activity may result from synergy among different compounds.⁴⁸ A crude extract may be more active than its isolated constituents. Such synergy is relevant both for plant-based preparations themselves and for their combinations with conventional antibiotics. Finally, safety must be considered because natural origin does not guarantee low toxicity.⁴⁹ Some natural substances may irritate the skin or mucous membranes and may exhibit cytotoxicity. Thus, antibacterial activity must be balanced against toxicity, pharmacokinetics, formulation stability, and the intended route of administration.

4.1. Dominant Antibacterial Mechanisms

Analysis of the literature indicates that the effects of phytochemicals on pathogenic bacteria can be described through ten distinct pathogen–compound interaction mechanisms. These mechanisms can be further grouped into four major categories according to the level at which they affect the bacterial cell.

Additional qualitative information can be obtained by ranking individual mechanisms according to their relative importance, assigning each a score from 10 (strongest contribution) to 1 (weakest contribution), based on the literature. In this approach, the scores form an ordinal scale; that is, the

⁴⁶ Peng Z., Wang X., Huang J., Li B. Pathogenic *Escherichia coli*. *Molecular Medical Microbiology* / ed. by Y. W. Tang et al. 3rd ed. Academic Press, 2024. P. 1065–1096. DOI: 10.1016/B978-0-12-818619-0.00069-1

⁴⁷ Li Y., Zidorn C. Seasonal variations of natural products in European herbs. *Phytochemistry Reviews*. 2022. Vol. 21. P. 1549–1575. DOI: 10.1007/s11101-021-09797-7

⁴⁸ Chaachouay N. Synergy, additive effects, and antagonism of drugs with plant bioactive compounds. *Drugs and Drug Candidates*. 2025. Vol. 4, No 1. Art. 4. DOI: 10.3390/ddc4010004

⁴⁹ Balkrishna A. et al. Exploring the safety, efficacy, and bioactivity of herbal medicines: bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine*. 2024. Vol. 3, No 1. P. 35–49. DOI: 10.14218/FIM.2023.00086

distance between neighbouring values is not constant, but the scale allows mechanisms to be ordered according to their probable importance within each phytochemical class.

The individual mechanisms can be grouped into four major categories according to the level of phytochemical action on the bacterial cell: structural, metabolic, regulatory, and synergistic. The first group, the structural group, includes mechanisms that disrupt bacterial cell structures: membrane damage, disruption of the cell envelope, and interference with cell wall synthesis. The second group, the metabolic group, includes mechanisms that inhibit intracellular processes: inhibition of nucleic acid synthesis, inhibition of bacterial enzymes and protein synthesis, and disruption of energy metabolism. The third group, the regulatory group, includes antivirulence and anti-adaptive mechanisms: induction or modulation of oxidative stress, inhibition of efflux pumps, suppression of quorum sensing, and antibiofilm activity. The fourth group, the synergistic group, refers to the enhancement of antimicrobial therapy through synergy with conventional antibiotics. The results of the assessment of each mechanism's contribution and each phytochemical are presented in Figure 1.

The most important mechanisms of flavonoids are inhibition of nucleic acid synthesis, observed for quercetin, catechins, and related flavonoids, as well as inhibition of efflux pumps and antibiofilm activity. Thus, flavonoids should primarily be regarded as multitarget regulatory and intracellular inhibitors.

For phenols and polyphenols, the dominant mechanisms are membrane damage, as demonstrated for eugenol and phenolic acids, and inhibition of proteins and enzymes, particularly with tannins. Many phenols contain hydroxyl groups that enable hydrogen bonding with proteins and membranes. Antibiofilm activity is also important, as shown for curcumin, because phenols can interfere with bacterial adhesion, matrix production, and survival of surface-associated bacteria. So, phenols should be considered membrane-active and protein-reactive antibacterial agents.

Terpenoids are the class most closely associated with membrane disruption. Compounds such as thymol, carvacrol, terpinen-4-ol, citral, and linalool are hydrophobic and can penetrate bacterial lipid bilayers. It disrupts membrane packing, increases permeability, and causes leakage of potassium ions, ATP, proteins, and other intracellular contents. Since bacterial energy production depends strongly on membrane integrity, terpenoid-induced membrane damage leads to collapse of the proton motive force, reduced ATP synthesis, and impaired nutrient transport. Therefore, disruption of energy metabolism ranks second among terpenoid-associated mechanisms, although it is often a consequence of membrane damage. Terpenoids may also improve antibiotic penetration into bacteria, especially Gram-negative microorganisms such as *E. coli*, where the

outer membrane limits compound entry. Thus, terpenoids are best considered membrane-disrupting permeability enhancers.

Alkaloids often form strong interactions with intracellular macromolecules. Several planar alkaloids, such as berberine- and sanguinarine-type compounds, can interact with DNA or interfere with DNA-associated processes. Thus, interference with nucleic acid metabolism is one of their most important mechanisms. Alkaloids such as berberine may also directly inhibit efflux pumps or act synergistically with other compounds. In addition, alkaloids can inhibit proteins involved in cell division, particularly FtsZ, and interfere with bacterial enzymes. Alkaloids are therefore best regarded as inhibitors of intracellular targets and resistance-modifying agents.

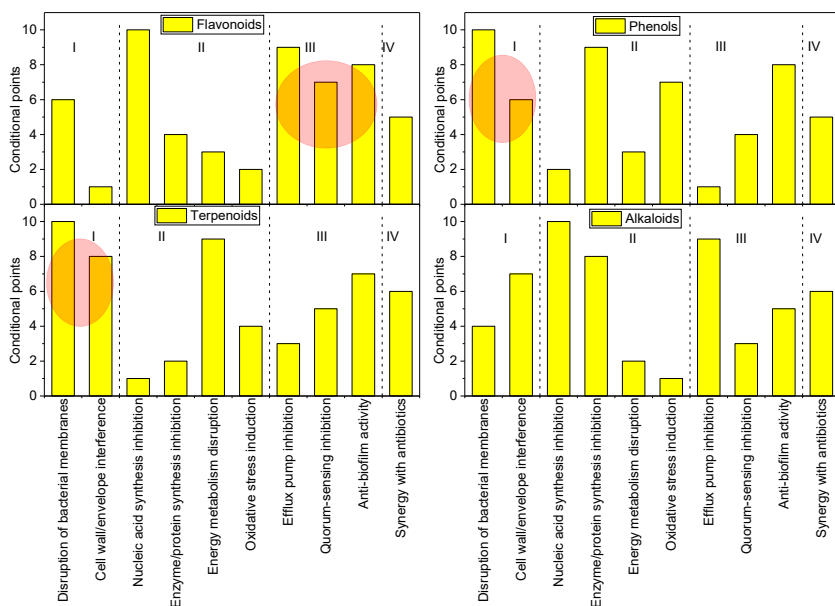


Fig. 1. Contribution of antibacterial mechanisms, scored from 1 to 10, to the effectiveness of four phytochemical classes. Mechanism groups:

I – structural; II – metabolic; III – regulatory; IV – synergistic.

Dominant mechanisms for each class are highlighted in pink

Additional information can be obtained by analysing mean scores across the four mechanism groups for each phytochemical class (Figure 2). The most balanced distribution among mechanism groups is characteristic of

alkaloids, with mean scores ranging between 5 and 6. Flavonoids show a clear predominance of regulatory mechanisms, with an average score of 8, compared with structural mechanisms, which average 3.5. For phenols and terpenoids, structural factors are more important, with scores of 8 and 9, respectively, than regulatory mechanisms, which score 4–5.

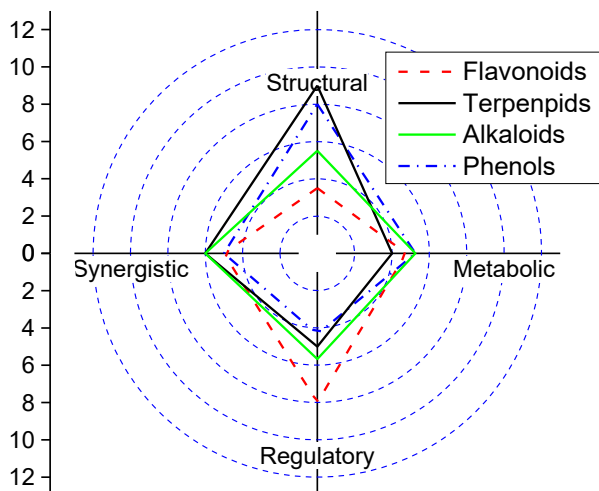


Fig. 2. Relative contribution, expressed as scores, of the four mechanism groups for flavonoids, phenols, terpenoids, and alkaloids

4.2. Key Pathogen-Specific Effects

The previous section assessed the effectiveness of antibacterial mechanisms across different phytochemical classes without considering specific pathogens. However, phytochemicals from medicinal plants exhibit different effects depending on the pathogen. Against *S. aureus*, the most relevant effects include membrane disruption, antibiofilm activity, efflux pump inhibition, and antibiotic potentiation. Flavonoids such as EGCG and baicalin are especially important as modulators of MRSA resistance. Against *E. coli*, activity is limited by the Gram-negative outer membrane and efflux systems. Effective approaches require compounds that increase permeability, inhibit efflux, or are delivered using improved formulations. Against *C. acnes*, phytochemicals combine antibacterial, antibiofilm, antioxidant, and anti-inflammatory properties. Compounds that can simultaneously reduce bacterial load and inflammatory signalling are particularly important.

Overall, the most promising therapeutic direction is not necessarily the replacement of antibiotics with isolated phytochemicals. However, the use of plant-derived preparations as multitarget adjuvants that weaken bacterial defences, reduce virulence, inhibit biofilms, and increase antibiotic susceptibility.

Analysis of the literature enables a qualitative 5-point rating of pathogen-specific activity. A score of 5 out of 5 stars corresponds to excellent, well-documented activity; 4 stars means good and consistent activity; 3 stars – moderate or variable activity; 2 stars – limited, weak, or inconsistent activity; and 1 star indicates low, rarely effective, or insufficiently studied activity. The results of this analysis, including dominant mechanisms for each phytochemical class and pathogen, are summarised in Table 2.

Table 2

Dominant antibacterial mechanisms with qualitative assessment of activity by pathogen and phytochemical class

Pathogen	Dominant antibacterial mechanisms with activity rating by a 5-star scale
Flavonoids	
<i>S. aureus</i>	Inhibition of quorum sensing, especially the <i>agr</i> system; inhibition of efflux pumps, particularly NorA – 4★
<i>E. coli</i>	Inhibition of efflux pumps, especially AcrAB–TolC; inhibition of DNA gyrase – 3★
<i>C. acnes</i>	Inhibition of lipases/enzymes; antibiofilm activity; inhibition of DNA gyrase – 4★
Phenols/polyphenols	
<i>S. aureus</i>	Membrane disruption; binding to proteins/enzymes; antibiofilm activity 5★
<i>E. coli</i>	Membrane disruption; disturbance of the cell wall/cell envelope – 4★
<i>C. acnes</i>	Membrane disruption; inhibition of sebum-associated enzymes; antibiofilm activity – 4★
Terpenoids	
<i>S. aureus</i>	Membrane disruption → ATP leakage → cell death – 5★
<i>E. coli</i>	Membrane disruption; increased outer membrane permeability; synergy with antibiotics – 4★
<i>C. acnes</i>	Membrane disruption; modulation of inflammatory signalling 0 5★
Alkaloids	
<i>S. aureus</i>	Inhibition of FtsZ and cell division; DNA interaction; involvement in efflux-related mechanisms – 4★
<i>E. coli</i>	Interaction with DNA/topoisomerases; involvement in efflux-related mechanisms – 3★
<i>C. acnes</i>	Enzyme inhibition; antibiofilm activity, although data remain moderate – 3★

The absence of an outer membrane in *S. aureus* makes this bacterium more susceptible to both membrane-active and intracellularly acting compounds.

Flavonoids received a high rating against this pathogen because *S. aureus* virulence strongly depends on quorum sensing, while efflux pump-mediated resistance is clinically relevant. Phenols and terpenoids received the highest ratings. Phenolic compounds can damage membranes, bind proteins, inhibit enzymes, and influence virulence factors. The absence of an outer membrane allows terpenoids to act rapidly and effectively against *S. aureus*. Alkaloids also received a high rating because FtsZ and DNA are accessible targets in *S. aureus*, and efflux-mediated resistance is common.

For *E. coli*, flavonoids received a moderate rating because the outer membrane and efflux systems significantly reduce flavonoid entry and effectiveness. Their activity increases significantly when combined with compounds that enhance outer membrane permeability. Phenols and polyphenols received a good rating because phenolic compounds with multiple hydroxyl groups can partially overcome the outer membrane barrier, especially tannins and gallic acid derivatives. Terpenoids also received a good rating because they are among the few phytochemical classes capable of substantially disturbing the outer membrane of Gram-negative bacteria. Their activity is somewhat lower than against *S. aureus* because of the structural complexity of the outer membrane, but their synergistic potential is considerable. Alkaloids received a moderate rating because the outer membrane barrier and efficient efflux systems strongly restrict their bioavailability and intrinsic activity against *E. coli*.

C. acnes is a Gram-positive, anaerobic/aerotolerant, slow-growing commensal that becomes pathogenic in sebaceous follicles. It contributes to acne vulgaris by promoting follicular colonisation, biofilm formation, lipase production, inflammation, and activation of innate immune pathways.

Flavonoids received a high rating because many target the virulence cascade of *C. acnes* rather than merely inhibiting growth. Phenols and polyphenols also received a high rating because anti-inflammatory mechanisms are clinically as important as direct antibacterial activity in acne pathogenesis. Terpenoids received the highest rating because they combine direct bactericidal activity, antibiofilm effects, anti-inflammatory properties, and pharmacokinetic suitability, as their lipophilicity is compatible with the sebaceous follicular environment. Alkaloids received a moderate rating because specific mechanistic data for *C. acnes* are less extensive than for other bacteria. Alkaloids do not appear to affect the lipase/porphyrin/sebum axis as effectively as flavonoids, and the available evidence remains limited.

In summary, the key patterns of pathogen-specific activity are as follows:

1. The barrier function of the outer membrane is the dominant factor reducing the susceptibility of *E. coli* to all phytochemical classes.

2. Target accessibility is greater in Gram-positive bacteria; thus, *S. aureus* and *C. acnes* demonstrate higher susceptibility because they lack an outer membrane.

3. Pathogen-specific virulence targets include the *agr* quorum-sensing system, NorA efflux pumps, biofilm-associated *icaADBC* genes (all for *S. aureus*); Lip1/Lip2 lipases, porphyrin metabolism, 5 α -reductase-associated pathways (all for *C. acnes*); AcrAB–TolC efflux system and the outer membrane (all for *E. coli*).

4. Synergistic combinations outperform monotherapy for all three pathogens.

4.3. Comparative Susceptibility of the Three Pathogens to Phytochemicals

Influence of Gram-positive and Gram-negative Cell Envelopes. The most consistent pattern across numerous studies is the greater susceptibility of Gram-positive bacteria to hydrophobic phytochemicals than Gram-negative bacteria. *S. aureus* and *C. acnes* lack an outer membrane; therefore, phytochemicals can more easily reach the cytoplasmic membrane. In contrast, *E. coli* possesses an LPS-rich outer membrane that prevents the penetration of many hydrophobic molecules. Their entry into the cell depends on porin channels, disruption of outer membrane integrity, or the use of formulations that improve active compound penetration. In addition, efflux pumps reduce intracellular accumulation of these substances.

Therefore, the approximate susceptibility ranking for membrane-active phytochemicals is: *C. acnes* $\approx$ *S. aureus* > *E. coli*.

This ranking is conditional and may vary depending on the bacterial strain, compound polarity, formulation, and experimental conditions.

Biofilm Context. Bacterial susceptibility within biofilms differs substantially from the susceptibility of planktonic cells. In planktonic culture studies, many essential oils and phenolic compounds demonstrate strong antibacterial activity. In biofilms, bacteria are embedded in an extracellular matrix, grow more slowly, and activate stress-response mechanisms. All these reduce susceptibility to antimicrobial agents. Several general patterns can be identified:

- prevention of biofilm formation is more effective and easier than the destruction of already established biofilms;
- terpenoids and phenols are useful for damaging biofilm-associated cells;
- tannins and flavonoids are effective in inhibiting bacterial adhesion and processes associated with extracellular matrix formation and maintenance;
- alkaloids and efflux pump inhibitors may increase intracellular stress in biofilm-associated bacteria.

Modification of Antibiotic Resistance by Phytochemicals. Phytochemicals are of interest as modifiers of antibiotic resistance. Due to their multitarget

mechanisms of action, they make it more difficult for bacteria to develop resistance, because a single mutation is usually insufficient to overcome their effects.

For *E. coli*, modifying antibiotic resistance is more challenging because the outer membrane and the AcrAB–TolC efflux system provide effective protection against many antimicrobial agents. Nevertheless, the combined use of phytochemicals with membrane-permeabilising agents, nanoparticles, or antibiotics may partially overcome this barrier and increase the effectiveness of antimicrobial therapy.

4.4. From Laboratory Data to Clinical Application

Despite strong *in vitro* antibacterial potential, translation into clinical practice remains limited. Laboratory conditions do not fully reproduce the environment of human tissues, including wounds, biofilms, and anaerobic follicles.

For example, *S. aureus* in wounds may be embedded in pus, necrotic tissue, or a biofilm matrix. *E. coli* in urinary or intestinal infections encounters immune factors and pH fluctuations. *C. acnes* grows slowly in anaerobic, lipid-rich follicles, which are difficult to reproduce in standard laboratory assays.

Chemical variability in plant extracts and essential oils is a major challenge. The composition of medicinal plant extracts depends on many factors, including plant species, geographical origin, soil and climate, harvesting season, plant part used, solvent, drying conditions, and storage conditions. Without strict chemical standardisation, antibacterial results are difficult to compare across studies.

Isolated Compounds versus Whole Extracts. Some studies test purified compounds, while others examine crude extracts. These results are not equivalent. Crude extracts may demonstrate: additive or synergistic effects; antagonistic effects; improved solubility of active components; reduced toxicity due to balancing among components; weaker activity due to dilution of active substances.

Bioavailability and Solubility Problems. Many antibacterial phytochemicals have low water solubility or limited bioavailability. These limitations are especially relevant for systemic infections. For topical infections, such as acne, achieving high local concentrations is more feasible, making topical administration more realistic.

Safety, Toxicity, and Irritation. «Natural» is not synonymous with «safe.» Some phytochemicals may cause irritation, allergy, cytotoxicity, or drug interactions. Therefore, safety studies are essential, especially for repeated topical application or systemic administration.

Limited Clinical Evidence. For many phytochemicals, clinical evidence remains limited, and additional clinical studies are required before therapeutic

use. These studies should include standardised composition, appropriate manufacturing control points, defined bacterial endpoints, and monitoring of toxicity and irritation.

4.5. Future Research Directions

Standardised Phytochemical Profiling. Future studies should use chemically well-characterised extracts. Modern analytical methods, including different combinations of chromatography, mass spectrometry, and NMR spectroscopy, should be used to identify and quantify active components. It would improve reproducibility and facilitate comparison among studies.

Improved Delivery Systems. Since many phytochemicals are poorly soluble or unstable, pharmaceutical formulation is a key research direction. For *C. acnes*, lipid-compatible topical formulations may improve penetration into sebaceous follicles. Phytochemical-loaded hydrogels or dressings may help target *S. aureus* biofilms; formulations that enhance outer membrane penetration may be valuable for *E. coli*.

Combination Therapy with Antibiotics. One of the most promising applications of phytochemicals is their use as adjuvant agents in antibiotic therapy. Combined use of phytochemicals and antibiotics may help reduce therapeutic antibiotic doses, restore susceptibility in resistant strains, and decrease selective pressure on microorganisms. However, synergistic interactions must be confirmed by microbial time-kill kinetics and *in vivo* experimental studies.

Antivirulence Strategies and Microbiome Preservation. Not all effective antibacterial therapeutic strategies need to kill bacteria directly. Some phytochemicals reduce microbial pathogenicity by inhibiting toxin production, quorum sensing, adhesion, motility, biofilm formation, inflammatory stimulation, and lipase activity. It is particularly important in *C. acnes* acne, because it is not only a potential pathogen but also a normal member of the resident skin microbiota. Complete eradication of this microorganism may disrupt the skin microbiome's balance. Therapeutic approaches aimed at reducing inflammation, virulence, and biofilm-associated persistence may be more appropriate than strategies focused solely on bacterial elimination.

CONCLUSIONS

Phytochemicals from medicinal herbs exhibit antibacterial activity against *S. aureus*, *E. coli*, and *C. acnes* via multiple mechanisms. These include membrane disruption, interference with the cell wall, inhibition of DNA replication, inhibition of enzymes, collapse of energy metabolism, inhibition of efflux pumps, antibiofilm activity, and suppression of virulence.

The strongest activity is generally observed against Gram-positive bacteria, such as *S. aureus* and *C. acnes*, because they lack the protective outer membrane of Gram-negative bacteria, such as *E. coli*. Terpenoids and phenolic compounds are particularly effective membrane-disrupting agents. Flavonoids act through enzyme inhibition, membrane effects, anti-inflammatory activity, and antibiotic potentiation. Alkaloids show promise, especially in combination with efflux pump inhibitors.

Against *S. aureus*, phytochemicals are particularly valuable as antibiofilm agents, efflux pump inhibitors, and modulators of MRSA resistance. Against *E. coli*, the main challenge is overcoming the Gram-negative outer membrane and the multidrug efflux system. In the treatment of *C. acnes*, phytochemicals that combine antibacterial and anti-inflammatory effects are particularly attractive, as they are highly relevant to acne pathogenesis.

Overall, phytochemicals derived from medicinal herbs should not be viewed simply as direct replacements for antibiotics. The greatest potential lies in their use as multitarget adjuvant agents that weaken bacterial defences, reduce virulence, disrupt biofilms, increase susceptibility to antibiotics, and support topical or localised therapy.

SUMMARY

The increasing prevalence of antibiotic-resistant pathogens underscores the need to discover new plant-derived antimicrobial agents. This study analyses the mechanisms of antibacterial action of flavonoids, terpenoids, phenols, and alkaloids against clinically significant pathogens: *Staphylococcus aureus*, *Escherichia coli*, and *Cutibacterium acnes*. Ten major ways of action of phytochemicals were identified and grouped into structural, metabolic, regulatory, and synergistic mechanisms. Terpenoids and phenols mainly exert antibacterial activity by damaging bacterial membranes and cell envelopes. Flavonoids exhibit pronounced regulatory effects. Alkaloids demonstrate the most balanced spectrum of activity. The sensitivity of bacteria to phytochemical compounds depends on the structure of the cell envelope: Gram-positive *S. aureus* and *C. acnes* are more vulnerable to membrane-active substances than Gram-negative *E. coli*. For *S. aureus*, compounds with antibiofilm activity that inhibit quorum sensing and efflux pumps are the most promising, particularly for combating MRSA. Substances that combine antibacterial and anti-inflammatory effects are particularly important for *C. acnes*. For *E. coli*, the effectiveness of phytochemicals requires increased outer membrane permeability or the use of advanced delivery systems. Phytochemicals should be considered not as direct substitutes for antibiotics, but as multifunctional adjuvant agents

capable of reducing bacterial virulence, inhibiting biofilms, and enhancing the effectiveness of antibiotic therapy. Further research should focus on standardised phytochemical profiling of extracts, active compound delivery systems, and synergy with conventional drugs.

Bibliography

1. Newman D. J., Cragg G. M. Natural products as sources of new drugs from 1981 to 2019. *Journal of Natural Products*. 2020. Vol. 83, No 3. P. 770–803. DOI: 10.1021/acs.jnatprod.9b01285
2. Taguri T., Tanaka T., Kouno I. Antimicrobial activity of 10 different plant polyphenolic compounds against bacteria associated with periodontal disease in vitro. *Journal of Periodontal Research*. 2024. Vol. 59, No 3. P. 457–469. DOI: 10.1248/bpb.27.1965
3. Cushnie T. P., Lamb A. B. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*. 2021. Vol. 26, No 5. P. 343–356. DOI: 10.1016/j.ijantimicag.2005.09.002
4. Tian F. et al. Antifungal activity of essential oil and plant-derived natural compounds against *Aspergillus flavus*. *Antibiotics*. 2022. Vol. 11, No 12. Art. 1727. DOI: 10.3390/antibiotics11121727
5. Zhang X.Z. et al. Natural alkaloids as antimicrobial agents: mechanisms, potentials and challenges. *Molecules*. 2026. Vol. 31, No 7. Art. 1204. DOI: 10.3390/molecules31071204
6. Fais A., Era B. Phytochemical composition and biological activity. *Plants*. 2024. Vol. 13, No 3. Art. 331. DOI: 10.3390/plants13030331
7. Sutton J. A. F. et al. Staphylococcus aureus cell wall structure and dynamics during host-pathogen interaction. *PLoS Pathogens*. 2021. Vol. 17, No 3. Art. e1009468. DOI: 10.1371/journal.ppat.1009468
8. Chalmers S. J., Wylam M. E. Methicillin-resistant Staphylococcus aureus infection and treatment options. *Methods in Molecular Biology*. 2020. Vol. 2069. P. 229–251. DOI: 10.1007/978-1-4939-9849-4_16
9. Peng Q. et al. A review of biofilm formation of Staphylococcus aureus and its regulation mechanism. *Antibiotics*. 2023. Vol. 12, No 1. Art. 12. DOI: 10.3390/antibiotics12010012
10. Naidoo N., Zishiri O. T. Presence, Pathogenicity, Antibiotic Resistance, and Virulence Factors of Escherichia coli: A Review. *Bacteria*. 2025. Vol. 4, No 1. Art. 16. DOI: 10.3390/bacteria4010016
11. Cogen A. L., Nizet V., Gallo R. L. Skin microbiota: A source of disease or defence? *British Journal of Dermatology*. 2023. Vol. 189, No 3. P. 299–309. DOI: 10.1111/j.1365-2133.2008.08437.x

12. Mayslich C., Grange P.A., Dupin N. Cutibacterium acnes as an opportunistic pathogen: an update of its virulence-associated factors. *Microorganisms*. 2021. Vol. 9, No 2. Art. 303. DOI: 10.3390/microorganisms9020303
13. Farhadi F. et al. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. *Phytotherapy Res.* 2019. Vol. 33, No 1. P. 13–40. DOI:10.1002/ptr.6208
14. Górniak I., Bartoszewski R., Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*. 2019. Vol. 18, No 1. P. 241–272. DOI: 10.1007/s11101-018-9591-z
15. Yamaguchi T. Antibacterial effect of the combination of terpenoids. *Archives of Microbiology*. 2022. Vol. 204. Art. 520. DOI: 10.1007/s00203-022-03142-y
16. De Rossi L., Rocchetti G., Lucini L., Rebecchi A. Antimicrobial potential of polyphenols: mechanisms of action and microbial responses – a narrative review. *Antioxidants*. 2025. Vol. 14, No 2. Art. 200. DOI: 10.3390/antiox14020200
17. Li G.Q. et al. Plant polyphenols: antibacterial activity and structural insights. *Fitoterapia*. 2025. Vol. 185. Art. 106763. DOI: 10.1016/j.fitote.2025.106763
18. Faisal S. et al. Alkaloids as potential antivirals: a comprehensive review. *Natural Products and Bioprospecting*. 2023. Vol. 13. Art. 4. DOI: 10.1007/s13659-022-00366-9
19. Ghosh S. et al. A comprehensive review of antimicrobial agents against clinically important bacterial pathogens: prospects for phytochemicals. *Phytotherapy Research*. 2025. Vol. 39. P. 138–161. DOI: 10.1002/ptr.8365
20. Kumar G., Engle K. Natural products acting against *S. aureus* through membrane and cell wall disruption. *Natural Product Reports*. 2023. Vol. 40, No 10. P. 1608–1646. DOI: 10.1039/D2NP00084A
21. Lou Z. et al. The antibacterial mechanism and cell damage mechanism of natural antibacterial agents from different sources and their applications in food. *Journal of Food Measurement & Characterization*. 2026. Vol. 20. P. 1362–1386. DOI: 10.1007/s11694-025-03794-6
22. Mu L. et al. The antibacterial activity, action mechanisms and prospects of baicalein as an antibacterial adjuvant. *World Journal of Microbiology and Biotechnology*. 2025. Vol. 41. Art. 304. DOI: 10.1007/s11274-025-04508-2
23. Boberek J. M., Stach J., Good L. Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. *PLoS One*. 2010. Vol. 5, No 10. Art. e13745. DOI: 10.1371/journal.pone.0013745

24. Rai D. et al. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochemical Journal*. 2008. Vol. 410, No 1. P. 147–155. DOI: 10.1042/BJ20070891
25. Xu S. et al. Plant flavonoids with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *ACS Infectious Diseases*. 2024. Vol. 10, No 9. P. 3086–3097. DOI: 10.1021/acsinfecdis.4c00292
26. Allison D. G., Lambert P. A. Modes of action of antibacterial agents. *Molecular Medical Microbiology* / ed. by Y. W. Tang et al. 3rd ed. Academic Press, 2024. P. 597–614. DOI: 10.1016/B978-0-12-818619-0.00133-7
27. Davidova S., Galabov A. S., Satchanska G. Antibacterial, antifungal, antiviral activity, and mechanisms of action of plant polyphenols. *Microorganisms*. 2024. Vol. 12, No 12. Art. 2502. DOI: 10.3390/microorganisms12122502
28. de Souza Nascimento A. M. et al. Antibacterial action mechanisms and mode of trypsin inhibitors: a systematic review. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2022. Vol. 37, No 1. P. 749–759. DOI: 10.1080/14756366.2022.2039918
29. Liu Y. et al. Flavonoids as promising natural compounds for combating bacterial infections. *International Journal of Molecular Sciences*. 2025. Vol. 26, No 6. Art. 2455. DOI: 10.3390/ijms26062455
30. Bae J. Y., Seo Y. H., Oh S. W. Antibacterial activities of polyphenols against foodborne pathogens and their application as antibacterial agents. *Food Science and Biotechnology*. 2022. Vol. 31, No 8. P. 985–997. DOI: 10.1007/s10068-022-01058-3
31. Bellanti F. et al. Redox imbalance in inflammation: the interplay of oxidative and reductive stress. *Antioxidants*. 2025. Vol. 14, No 6. Art. 656. DOI: 10.3390/antiox14060656
32. Bhattacharya S. et al. Mitigating ROS signalling pathway-mediated defence mechanism: a novel approach to counteract bacterial resistance using natural antioxidant-based antibiotics. *Phytochemistry Reviews*. 2025. DOI: 10.1007/s11101-025-10179-6
33. Ramalingam S. et al. Plant-based natural products as inhibitors for efflux pumps to reverse multidrug resistance in *Staphylococcus aureus*: a mini review. *Mini-Reviews in Medicinal Chemistry*. 2024. Vol. 24, No 3. P. 272–288. DOI: 10.2174/1389557523666230406092128
34. Zhang L. et al. Bacterial efflux pump inhibitors reduce antibiotic resistance. *Pharmaceutics*. 2024. Vol. 16, No 2. Art. 170. DOI: 10.3390/pharmaceutics16020170
35. Bouyahya A. et al. Mechanisms, anti-quorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria:

a comprehensive review. *Molecules*. 2022. Vol. 27, No 5. Art. 1484. DOI: 10.3390/molecules27051484

36. Zhang M. et al. Recent advances on the regulation of bacterial biofilm formation by herbal medicines. *Frontiers in Microbiology*. 2022. Vol. 13. Art. 1039297. DOI: 10.3389/fmicb.2022.1039297

37. Liu Y. et al. Eradicating biofilms: decoding persistent mechanisms and combating therapeutic strategies. *Advanced Healthcare Materials*. 2026. Vol. 15, No 6. Art. e02707. DOI: 10.1002/adhm.202502707

38. Alam M. et al. Synergistic role of plant extracts and essential oils against multidrug resistance and gram-negative bacterial strains producing extended-spectrum β -lactamases. *Antibiotics*. 2022. Vol. 11, No 7. Art. 855. DOI: 10.3390/antibiotics11070855

39. Dai C., Liu Y., Lv F., Cheng P., Qu S. An alternative approach to combat multidrug-resistant bacteria: new insights into traditional Chinese medicine monomers combined with antibiotics. *Advanced Biotechnology*. 2025. Vol. 3. Art. 6. DOI: 10.1007/s44307-025-00059-7

40. Ullah S. et al. Important flavonoids and their role as a therapeutic agent. *Molecules*. 2020. Vol. 25, No 22. Art. 5243. DOI: 10.3390/molecules25225243

41. Dias M. C., Pinto D. C. G. A., Silva A. M. S. Plant flavonoids: chemical characteristics and biological activity. *Molecules*. 2021. Vol. 26, No 17. Art. 5377. DOI: 10.3390/molecules26175377

42. Rajput A., Sharma R., Bharti R. Pharmacological activities and toxicities of alkaloids on human health. *Materials Today: Proceedings*. 2022. Vol. 48, pt. 5. P. 1407–1415. DOI: 10.1016/j.matpr.2021.09.189

43. Villanueva X. et al. Effect of chemical modifications of tannins on their antimicrobial and antibiofilm effect against Gram-negative and Gram-positive bacteria. *Frontiers in Microbiology*. 2023. Vol. 13. Art. 987164. DOI: 10.3389/fmicb.2022.987164

44. Huang P. Y., Hsu C. K., Tang H. J., Lai C. C. Eravacycline: a comprehensive review of in vitro activity, clinical efficacy, and real-world applications. *Expert Review of Anti-infective Therapy*. 2024. Vol. 22, No 6. P. 387–398. DOI: 10.1080/14787210.2024.2351552

45. Pannakal S. T. et al. Acnocure, a synergistic antimicrobial and anti-inflammatory combination of thymol and Curcuma turmerones, formulation and time-kill studies against *C. acnes*. *Cosmetics*. 2025. Vol. 12, No 2. Art. 37. DOI: 10.3390/cosmetics12020037

46. Peng Z., Wang X., Huang J., Li B. Pathogenic *Escherichia coli*. *Molecular Medical Microbiology* / ed. by Y. W. Tang et al. 3rd ed. Academic Press, 2024. P. 1065–1096. DOI: 10.1016/B978-0-12-818619-0.00069-1

47. Li Y., Zidorn C. Seasonal variations of natural products in European herbs. *Phytochemistry Reviews*. 2022. Vol. 21. P. 1549–1575. DOI: 10.1007/s11101-021-09797-7

48. Chaachouay N. Synergy, additive effects, and antagonism of drugs with plant bioactive compounds. *Drugs and Drug Candidates*. 2025. Vol. 4, No 1. Art. 4. DOI: 10.3390/ddc4010004

49. Balkrishna A. et al. Exploring the safety, efficacy, and bioactivity of herbal medicines: bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine*. 2024. Vol. 3, No 1. P. 35–49. DOI: 10.14218/FIM.2023.00086

Information about the author:

Derkach Tetiana,

Doctor of Pedagogical Science, PhD in Chemistry, Professor,
Dean of the Faculty of Chemical and Biopharmaceutical Technologies
Kyiv National University of Technologies and Design
2, Mala Shyianovska Street, Kyiv, Ukraine

DECISION FRAMEWORK FOR ASSESSING THE ENVIRONMENTAL SAFETY OF RENEWABLE ENERGY IN AGRI-FOOD SYSTEMS

Kryshtop Yevhen

DOI <https://doi.org/10.30525/978-9934-26-714-7-4>

INTRODUCTION

Climate change, resource degradation and the commitment to climate neutrality have turned the energy base of agricultural production into a structural problem rather than a technical one. Global renewable capacity passed 3,870 GW in 2023, after a record annual addition of 473 GW¹. The Net Zero by 2050 scenario of the International Energy Agency requires that pace to triple, to roughly 1,100 GW a year by 2030². Agri-food systems sit at the centre of this trajectory: they are at once a major energy consumer, a significant source of greenhouse gas emissions, and an important arena for renewable deployment³.

The relationship between renewable energy and agri-food systems is therefore bidirectional, and more intricate than a simple substitution of fossil fuels. Agrivoltaics, biogas from agricultural residues, small wind installations on farmland and distributed rooftop photovoltaics form a technology set with an environmental signature of its own – and with methodological problems to match. Impact allocation between the agricultural and the energy function remains unresolved in Agri-PV; the global warming potential (GWP) of bioenergy swings with the accounting convention adopted for avoided methane; and large-scale infrastructure raises the risk of landscape fragmentation⁴.

¹ International Renewable Energy Agency. Renewable capacity statistics 2024. Abu Dhabi : IRENA, 2024. URL: <https://www.irena.org/Publications/2024/Mar/Renewable-capacity-statistics-2024>

² International Energy Agency. Net Zero Roadmap: A Global Pathway to Keep the 1.5 °C Goal in Reach. 2023 Update. Paris : IEA, 2023. URL: <https://www.iea.org/reports/net-zero-roadmap-a-global-pathway-to-keep-the-15-c-goal-in-reach>

³ Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by P. R. Shukla et al. Cambridge ; New York : Cambridge University Press, 2022. DOI: 10.1017/9781009157926

⁴ Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by P. R. Shukla et al. Cambridge ; New York : Cambridge University Press, 2022. DOI: 10.1017/9781009157926 ; Chatzipanagi A., Taylor N., Jaeger-Waldau A. Overview of the potential and challenges for agri-photovoltaics in the European Union. Luxembourg : Publications Office of the European Union, 2023. EUR 31482 EN. DOI: 10.2760/208702

The state of the art addresses these problems through three method families grown in largely separate traditions. Life Cycle Assessment (LCA) quantifies environmental burdens across the full life cycle; multi-criteria decision-making (MCDM) ranks competing alternatives under heterogeneous criteria; system dynamics (SD) models long-term feedback behaviour. Reviews of energy planning confirm that all three are applied to renewable systems⁵. What the literature withholds is an account of when each should be used: comparative studies describe the methods but rarely formalise the choice between them.

The unresolved question is not whether LCA, MCDM or SD is superior, but under which combinations of data quality, analytical purpose and regulatory context each becomes defensible, and how conflicting signals should be resolved. Without explicit criteria, method choice may follow disciplinary habit or software availability, reducing comparability. This problem is acute in transition economies, where incomplete verified and representative LCI coverage limits the transferability of Western European models. In Ukraine, EU integration requires methodological alignment, while the armed conflict has disrupted statistical continuity and primary data collection.

This chapter proposes a decision framework for choosing among LCA, MCDM and SD when the environmental safety of renewable energy projects in agri-food systems is at issue. Two questions guide it: how does the choice of methodological framework shape assessment outcomes for such projects in transition economies, and how can that choice be made explicit and auditable?

The chapter therefore develops a three-parameter selection procedure that distinguishes attributional from consequential LCA and derives hybridisation from diagnosed data, purpose and regulatory conditions.

1. Conceptual and Methodological Foundations

Scope and technological categories. This study takes green energy narrowly: electricity and heat generated from renewable sources whose life cycle greenhouse gas emissions are substantially lower than those of fossil alternatives. Nuclear power falls outside the scope, since its assessment belongs to a separate discourse on long-lived waste and low-probability, high-consequence risk that would blur the selection problem at issue here. Within agri-food systems, green energy spans five categories: ground-mounted agrivoltaics (Agri-PV); biogas from agricultural residues; small wind installations on farmland; distributed

⁵ Pohekar S. D., Ramachandran M. Application of multi-criteria decision making to sustainable energy planning : a review. *Renewable and Sustainable Energy Reviews*. 2004. Vol. 8. № 4. P. 365–381. DOI: 10.1016/j.rser.2003.12.007 ; Strantzali E., Aravossis K. Decision making in renewable energy investments : a review. *Renewable and Sustainable Energy Reviews*. 2016. Vol. 55. P. 885–898. DOI: 10.1016/j.rser.2015.11.021

rooftop photovoltaics; and solid biomass from dedicated energy crops. Each engages agroecosystems through a different mechanism, and that mechanism conditions which assessment method fits.

The dual function of the agricultural landscape. Renewable energy in agri-food systems turns on a single conceptual feature: the agricultural landscape does two jobs at once, the same hectare serving as a productive resource for food and as a physical carrier for energy infrastructure. In Agri-PV this duality creates an allocation problem that the ISO 14040/14044 framework does not settle by prescribed rule. No standardised convention divides the environmental burden of the installation between the crop and the kilowatt-hour, and different allocation choices yield materially different GWP values for one and the same physical system.

In bioenergy the duality runs along the chain agricultural residues → biogas + digestate → electricity + organic fertiliser, where the choice of system boundary changes the result outright. Digestate treated as a co-product carrying its own burden share, as an avoided-burden credit against mineral fertiliser, or as a waste stream yields three different verdicts on one plant; comparable sensitivity is documented across bioenergy systems, where energy-based, economic and system-expansion allocation diverge significantly in GWP and other impact categories⁶. The same chain shows why the discussion cannot remain within a purely energetic frame. Converting residues into energy and a nutrient-bearing soil amendment is a waste valorisation pathway that closes a nutrient loop at farm or community scale – in circular economy terms, industrial symbiosis between agricultural production and energy generation. Methods that register only the energy output will misrepresent such systems systematically.

Environmental safety as an umbrella construct. *Environmental safety* is polysemic: depending on disciplinary tradition it denotes the absence of threats to human health, the preservation of ecosystem functionality, or compliance with regulatory limits, and in the international LCA literature the neighbouring notions – environmental performance, environmental impacts, environmental risk – are none of them exact synonyms. The term is retained here as an author-defined umbrella construct whose content is fixed explicitly rather than assumed, namely a multidimensional construct of four dimensions:

- 1) climatic – global warming potential over a 100-year horizon, expressed in g CO₂-eq. kWh⁻¹;
- 2) ecosystem – eutrophication, acidification, land use and freshwater ecotoxicity;

⁶ Life cycle assessment and techno-economic analysis of sustainable bioenergy production: a review / A. I. Osman [et al.]. *Environmental Chemistry Letters*. 2024. Vol. 22, no. 3. P. 1115–1154. DOI: 10.1007/s10311-023-01694-z.

- 3) resource – abiotic resource depletion and freshwater consumption;
- 4) toxicological – human toxicity.

These are conceptual categories, not the impact categories of any single characterisation method. They are operationalised at the goal and scope stage by selecting the corresponding indicators from a clearly identified and established life cycle impact assessment (LCIA) method⁷. Because impact-category definitions and indicators vary across LCIA methods, every application must name the method used and the indicator representing each dimension. Mixing terminology drawn from different methods within one indicator set is a reporting error, not a stylistic choice.

Expressing the construct through life cycle indicators secures comparability across dimensions and guards against the *single-target trap*, in which optimising one indicator conceals deterioration in another: attention confined to GWP can mask rising eutrophication from digestate application, or soil degradation under monocultural energy cropping. The hazard is acute in agri-food systems, where soil and water are at once the object of protection and the productive base; the diagnostic limits of single-indicator screening are well established⁸. Because the four dimensions differ in measurability, time constant and policy relevance, no one method captures them all. LCA locates life cycle hotspots and supports targeted mitigation within a systemic view of agroecosystems⁹, yet it neither weighs social acceptability nor models feedback over decades. The case for comparative systematisation follows from the construct itself.

Positioning the technological approach. Earlier work systematised approaches to sustainable resource use in agri-food systems along five directions¹⁰. This chapter develops the innovation-technological direction through a comparative typology of the three assessment methods (Table 1).

⁷ Guinée J. B. (ed.). Handbook on life cycle assessment : operational guide to the ISO standards. Dordrecht : Kluwer Academic Publishers, 2002. DOI: 10.1007/0-306-48055-7

⁸ Laurent A., Olsen S. I., Hauschild M. Z. Limitations of carbon footprint as indicator for environmental sustainability and LCA-based screening tools. *Environmental Science & Technology*. 2012. Vol. 46. № 8. P. 4100–4108. DOI: 10.1021/es204163f

⁹ Lulovicova A., Bouissou S. Life cycle assessment as a prospective tool for sustainable agriculture and food planning at a local level. *Geography and Sustainability*. 2024. Vol. 5. № 2. P. 251–264. DOI: 10.1016/j.geosus.2024.01.008

¹⁰ Пузік В. К., Криштон Є. А. Системний підхід до екологічно збалансованого природокористування: концептуальні моделі, аналітичні рамки та індикатори. *Методологічні підходи та практичне обґрунтування екологічно збалансованого природокористування і шляхи зниження антропогенного навантаження на природні і штучні екосистеми у сфері АПК* : звіт про НДР (остаточний) / Державний біотехнологічний університет. Харків, 2025. Розд. VII. С. 213–244. № держреєстрації 0121U107701. URL: <https://repo.btu.kharkiv.ua/server/api/core/bitstreams/4d9ea1b4-3100-42e0-80f8-f53ff23f8ed5/content>

Table 1

Conceptual typology of methodological approaches to assessing sustainable resource use in agri-food systems

<i>Criterion</i>	<i>Systems approach (DPSIR / SES)</i>	<i>Ecosystem approach</i>	<i>Landscape-ecological approach</i>	<i>Technological approach (LCA / MCDM / SD)</i>
Object of analysis	The agricultural system as a socio-ecological-technical system; actors, resources, rules	Ecosystem functionality; services and their interlinkages	Spatial organisation of agricultural landscapes; fragmentation, connectivity	Product, technology or project; material and energy flows
Analytical framework	DPSIR; SES framework (Ostrom); planetary boundaries	CBD, IPBES, TEEB; ecosystem services concept	Landscape metrics (FRAGSTATS); GIS analysis	LCA (ISO 14040/14044); MCDM (AHP, TOPSIS); system dynamics
Spatial scale	Field or farm to macro-policy	Plot to catchment	Landscape or region; agroclimatic zone	Product to system; cradle-to-grave or cradle-to-gate
Time horizon	Medium to long term; iterative	Long term (succession, climatic cycles)	Medium term (10–50 years)	SD: 15–50 years; LCA: static; MCDM: present state
Principal strength	Comprehensive representation of cause–effect relations	Attention to systemic integrity and services	Spatial diagnosis of degradation and restoration	Quantitative impact assessment; comparison of alternatives
Principal limitation	Difficulty of collecting multidimensional data	Non-standardised valuation of services	Dependence on detailed GIS data	Scarcity of local inventories in transition economies

Source: developed by the author on the basis of¹¹.

¹¹ Strantzali E., Aravossis K. Decision making in renewable energy investments : a review. *Renewable and Sustainable Energy Reviews*. 2016. Vol. 55. P. 885–898. DOI: 10.1016/j.rser.2015.11.021 ; Guinée J. B. (ed.). *Handbook on life cycle assessment : operational guide to the ISO standards*. Dordrecht : Kluwer Academic Publishers, 2002. DOI: 10.1007/0-306-48055-7 ; Пузік В. К., Криштоп Є. А. Системний підхід до екологічно збалансованого природокористування: концептуальні моделі, аналітичні рамки та індикатори. *Методологічні підходи та практичне обґрунтування екологічно збалансованого природокористування і шляхи зниження антропогенного навантаження на природні і штучні екосистеми у сфері АПК* : звіт про НДР (остаточний) / Державний біотехнологічний університет. Харків, 2025. Розд. VII. С. 213–244. № держреєстрації 0121U107701. URL: <https://repo.btu.kharkiv.ua/server/api/core/bitstreams/4d9ea1b4-3100-42e0-80f8-f53ff23f8ed5/content> ; Forrester J. W. *Industrial dynamics*. Cambridge, MA : MIT Press, 1961.

Table 1 shows the technological approach complementing rather than displacing the systems and landscape-ecological traditions: DPSIR and the SES framework supply the criteria structure and the governance context, while LCA, MCDM and SD deliver quantitative assessment of specific options.

Ecosystem interactions of green energy technologies. The complementarity of methodological approaches must be considered in relation to the ecological processes that actually unfold in agroecosystems. Empirical evidence shows that ground-mounted photovoltaic systems alter local air and soil microclimates, soil moisture, vegetation biomass and plant-species diversity, with effects varying across seasons and management regimes¹². Habitat-enhancing agrivoltaic configurations may improve pollinator supply, water retention, sediment retention and carbon storage relative to conventional agricultural practice, although these findings are model-based and require field validation¹³.

More broadly, territorial LCA can support environmental planning in local agri-food systems by identifying environmental impacts and trade-offs across alternative scenarios¹⁴. Taken together, these studies indicate that environmental outcomes are context- and management-dependent: appropriate management can enhance ecosystem functions, whereas poorly adapted siting and management practices may create localised risks. Table 2 summarises these interactions by technology.

Table 2

**Ecological interactions of green energy technologies
in agricultural landscapes**

<i>Renewable technology</i>	<i>Soil resources</i>	<i>Water resources</i>	<i>Biodiversity</i>	<i>Landscape structure</i>
Agri-PV (ground-mounted)	Panel shading alters soil temperature and moisture; summer cooling and local drying have been observed beneath PV arrays ¹ .	Water-retention effects depend on system configuration and vegetation management; habitat-enhancing scenarios have shown modelled gains in water retention ² .	Plant biomass and species diversity may decline beneath conventional PV arrays ³ , whereas habitat-enhancing agrivoltaic configurations may increase pollinator supply ⁴ .	Altered visual structure; requires GIS-based connectivity assessment

¹² Armstrong A., Ostle N. J., Whitaker J. Solar park microclimate and vegetation management effects on grassland carbon cycling. *Environmental Research Letters*. 2016. Vol. 11. № 7. Article 074016. DOI: 10.1088/1748-9326/11/7/074016

¹³ Enhancing ecosystem services and biodiversity in agrivoltaics through habitat-enhancing strategies / A. Ludzuweit et al. *Renewable and Sustainable Energy Reviews*. 2025. Vol. 212. Article 115380. DOI: 10.1016/j.rser.2025.115380

¹⁴ Lulovicova A., Bouissou S. Life cycle assessment as a prospective tool for sustainable agriculture and food planning at a local level. *Geography and Sustainability*. 2024. Vol. 5. № 2. P. 251–264. DOI: 10.1016/j.geosus.2024.01.008

Table 2 (Continued)

<i>Renewable technology</i>	<i>Soil resources</i>	<i>Water resources</i>	<i>Biodiversity</i>	<i>Landscape structure</i>
Biogas from agricultural residues	Digestate can affect soil organic matter and improve selected soil physical properties, although outcomes are site- and management-dependent ⁵ .	Nitrate-leaching risk depends on digestate characteristics, soil type, application rate and the synchrony of nitrogen supply with crop demand; timely nutrient characterisation and crop-matched application reduce over-fertilisation risk ⁶ .	Context-dependent effects on soil biota: possible short-term earthworm mortality or avoidance, but neutral or positive longer-term responses ⁷ .	Localised landscape effects associated with digesters and storage facilities
Wind installations on farmland	Minimal permanent land withdrawal, although construction and access roads may cause local soil disturbance and compaction.	No substantial direct effect on the water regime under normal operation; local construction-related effects remain site-specific.	Site- and species-specific collision, disturbance, displacement and barrier risks for birds and bats; reduced through ecological screening, avoidance of sensitive habitats and major flyways, appropriate turbine siting and, where necessary, operational curtailment ⁸ .	Altered landscape silhouette; spatial suitability and social acceptability require site-specific assessment.
Distributed rooftop PV	No direct effect; no agricultural land occupation	No direct interaction with agricultural water resources during normal operation	Limited direct effect; no additional land occupation	Minimal landscape effect; potential building-level visual considerations
Energy crops (miscanthus, willow)	Perennial Miscanthus and willow can increase soil organic carbon when replacing annual arable crops, although outcomes depend on previous land use, soil conditions and plantation age ⁹ .	Water use may exceed that of annual arable crops, particularly for willow short-rotation coppice; effects on evapotranspiration and local water balance are site-specific and depend on precipitation, soil conditions, groundwater availability, crop species and stand age ¹⁰ .	Relative to intensive arable cropping, Miscanthus and willow can provide additional habitat and support greater biodiversity, although outcomes depend on crop structure, management, plantation age and the surrounding landscape ¹¹ .	Plantation size and spatial configuration alter landscape structure; appropriately located perennial energy crops can increase heterogeneity in intensively arable landscapes, although effects vary by crop and spatial scale ¹² .

Source: developed by the author from the sources cited in the table.

Three regularities emerge. Agri-PV and bioenergy require assessment beyond the climatic dimension; rooftop PV has comparatively limited direct interaction with agroecosystems; and no technology is preferable independently

of context. The framework must therefore represent both local ecological risks and circular-economy benefits, including nutrient-loop closure, residue valorisation and avoided agricultural land occupation.

Figure 1 shows renewable technologies generating distinct yet overlapping challenges. Agrivoltaics and residue-based biogas are associated primarily with impact allocation, while biogas additionally exhibits substantial GWP variability arising from system-boundary choices, methane-accounting conventions and LCA orientation; wind installations and dedicated energy crops raise landscape and spatial concerns, and energy crops introduce a direct food-energy trade-off. Limited coverage of verified, representative Ukrainian LCI data acts as a cross-cutting constraint under transition-economy conditions. The terminal node, “Need for structured method selection”, expresses the requirement to reconcile technology-specific challenges with data conditions and assessment requirements; the corresponding framework is developed in Section 3.

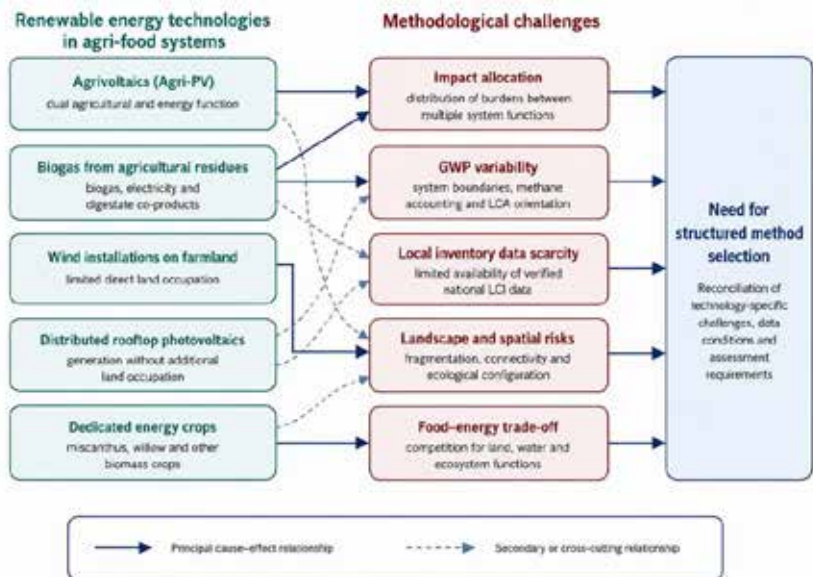


Fig. 1. Problem architecture linking renewable energy technologies in agri-food systems to technology-specific methodological challenges and the resulting need for explicit method selection. Solid arrows indicate principal cause-effect relationships; dashed arrows indicate secondary or cross-cutting relationships

Source: developed by the author from the literature synthesis presented in Section 1 and Table 2.

The regulatory determinants of method choice. Analytical considerations do not govern method selection alone. Three regulatory frameworks bear on Ukraine. The United Nations Sustainable Development Goals, SDG 7 and SDG 13 in particular, set the normative baseline. The European Green Deal, with the Fit for 55 package and the Farm to Fork and Bioeconomy strategies, supplies the operative instruments: the EU Taxonomy requires demonstration of substantial contribution and compliance with the do no significant harm (DNSH) principle across six environmental objectives, its technical screening criteria set activity by activity and its evidence requirements varying accordingly¹⁵; the Environmental Footprint methods furnish a standardised life-cycle basis for environmental claims, grounded in attributional LCA¹⁶; and the Corporate Sustainability Reporting Directive obliges large undertakings to disclose taxonomy-aligned data. The EU – Ukraine Association Agreement and candidate status granted in 2022 turn methodological harmonisation from an option into a policy requirement.

Life cycle thinking is thus entering decision processes through these methods¹⁷, while global decarbonisation strategies press for integrated systems analysis¹⁸. Method selection must therefore satisfy scientific and regulatory criteria at once – a demand that Section 2 tests against the three candidate methods.

2. Comparative Analysis of Assessment Methods

Review protocol. The targeted methodological review was reported using the applicable items and flow diagram of the PRISMA 2020 statement¹⁹, which updates the 2009 statement²⁰ and supports transparent reporting of record

¹⁵ Commission Delegated Regulation (EU) 2021/2139 of 4 June 2021 supplementing Regulation (EU) 2020/852 by establishing technical screening criteria. *Official Journal of the European Union*. 2021. L 442. P. 1–349. URL: https://eur-lex.europa.eu/eli/reg_del/2021/2139/oj

¹⁶ Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations. *Official Journal of the European Union*. 2021. L 471. P. 1–396. URL: <https://eur-lex.europa.eu/eli/reco/2021/2279/oj>; European Commission, Joint Research Centre. Environmental Footprint methods. URL: <https://eplca.jrc.ec.europa.eu/EnvironmentalFootprint.html>

¹⁷ Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations. *Official Journal of the European Union*. 2021. L 471. P. 1–396. URL: <https://eur-lex.europa.eu/eli/reco/2021/2279/oj>; European Commission, Joint Research Centre. Environmental Footprint methods. URL: <https://eplca.jrc.ec.europa.eu/EnvironmentalFootprint.html>

¹⁸ International Energy Agency. Net Zero Roadmap: A Global Pathway to Keep the 1.5 °C Goal in Reach. 2023 Update. Paris : IEA, 2023. URL: <https://www.iea.org/reports/net-zero-roadmap-a-global-pathway-to-keep-the-15-c-goal-in-reach>

¹⁹ The PRISMA 2020 statement: an updated guideline for reporting systematic reviews / M. J. Page et al. *BMJ*. 2021. Vol. 372. Article n71. DOI: 10.1136/bmj.n71

²⁰ Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement / D. Moher et al. *PLoS Medicine*. 2009. Vol. 6. № 7. Article e1000097. DOI: 10.1371/journal.pmed.1000097

identification, screening and inclusion. PRISMA was used as a reporting framework rather than as a claim that the review constituted an exhaustive global systematic review. The review sought to characterise approaches retrieved through the predefined targeted strategy, identify methodological imbalances within that sample, and establish the case for integration. The review protocol was not prospectively registered.

The searches were run in Scopus and the Web of Science Core Collection on 15 January 2026. Filters were applied for document type (article, review) and subject area (environmental science, energy, agriculture); the publication window ran from 2019 to 2024, and languages were English and Ukrainian. One conceptual strategy was executed in both databases, the field tag adapted to each: TITLE-ABS-KEY in Scopus, Topic (TS) in Web of Science. An outer bracket encloses the three method blocks, so that every retrieved record satisfies the method condition together with each of the four conditions that follow:

((“life cycle assessment” OR LCA OR “attributional LCA” OR “consequential LCA”) OR (“multi-criteria decision making” OR MCDM OR AHP OR TOPSIS OR VIKOR) OR (“system dynamics”)) AND (“renewable energy” OR RES OR bioenergy OR biogas OR agrivoltaic* OR “agri-PV” OR wind OR photovoltaic*) AND (“environmental safety” OR “environmental impact” OR sustainability) AND (agricultur* OR “agro-industrial complex” OR agri-food OR agroecosystem*) AND (“transition econom*” OR Ukraine OR “Eastern Europe”)

Deduplication ran in two passes: records were matched automatically on DOI, and those lacking a DOI or registered divergently were then matched manually on title, first author and publication year, the journal version being retained wherever a study appeared as both preprint and article. The geographical component was included deliberately to retrieve studies potentially relevant to transition-economy, Ukrainian or Eastern European contexts. The occurrence of one or more of these terms in the searchable bibliographic fields was a retrieval condition but did not necessarily mean that a study was empirically focused on a transition economy; geographical focus was subsequently coded from the empirical study area rather than from the matched search term. The review is therefore a targeted methodological review rather than a statistically representative mapping of the global literature: its aim was not to quantify worldwide publication patterns but to identify approaches potentially applicable under transition-economy conditions. Accordingly, the frequencies reported below characterise the targeted analytical sample rather than the global research field.

The PRISMA analytical sample and the supplementary evidence base were kept separate. All quantitative analyses used only the 87 eligible peer-reviewed studies published within the predefined 2019–2024 window. Publications

from 2025 identified during post-review verification, including²¹, as well as institutional reports, foundational or other out-of-window publications, and technology-specific sources²², were used only for contextual interpretation and did not enter the PRISMA counts, cluster frequencies or identification of the research gap. Consequently, the analytical sample does not capture the complete 2025 literature and may underrepresent the most recent methodological developments. Sources²³ remained outside the analytical sample because they

²¹ Enhancing ecosystem services and biodiversity in agrivoltaics through habitat-enhancing strategies / A. Ludzuweit et al. *Renewable and Sustainable Energy Reviews*. 2025. Vol. 212. Article 115380. DOI: 10.1016/j.rser.2025.115380 ; Tighnavard Balasbanch A., Aldrovandi S., Sher W. A systematic review of implementing multi-criteria decision-making (MCDM) approaches for the circular economy and cost assessment. *Sustainability*. 2025. Vol. 17. № 11. Article 5007. DOI: 10.3390/su17115007 ; Mlybari E. A. Smart sustainability in construction: an integrated LCA–MCDM framework for climate-adaptive material selection in educational buildings. *Sustainability*. 2025. Vol. 17. № 21. Article 9650. DOI: 10.3390/su17219650 ; A critical review of life cycle assessment of renewable agricultural systems / M. Kiehbadrounizhad et al. *Sustainable Energy Technologies and Assessments*. 2025. Vol. 73. Article 104100. DOI: 10.1016/j.seta.2024.104100

²² Long-term application of biogas digestate improves soil physical properties / M. Mayerová et al. *Soil and Tillage Research*. 2023. Vol. 231. Article 105715. DOI: 10.1016/j.still.2023.105715 ; Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching / T. A. Sogn et al. *International Journal of Recycling of Organic Waste in Agriculture*. 2018. Vol. 7. P. 49–58. DOI: 10.1007/s40093-017-0188-0 ; Digestate in replacement of synthetic fertilisers: a comparative 3-year field study of the crop performance and soil residual nitrates in West-Flanders / G. Reuland et al. *European Journal of Agronomy*. 2024. Vol. 161. Article 127380. DOI: 10.1016/j.eja.2024.127380 ; Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils / V. Moinard et al. *Applied Soil Ecology*. 2021. Vol. 168. Article 104149. DOI: 10.1016/j.apsoil.2021.104149 ; European Commission. Directorate-General for Environment. Guidance document on wind energy developments and EU nature legislation. Luxembourg : Publications Office of the European Union, 2020. DOI: 10.2779/095188 ; Harris Z. M., Spake R., Taylor G. Land use change to bioenergy: a meta-analysis of soil carbon and GHG emissions. *Biomass and Bioenergy*. 2015. Vol. 82. P. 27–39. DOI: 10.1016/j.biombioe.2015.05.008 ; A review of the impacts of Short Rotation Coppice cultivation on water issues / I. Dimitriou et al. *Landbauforschung*. 2009. Vol. 59. № 3. P. 197–206 ; Dedicated biomass crops can enhance biodiversity in the arable landscape / A. J. Haughton et al. *GCB Bioenergy*. 2016. Vol. 8. № 6. P. 1071–1081. DOI: 10.1111/gcbb.12312 ; Landscape metrics and land-use patterns of energy crops in the agricultural landscape / X. Xu et al. *BioEnergy Research*. 2023. Vol. 16. P. 2178–2191. DOI: 10.1007/s12155-023-10584-9

²³ Long-term application of biogas digestate improves soil physical properties / M. Mayerová et al. *Soil and Tillage Research*. 2023. Vol. 231. Article 105715. DOI: 10.1016/j.still.2023.105715 ; Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching / T. A. Sogn et al. *International Journal of Recycling of Organic Waste in Agriculture*. 2018. Vol. 7. P. 49–58. DOI: 10.1007/s40093-017-0188-0 ; Digestate in replacement of synthetic fertilisers: a comparative 3-year field study of the crop performance and soil residual nitrates in West-Flanders / G. Reuland et al. *European Journal of Agronomy*. 2024. Vol. 161. Article 127380. DOI: 10.1016/j.eja.2024.127380 ; Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils / V. Moinard et al. *Applied Soil Ecology*. 2021. Vol. 168. Article 104149. DOI: 10.1016/j.apsoil.2021.104149 ; European Commission. Directorate-General for Environment. Guidance document on wind energy developments and EU nature legislation. Luxembourg : Publications Office of the European Union, 2020. DOI: 10.2779/095188 ; Harris Z. M., Spake R., Taylor G. Land use change to bioenergy: a meta-analysis of soil carbon and GHG emissions. *Biomass and Bioenergy*. 2015. Vol. 82. P. 27–39. DOI: 10.1016/j.biombioe.2015.05.008 ; A review of the impacts of Short Rotation Coppice cultivation on water issues / I. Dimitriou et al. *Landbauforschung*. 2009. Vol. 59. № 3. P. 197–206 ; Dedicated biomass crops can enhance biodiversity in the arable landscape / A. J. Haughton et al. *GCB Bioenergy*. 2016. Vol. 8. № 6. P. 1071–1081. DOI: 10.1111/gcbb.12312 ; Landscape metrics and land-use patterns of energy crops in the agricultural landscape / X. Xu et al. *BioEnergy Research*. 2023. Vol. 16. P. 2178–2191. DOI: 10.1007/s12155-023-10584-9

fell outside the publication window or did not use LCA, MCDM or SD as the principal analytical instrument.

Selection process and sample. The search identified 512 records across the two databases. Duplicate removal ($n = 170$) left 342 unique records for title and abstract screening, from which 164 full-text reports were assessed for eligibility and 87 peer-reviewed articles and reviews entered the final analytical sample (Figure 2).

A single reviewer conducted the screening and reassessed borderline cases through repeated full-text examination against the predefined criteria; no independent second reviewer took part – a limitation of the review, revisited among the validity considerations below. Title-and-abstract screening excluded 178 records on thematic or language grounds, leaving 164 reports to be retrieved. Full-text assessment then excluded 77 on three grounds: no explicit methodological approach ($n = 32$); a technical focus without substantive environmental analysis ($n = 21$); and no relevance to agri-food systems ($n = 24$).

Inclusion required relevance to renewable energy assessment in agri-food systems; LCA, MCDM or SD as the principal analytical instrument rather than a descriptive element; an environmental or systems perspective; peer-reviewed article or review status; an available full text; and publication within 2019–2024. Records failing any of these conditions, or duplicating another record, were excluded.

Data extraction and synthesis. Data were extracted from each included study on a structured coding form built for this review. The variables were publication year, geographical focus, renewable energy technology, principal methodological approach, LCA orientation and MCDM technique where applicable, analytical scale, temporal perspective, data characteristics, and reported treatment of uncertainty. Every study was assigned to one of three mutually exclusive principal methodological clusters: LCA, MCDM, or system dynamics and integrated approaches. Secondary methodological components were coded separately. Technologies were classified by the primary technology investigated, and geographical context was coded from the empirical study area rather than from author affiliation. Descriptive frequencies and proportions were calculated only for the mutually exclusive principal methodological and renewable-energy technology classifications. The remaining variables informed qualitative methodological comparison and were not summarised as frequencies because they were conditional, non-mutually exclusive or incompletely reported across studies. Reported treatment of uncertainty informed the qualitative discussion of limitations rather than a separate frequency estimate. No formal risk-of-bias assessment or quality scoring was undertaken, since the purpose

was methodological mapping rather than quantitative evidence synthesis. The structured coding form and the study-level list of the 87 included publications are retained in the author’s working materials and are available from the author upon request.

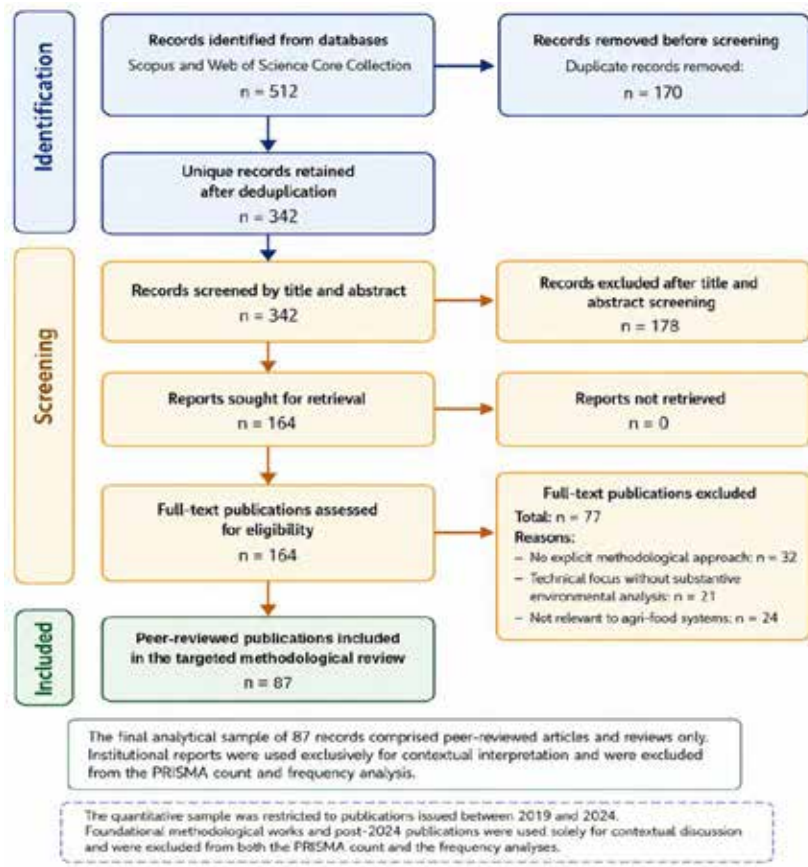


Fig. 2. PRISMA 2020 flow diagram documenting record identification, deduplication, title-and-abstract screening, report retrieval, full-text eligibility assessment and formation of the final analytical sample of 87 peer-reviewed articles and reviews

Source: developed by the author in accordance with the PRISMA 2020 statement²⁴

²⁴ The PRISMA 2020 statement: an updated guideline for reporting systematic reviews / M. J. Page et al. *BMJ*. 2021. Vol. 372. Article n71. DOI: 10.1136/bmj.n71

Structure of the targeted analytical sample. Analysis of the final sample (n = 87) identified three mutually exclusive principal methodological clusters:

- LCA – n = 32 (36.8%);
- MCDM – n = 30 (34.5%);
- system dynamics and integrated approaches – n = 25 (28.7%).

Across the full analytical sample, the dominant renewable-energy technologies were photovoltaics – n = 37 (42.5%), wind energy – n = 27 (31.0%), bioenergy – n = 16 (18.4%), and other or mixed technologies – n = 7 (8.0%).

Cluster and technology percentages use n = 87 and mutually exclusive study classifications. MCDM technique was coded as a conditional, non-mutually exclusive attribute and was used only for qualitative methodological comparison.

Studies focused on EU Member States and the United Kingdom predominated within the targeted sample, whereas only 10 studies (11.5%) explicitly addressed transition-economy contexts. Despite the inclusion of transition-economy terminology in the search strategy, few studies offered empirical analysis directly applicable to such settings. Within this targeted sample, the distribution indicates a continuing shortage of methodological work on environmental assessment under transition-economy conditions, limiting the transferability of existing approaches to Ukraine and supporting the need for context-specific development. Related reviews identify methodological and implementation constraints in sustainable construction in developing countries²⁵ and in the application of MCDM to circular-economy and cost-assessment problems²⁶. These studies provide relevant contextual evidence of broader implementation challenges, but neither directly documents the availability or representativeness of Ukrainian LCI data.

Recent research reports growing integration of LCA and MCDM in sustainability assessment, including renewable-energy and waste-management applications²⁷. These studies, however, do not provide an explicit rule for

²⁵ Establishing the limitations of sustainable construction in developing countries: a systematic literature review using PRISMA / O. E. Ogunmakinde et al. *Smart and Sustainable Built Environment*. 2024. Vol. 13. № 3. P. 609–624. DOI: 10.1108/SASBE-10-2022-0223

²⁶ Tighnavard Balasbانه A., Aldrovandi S., Sher W. A systematic review of implementing multi-criteria decision-making (MCDM) approaches for the circular economy and cost assessment. *Sustainability*. 2025. Vol. 17. № 11. Article 5007. DOI: 10.3390/su17115007

²⁷ A critical review of life cycle assessment of renewable agricultural systems / M. Kiehadrouinezhad et al. *Sustainable Energy Technologies and Assessments*. 2025. Vol. 73. Article 104100. DOI: 10.1016/j.seta.2024.104100 ; Integrating life cycle assessment and multi criteria decision making for sustainable waste management: key issues and recommendations for future studies / A. E. Torkayesh et al. *Renewable and Sustainable Energy Reviews*. 2022. Vol. 168. Article 112819. DOI: 10.1016/j.rser.2022.112819

deciding when integration is warranted. Integration is treated as a design assumption rather than the outcome of a diagnostic step, so hybrids may be adopted where one method would suffice, or a single method may be retained where the data cannot support it. Universal integrated models also remain narrow in scope and require adaptation²⁸.

Existing frameworks also remain calibrated on a data environment that transition economies do not share. The integrated LCA – MCDM designs in the sample presuppose an inventory complete enough to run the LCA component at full resolution; where that presupposition fails, they offer no fallback and no procedure for documenting the resulting bias. That is the Ukrainian situation, and it is why importing an existing hybrid design solves nothing.

Three findings summarise the review. First, life-cycle greenhouse gas emissions from renewable electricity technologies are substantially lower than those from unabated coal generation: IPCC AR5 reports median values of approximately 41–48 g CO₂-eq. kWh⁻¹ for solar photovoltaics and 11–12 g CO₂-eq. kWh⁻¹ for wind, compared with 820 g CO₂-eq. kWh⁻¹ for pulverised coal²⁹. Second, LCA results vary considerably with system boundaries and with attributional or consequential orientation – a material methodological risk that demands explicit documentation³⁰. Third, and decisively for this chapter, no explicitly validated method-selection framework meeting all predefined criteria was identified within the reviewed sample for renewable energy in the agri-food systems of transition economies.

LCA-only studies achieve considerable depth for individual technologies but treat socio-economic and governance factors thinly³¹, whereas integrated approaches acknowledge that no single instrument covers the problem, recent work on renewable integration in agriculture supporting the combination of LCA with multi-criteria methods³². This chapter departs from that literature in one respect: it treats the combination itself as a decision to be justified rather

²⁸ Mlybari E. A. Smart sustainability in construction: an integrated LCA – MCDM framework for climate-adaptive material selection in educational buildings. *Sustainability*. 2025. Vol. 17. № 21. Article 9650. DOI: 10.3390/su17219650

²⁹ Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by O. Edenhofer et al. Cambridge ; New York : Cambridge University Press, 2014. DOI: 10.1017/CBO9781107415416

³⁰ Laurent A., Olsen S. I., Hauschild M. Z. Limitations of carbon footprint as indicator for environmental sustainability and LCA-based screening tools. *Environmental Science & Technology*. 2012. Vol. 46. № 8. P. 4100–4108. DOI: 10.1021/es204163f

³¹ Dec K. Life cycle assessment of renewable energy sources – key issues. Bibliometric analysis of the literature. *Economics and Environment*. 2024. Vol. 89. № 2. Article 839. DOI: 10.34659/eis.2024.89.2.839

³² A critical review of life cycle assessment of renewable agricultural systems / M. Kiehadrouinezhad et al. *Sustainable Energy Technologies and Assessments*. 2025. Vol. 73. Article 104100. DOI: 10.1016/j.seta.2024.104100

than assumed. Fragmentation is not cured by adding methods, but by specifying the conditions under which each applies.

Life Cycle Assessment. LCA quantifies the environmental burden of products, processes or systems across the full life cycle, either cradle-to-grave or cradle-to-gate. ISO 14040:2006³³ and ISO 14044:2006³⁴ set out its principles, framework and procedural requirements, and the method proceeds in four phases: definition of goal, scope and functional unit; life cycle inventory analysis (LCI); life cycle impact assessment (LCIA); and interpretation. The standards prescribe no fixed list of impact categories; these are selected in accordance with the goal and scope of the study using a documented LCIA method, such as CML-IA³⁵ or the Environmental Footprint methods³⁶. Practice rests on widely used commercial databases such as ecoinvent and Sphera/GaBi, whose geographical coverage remains stronger for Western and Central Europe than for Ukraine.

One decision deserves particular emphasis, because it is so often left implicit. Attributional LCA describes average impacts within a defined technological system and underpins Environmental Product Declarations and the Environmental Footprint methods, which are oriented toward it for regulatory reporting. Consequential LCA models the changes in impacts across a wider system that follow from a decision and may be more appropriate where the assessment question concerns the system-wide consequences of renewable deployment in agri-food systems. Conflating the two is a frequent source of incomparable results.

Established LCA guidance already relates modelling choices to decision context. The ILCD Handbook distinguishes decision situations requiring different modelling approaches, while Ekvall and Weidema formalise consequential system boundaries and data selection³⁷. The contribution of the present framework therefore lies not in introducing the attributional – consequential

³³ ISO 14040:2006. Environmental management – Life cycle assessment – Principles and framework. Geneva : International Organization for Standardization, 2006.

³⁴ ISO 14044:2006. Environmental management – Life cycle assessment – Requirements and guidelines. Geneva : International Organization for Standardization, 2006.

³⁵ Guinée J. B. (ed.). Handbook on life cycle assessment: operational guide to the ISO standards. Dordrecht : Kluwer Academic Publishers, 2002. DOI: 10.1007/0-306-48055-7

³⁶ Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations. *Official Journal of the European Union*. 2021. L 471. P. 1–396. URL: <https://eur-lex.europa.eu/eli/reco/2021/2279/oj> ; European Commission, Joint Research Centre. Environmental Footprint methods. URL: <https://eplca.jrc.ec.europa.eu/EnvironmentalFootprint.html>

³⁷ International Reference Life Cycle Data System (ILCD) Handbook – General guide for Life Cycle Assessment – Detailed guidance / European Commission, Joint Research Centre, Institute for Environment and Sustainability. First edition. Luxembourg : Publications Office of the European Union, 2010. EUR 24708 EN. DOI: 10.2788/38479 ; Ekvall T., Weidema B. P. System boundaries and input data in consequential life cycle inventory analysis. *The International Journal of Life Cycle Assessment*. 2004. Vol. 9. № 3. P. 161–171. DOI: 10.1007/BF02994190

distinction itself, but in embedding it as an explicit choice dimension within a broader selection procedure spanning LCA, MCDM and SD under agri-food and transition-economy constraints.

The principal strength of LCA is standardisation: it offers the firmest life-cycle basis for Environmental Footprint studies and may support compliance assessment under the relevant EU Taxonomy technical screening criteria. The Taxonomy does not, however, prescribe a full LCA for every renewable energy activity – its criteria are activity-specific, and DNSH verification may rest on a range of indicators and documentation. A second strength is that impact coverage extends across several categories, which protects against the single-target trap.

Under transition-economy conditions, the critical limitation is dependence on inventory quality. The coverage of verified, geographically and technologically representative inventories for the Ukrainian processes required in renewable-energy assessment remains insufficient; where proxy datasets are used, they may introduce systematic geographical and technological deviation.

Multi-criteria decision-making. MCDM is a family of methods for structured comparison of alternatives against criteria measured in incommensurable units. For renewable projects in agri-food systems it brings LCA impact indicators, social criteria such as community acceptance and employment, and landscape indicators into a single decision.

The analytic hierarchy process³⁸ builds on pairwise comparisons. The criterion weight w_j is derived as the normalised principal eigenvector of the comparison matrix A , satisfying $A \cdot w = \lambda_{max} \cdot w$ with $\sum w_j = 1$, subject to the consistency condition $CR = CI/RI < 0.10$. TOPSIS³⁹ ranks alternatives by relative distance to the ideal solution: $CC_i = d_i^- / (d_i^+ + d_i^-)$, a higher CC_i marking a preferred alternative.

Sensitivity to weighting is a material risk. Pohekar and Ramachandran⁴⁰ showed that even moderate shifts in weights can reverse a ranking, which makes sensitivity analysis a mandatory component of any MCDM assessment rather than an optional refinement.

For transition economies MCDM offers three advantages: it tolerates imperfect data, accepting partial, ordinal and qualitative indicators; it admits the social and institutional dimension through stakeholder weighting, which matters for renewable decisions in rural communities; and it is compatible

³⁸ Saaty T. L. The analytic hierarchy process: planning, priority setting, resource allocation. New York : McGraw-Hill, 1980.

³⁹ Hwang C.-L., Yoon K. Multiple attribute decision making: methods and applications. Berlin ; Heidelberg : Springer, 1981. DOI: 10.1007/978-3-642-48318-9

⁴⁰ Pohekar S. D., Ramachandran M. Application of multi-criteria decision making to sustainable energy planning : a review. *Renewable and Sustainable Energy Reviews*. 2004. Vol. 8. № 4. P. 365–381. DOI: 10.1016/j.rser.2003.12.007

with the DPSIR framework, where drivers and pressures define the regulatory context, state and impact supply the environmental criteria, and responses represent the management options⁴¹.

System dynamics. Where LCA and MCDM yield static assessments or rankings, SD models complex systems through stocks, flows and feedback loops. Developed by Forrester⁴² and extended by Sterman⁴³, it represents behaviour beyond the reach of the other two. For agri-energy systems it captures three structures: a reinforcing loop reproducing the renewable learning curve, in which rising capacity lowers unit cost, attracts investment and raises capacity further; an illustrative balancing loop in which deteriorating emissions performance triggers policy intervention, investment or substitution responses that may reduce emissions after a delay; and explicit time delays between investment decisions and commissioning.

The principal barrier to SD in Ukraine is the shortage of time series long and internally consistent enough for calibration and validation: since 2022 the armed conflict has disrupted statistical continuity, altered the energy mix and changed production parameters. Models must therefore distinguish wartime from reconstruction regimes explicitly. Over the 2030–2050 horizon, however, SD remains valuable for grounding national renewable energy targets.

Comparative matrix. These differences call for systematic comparison of the domains of applicability and the limits of each approach. Table 3 sets them side by side.

Table 3

Comparative matrix of methodological approaches to assessing the environmental safety of renewable energy in agri-food systems

<i>Criterion</i>	<i>LCA (attributional / consequential)</i>	<i>MCDM (AHP, TOPSIS, VIKOR)</i>	<i>System dynamics (SD)</i>
Input data	Quantitative material and energy inventories; average values for attributional, marginal for consequential	Quantitative and qualitative; tolerates partial or aggregated data; admits expert judgement	Longitudinal data sufficient for calibration and validation; non-linear relations between variables
Scale and horizon	Product, process or project; full life cycle from extraction to disposal	Project, region or strategy; present state or short-term projection	System, region or country; strategic horizon of 15–50 years

⁴¹ Next generation application of DPSIR for sustainable policy implementation / S. A. Carnohan et al. *Current Research in Environmental Sustainability*. 2023. Vol. 5. Article 100201. DOI: 10.1016/j.crsust.2022.100201

⁴² Forrester J. W. *Industrial dynamics*. Cambridge, MA : MIT Press, 1961.

⁴³ Sterman J. D. *Business dynamics: systems thinking and modeling for a complex world*. Boston : Irwin/McGraw-Hill, 2000.

Table 3 (Continued)

<i>Criterion</i>	<i>LCA (attributional / consequential)</i>	<i>MCDM (AHP, TOPSIS, VIKOR)</i>	<i>System dynamics (SD)</i>
Impact coverage	Broad: categories selected at the goal and scope stage from a documented LCIA method (e.g., CML-IA or the Environmental Footprint methods)	Selective: determined by the chosen criteria set; may include social and economic dimensions	Dynamically integrated: resource flows, emissions, feedbacks between subsystems
Standardisation, methodological basis and tools	ISO 14040:2006 ¹³ ; ISO 14044:2006 ¹⁴ ; Environmental Footprint methods; may support EU Taxonomy screening where life-cycle evidence is required	No single standard; AHP after Saaty ¹⁵ ; TOPSIS after Hwang and Yoon ¹⁶	Methodology of Forrester ¹⁷ and Sterman ¹⁸ ; Vensim, AnyLogic
Fit for transition economies	Limited: scarce verified local inventories introduce systematic deviation	High: flexible under incomplete and aggregated data; adaptive criteria selection	Moderate: requires stable series; armed conflict substantially constrains application in Ukraine
Relevance to agri-food systems	Quantifies the environmental burden of renewable technologies; critical for Agri-PV and bioenergy	Compares alternatives across landscape, social and regulatory criteria	Models long-term sectoral development; learning and regulatory feedback loops
Resource intensity	High: ecoinvent/GaBi licences; 3–6 months for a full analysis	Moderate: 1–3 months depending on criteria and stakeholder numbers	High: 3–12 months; a team of 3–5 systems modellers

Source: developed by the author on the basis of⁴⁴.

⁴⁴ Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by P. R. Shukla et al. Cambridge ; New York : Cambridge University Press, 2022. DOI: 10.1017/9781009157926 ; Pohekar S. D., Ramachandran M. Application of multi-criteria decision making to sustainable energy planning : a review. *Renewable and Sustainable Energy Reviews*. 2004. Vol. 8. № 4. P. 365–381. DOI: 10.1016/j.rser.2003.12.007 ; Strantzali E., Aravossis K. Decision making in renewable energy investments : a review. *Renewable and Sustainable Energy Reviews*. 2016. Vol. 55. P. 885–898. DOI: 10.1016/j.rser.2015.11.021 ; Guinée J. B. (ed.). Handbook on life cycle assessment: operational guide to the ISO standards. Dordrecht : Kluwer Academic Publishers, 2002. DOI: 10.1007/0-306-48055-7 ; Forrester J. W. Industrial dynamics. Cambridge, MA : MIT Press, 1961 ; Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations. *Official Journal of the European Union*. 2021. L 471. P. 1–396. URL: <https://eur-lex.europa.eu/eli/reco/2021/2279/oj> ; European Commission, Joint Research Centre. Environmental Footprint methods. URL: <https://eplca.jrc.ec.europa.eu/EnvironmentalFootprint.html> ; Environmental management – Life cycle assessment – Principles and framework. Geneva : International Organization for Standardization, 2006 ; ISO 14044:2006. Environmental management – Life cycle assessment – Requirements and guidelines. Geneva : International Organization for Standardization, 2006 ; Sterman J. D. Business dynamics: systems thinking and modeling for a complex world. Boston : Irwin/McGraw-Hill, 2000.

Table 3 shows why method choice is context-dependent: LCA is standardised but inventory-intensive, MCDM is flexible but weight-sensitive, and SD is strategically valuable but dependent on sufficiently stable time series.

3. The Decision Framework and Its Application

Principles and structure. The framework rests on the *fit-for-purpose* principle: the choice of approach should follow from the analytical objective, data availability and regulatory context, not from disciplinary convention or tool availability. It treats renewable energy in agri-food systems as a complex socio-ecological system (SES) of biophysical, economic and governance interactions.

It is a structured, rule-based selection framework rather than a fully formalised model: it orders and documents the reasoning behind a method choice without replacing that reasoning with computation. Three analytically distinct but interacting parameters are evaluated in sequence.

The procedure evaluates three parameters sequentially: data quality (I_1), assessment purpose (I_2), and regulatory and institutional context (I_3). Their branch conditions and corresponding methodological outputs are specified in Figure 3 and Table 4.

Resolution rule. Where the signals from $I_1 - I_3$ conflict, priority belongs to the approach explicitly required by the applicable regulatory or financing instrument. Where that instrument calls for life-cycle evidence, LCA becomes the preferred method. Where data constraints make a fully representative LCA impracticable, the procedure directs the analyst to a transitional hybrid combining MCDM with environmental indicators derived through an appropriate LCIA method within a transparent proxy-based or streamlined LCA using adapted or proxy LCI datasets, together with explicit documentation of the resulting limitations. Adapted or proxy LCI datasets are defined here as geographically or technologically adjusted secondary inventory datasets derived from transparent source inventories and accompanied by an explicit assessment of their geographical, temporal and technological representativeness, assumptions and uncertainty. They are a transitional solution where verified national life cycle inventory data are unavailable or insufficiently representative for the relevant processes, and they are not substitutes for comprehensive country-specific LCI inventories.

The framework is advisory rather than algorithmic: it admits variation with context and data quality, and permits hybrid approaches where $I_1 - I_3$ conflict. Figure 3 sets out the logic of the three-step procedure.

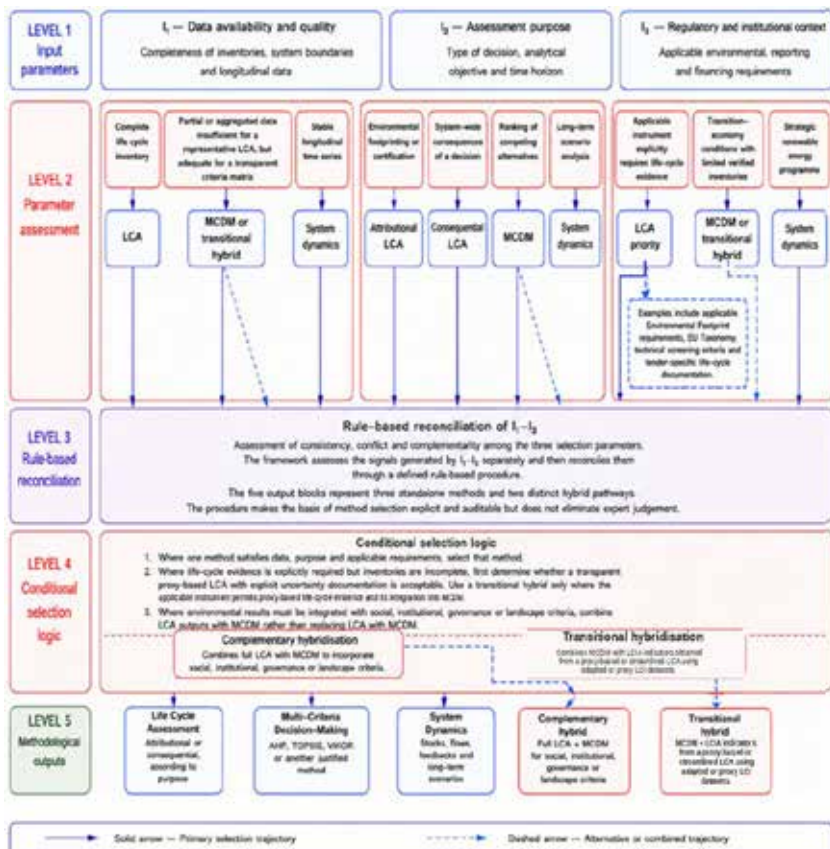


Fig. 3. Structured method-selection framework based on three analytically distinct input parameters – data availability and quality (I₁), assessment purpose (I₂), and regulatory and institutional context (I₃) – followed by rule-based reconciliation into five methodological outputs: LCA, MCDM, SD, complementary hybridisation, and transitional hybridisation. Solid arrows indicate primary selection trajectories; dashed arrows indicate alternative or combined trajectories

Source: developed by the author

The sequence reduces but does not eliminate expert judgement: it makes the basis of method selection explicit, auditable and open to review.

The decision matrix. To make the procedure operational, the three-step logic is systematised as a decision matrix (Table 4), which maps situational characteristics onto recommended approaches.

Table 4

Decision matrix for selecting a methodological approach to renewable energy assessment in agri-food systems

<i>Step</i>	<i>Parameter</i>	<i>Situational characteristic</i>	<i>Recommended method</i>	<i>Complementary assessment or integration</i>
1	I1: data quality	Complete material and energy inventories across the value chain; LCA type determined	LCA (attributional or consequential according to purpose)	LCA + MCDM for post-processing of social criteria
1	I1: data quality	Partial or aggregated data, insufficient for a representative LCA but adequate for a transparent criteria matrix	MCDM or transitional hybrid	MCDM + LCIA indicators from a proxy-based or streamlined LCA using adapted or proxy LCI datasets
1	I1: data quality	Longitudinal data sufficient for calibration and validation of the SD model	SD	SD + simplified LCA for steady-state verification
2	I2: purpose	Environmental certification; Environmental Footprint-compliant assessment; access to green finance	Attributional LCA	LCA evidence used within activity-specific EU Taxonomy screening
2	I2: purpose	Analysis of the systemic consequences of renewable programmes or agricultural bioenergy	Consequential LCA	Consequential LCA + SD for the long-term context
2	I2: purpose	Ranking of competing alternatives; support for a public decision	MCDM	MCDM + DPSIR as the criteria structure
2	I2: purpose	Strategic scenario analysis over a 15–50 year horizon	SD	SD + integrated assessment models
3	I3: regulatory context	A compliance or financing instrument specifying life-cycle evidence	Attributional LCA	LCA + MCDM for management criteria
3	I3: regulatory context	Transition-economy setting with limited institutional and inventory capacity	The method required by the assessment purpose, with transparent proxy data, staged assessment or a transitional hybrid where necessary	Transitional hybrid where appropriate
3	I3: regulatory context	Strategic renewable energy programme for agri-food systems	SD	SD + MCDM in iterative scenario-selection cycles
Output	–	Conflicting signals across I1–I3 → priority to the approach specified by the applicable instrument; where impracticable → transitional hybrid	Transitional hybrid: MCDM with LCIA indicators obtained from a proxy-based or streamlined LCA using adapted or proxy LCI datasets	Documentation of the uncertainty range is a necessary condition of correct application

Source: developed by the author. Notation: attributional LCA – average-impact orientation, basis of the Environmental Footprint methods; consequential LCA – system-consequence orientation; MCDM – multi-criteria decision-making; SD – system dynamics; transitional hybrid – MCDM with LCIA indicators obtained from a proxy-based or streamlined LCA using adapted or proxy LCI datasets

Originality of the framework. The framework's claim to originality rests on four departures from existing methodological reviews: first, its stepwise structure uses three analytically distinct but interacting parameters to exclude unsuitable methods sequentially and replace holistic judgement with documented, auditable inference, whereas earlier comparative reviews⁴⁵ merely set the methods side by side. Second, it treats the attributional – consequential distinction as an independent choice dimension, whereas most reviews collapse LCA into one category – a simplification with material consequences because the variants answer different questions and are not interchangeable for either regulatory or analytical purposes. Third, agri-food specificity – Agri-PV allocation and bioenergy accounting – and the European integration context enter the selection logic directly rather than as external caveats; frameworks developed for construction materials⁴⁶ or waste management⁴⁷ transfer the integration principle but not these domain constraints. Fourth, hybrid combinations are supported explicitly: while existing work presents hybridisation as a design choice, this procedure derives it from diagnosed conditions and identifies when a hybrid is an appropriate output rather than a compromise.

Illustrative application. Two hypothetical Ukrainian cases – a 3 MW agrivoltaic installation and a 0.5 MW biogas plant – use secondary data and assumed project parameters to illustrate the sequential evaluation of $I_1 - I_3$ (Table 5), demonstrating the selection logic rather than providing empirical validation. Because no complete decision matrix or criterion weights were constructed, no formal sensitivity analysis was performed; empirical applications should vary the weights systematically and report both baseline and perturbed rankings.

The cases show that a financing requirement can determine the required orientation of life-cycle evidence for Agri-PV, while insufficient representative inventories direct the assessment to a transitional hybrid rather than a full standalone attributional LCA. By contrast, comparison of community alternatives under limited data favours MCDM, with simplified attributional LCA used only as a complementary assessment. For biogas, results remain conditional on LCA orientation, digestate allocation, functional unit, system boundary, substrate

⁴⁵ Pohekar S. D., Ramachandran M. Application of multi-criteria decision making to sustainable energy planning : a review. *Renewable and Sustainable Energy Reviews*. 2004. Vol. 8. № 4. P. 365–381. DOI: 10.1016/j.rser.2003.12.007 ; Strantzali E., Aravossis K. Decision making in renewable energy investments : a review. *Renewable and Sustainable Energy Reviews*. 2016. Vol. 55. P. 885–898. DOI: 10.1016/j.rser.2015.11.021

⁴⁶ Mlybari E. A. Smart sustainability in construction: an integrated LCA–MCDM framework for climate-adaptive material selection in educational buildings. *Sustainability*. 2025. Vol. 17. № 21. Article 9650. DOI: 10.3390/su17219650

⁴⁷ Integrating life cycle assessment and multi criteria decision making for sustainable waste management: key issues and recommendations for future studies / A. E. Torkayesh et al. *Renewable and Sustainable Energy Reviews*. 2022. Vol. 168. Article 112819. DOI: 10.1016/j.rser.2022.112819

composition, methane yield and fugitive emissions; quantifying their effects requires plant-specific models.

Table 5

**Illustrative application to two hypothetical renewable energy projects
in Ukrainian agri-food systems**

<i>Parameter / selection step</i>	<i>Scenario A: Agri-PV, 3 MW (Kharkiv oblast)</i>	<i>Scenario B: Biogas plant, 0.5 MW (Poltava oblast)</i>
I1: data quality	Partial inventories: capacity, panel type (monocrystalline silicon) and site region known; manufacturing-chain LCI limited. Provisional proxy: ecoinvent datasets for Poland	Biogas yield (m ³ per tonne of manure) and substrate composition assumed known; transport and pipeline LCI limited; data insufficient for consequential LCA
I2: purpose	Hypothetical financing condition: the lender requires life-cycle environmental documentation aligned with EU methods	Internal community decision: public tender among competing technologies; MCDM ranking required
I3: regulatory context	Life-cycle evidence specified by the financing instrument → attributional life-cycle evidence required; because representative inventories are insufficient, the conflict with I1 is resolved through a transitional hybrid.	No instrument specifying an LCA type → MCDM methodologically sufficient
Framework output	Transitional hybrid: MCDM combined with environmental indicators derived via LCIA from adapted or proxy LCI datasets within a transparent proxy-based attributional LCA, with documented geographical, temporal and technological representativeness, assumptions and uncertainty.	MCDM across nine criteria (GWP, cost, employment, acceptance, nitrogen cycle, safety, energy independence, subsidy access and risk), with a simplified attributional LCA for GWP used as a complementary assessment.
Treatment of uncertainty	Proxy-related deviation is not quantifiable without a comparative regional inventory analysis and should be reported qualitatively; Agri-PV allocation is not standardised; an uncertainty statement is mandatory	GWP for biogas varies substantially with the accounting convention for avoided methane and with digestate allocation ¹⁹ ; sensitivity analysis of criterion weights is mandatory in any empirical application
Relation to the literature	IPCC AR5 reports median life-cycle GHG emissions of approximately 41–48 g CO ₂ -eq. kWh ⁻¹ for rooftop and utility-scale photovoltaics ²⁰ .	Hybrid MCDM with LCA elements is consistent with the broader renewable-energy decision-making literature ²¹ ; the choice of LCA type for bioenergy is decisive ²² .

Source: developed by the author. Project parameters are hypothetical and serve to illustrate the sequence of the framework. Polish datasets were chosen provisionally as a geographically proximate proxy; their technological and energy-system representativeness for Ukraine remains to be tested. Any empirical application will require data whose geographical, temporal and technological representativeness for the relevant Ukrainian processes is demonstrated

Validity, limitations and conditions of application. The illustrative application must be read against the limitations that define its validity.

On internal validity, two-stage screening under a predefined protocol constrained confirmation bias in source selection, but the absence of an independent second reviewer remains a limitation, as does the lack of any formal quality appraisal or risk-of-bias assessment, which limits the capacity to distinguish the frequency of an approach from the strength of the evidence behind it. Separating attributional from consequential approaches partly controlled LCA variability, and the subjectivity of MCDM would require parallel computation under AHP and TOPSIS with sensitivity analysis in any empirical application.

On external validity, geographical bias limits generalisability, since studies from the EU and the United Kingdom predominate in the sample. The wartime context constrains SD: modelling that covers 2022–2024 must label the wartime scenario explicitly and compute the reconstruction scenario separately. Participatory MCDM under martial law suffers limited stakeholder representativeness, which weakens the reliability of derived weights.

Nor does the sequential structure eliminate expert judgement, which survives at five points at least: deciding whether available data suffice for a given method; classifying the purpose of the assessment; setting criterion weights; selecting proxy datasets; and ranking competing regulatory requirements. What the framework contributes is that each judgement is made at an identified step and can therefore be documented, reviewed and challenged.

Four further limitations condition the framework: the coverage of verified, nationally representative inventories for the Ukrainian energy mix, agriculture, transport and renewable-energy technologies remains incomplete, and this study did not identify a comprehensive nationally coordinated LCI database covering all the required sectors; consequently, proxy datasets may still be necessary, with their geographical and technological representativeness and associated systematic deviation documented explicitly; wartime and post-war uncertainty affects both the energy balance and the production structure; and specific weight sets transfer poorly between contexts even where the selection logic transfers intact.

Revision will be required as broader and more representative Ukrainian LCI datasets become available, if the EU Taxonomy technical screening criteria for renewable energy are updated, if a standardised allocation convention for Agri-PV is established, or as machine-learning-assisted LCA matures.

Integration with complementary analytical traditions. The frameworks applied in earlier work⁴⁸ – DPSIR, SES and landscape metrics – complement LCA, MCDM and SD naturally, and integration operates on four levels. The diagnostic level (DPSIR) identifies drivers, pressures, states, impacts and responses, its components supplying the structure of the MCDM criteria set⁴⁹. The product level (LCA) quantifies the burden of specific technologies, attributionally for regulatory reporting and consequentially for systemic effects. The decision level (MCDM) compares alternatives, with weights derived through the SES framework. The strategic level (SD) represents reinforcing and balancing loops over the 2030–2050 horizon.

Landscape metrics can enter attributional LCA as land use indicators – a point that matters for Agri-PV, where spatial configuration affects landscape connectivity, insect habitat and microclimatic function. A fragmentation index such as patch density (FRAGSTATS) could be incorporated into the land use category of an LCIA method offering a compatible indicator, subject to verification of unit and characterisation consistency.

Practical implications. Implementation should begin with verified, nationally representative Ukrainian LCI datasets for electricity, agriculture, transport and renewable-energy technologies, together with documented market-response assumptions for consequential modelling. Where proxy datasets remain necessary, their geographical, temporal and technological representativeness must be documented explicitly. At regional and enterprise levels, MCDM can support decisions under incomplete data, while SD remains appropriate for strategic modelling provided that wartime and reconstruction regimes are treated separately.

Effective implementation also requires coordinated national data-governance arrangements assigning responsibility for dataset maintenance, methodological quality assurance and periodic revision. Pilot assessments should follow common protocols for metadata, uncertainty reporting and sensitivity analysis to ensure comparability across institutions, regions and financing programmes. Table 6 assigns the corresponding measures to the principal institutional and enterprise actors.

⁴⁸ Пузік В. К., Криштон Є. А. Системний підхід до екологічно збалансованого природокористування: концептуальні моделі, аналітичні рамки та індикатори. *Методологічні підходи та практичне обґрунтування екологічно збалансованого природокористування і шляхи зниження антропогенного навантаження на природні і штучні екосистеми у сфері АПК* : звіт про НДР (остаточний) / Державний біотехнологічний університет. Харків, 2025. Розд. VII. С. 213–244. № держреєстрації 0121U107701. URL: <https://repo.btu.kharkiv.ua/server/api/core/bitstreams/4d9ea1b4-3100-42e0-80f8-f53ff23f8ed5/content>

⁴⁹ Next generation application of DPSIR for sustainable policy implementation / S. A. Carnohan et al. *Current Research in Environmental Sustainability*. 2023. Vol. 5. Article 100201. DOI: 10.1016/j.crsust.2022.100201

Table 6

**Practical recommendations for key actors: green energy
and environmental safety in Ukrainian agri-food systems**

<i>Actor</i>	<i>Problem</i>	<i>Recommended measure</i>	<i>Expected outcome</i>
Universities and research institutes	Insufficient coverage of nationally representative Ukrainian LCI datasets and insufficiently developed market-response assumptions for consequential modelling	Develop nationally representative LCI datasets and consequential modelling assumptions with the EU Joint Research Centre or comparable international bodies	Methodologically sound LCA for renewable projects in agri-food systems; compatibility with European reporting requirements
Ministry of Environmental Protection	MCDM assessments incomparable across studies, owing to divergent criteria sets and algorithms	Standardise a minimum criteria set (≥ 3 environmental, 2 social, 2 economic); require parallel computation and sensitivity analysis	Comparability across studies; scope for meta-analysis and evidence-based programme design
Ministry of Agrarian Policy	No methodological requirements for the environmental assessment of renewable projects in agri-food systems	Write an LCA or MCDM requirement into the conditions of agri-energy grant competitions and support schemes	Higher project quality; exclusion of options carrying concealed environmental burdens
Ministry of Energy	No strategic SD model for recovery of the renewable sector	Build an SD model of renewable development in agri-food systems to 2050 with international energy agencies; label wartime and reconstruction scenarios explicitly	Evidence-based renewable targets; an instrument for post-conflict strategic planning
Agricultural holdings and renewable developers	No standard for voluntary environmental disclosure	Adopt a sectoral reporting standard compatible with European disclosure requirements; publish life-cycle declarations	Access to ESG investors; competitive advantage in European green finance markets

Source: developed by the author on the basis of⁵⁰.

⁵⁰ International Renewable Energy Agency. Renewable capacity statistics 2024. Abu Dhabi : IRENA, 2024. URL: <https://www.irena.org/Publications/2024/Mar/Renewable-capacity-statistics-2024> ; International Energy Agency. Net Zero Roadmap: A Global Pathway to Keep the 1.5 °C Goal in Reach. 2023 Update. Paris : IEA, 2023. URL: <https://www.iea.org/reports/net-zero-roadmap-a-global-pathway-to-keep-the-15-c-goal-in-reach> ; Пузік В. К., Криштоп Є. А. Системний підхід до екологічно збалансованого природокористування: концептуальні моделі, аналітичні рамки та індикатори. *Методологічні підходи та практичне обґрунтування екологічно збалансованого природокористування і шляхи зниження антропогенного навантаження на природні і штучні екосистеми у сфері АПК* : звіт про НДР (остаточний) / Державний біотехнологічний університет. Харків, 2025. Розд. VII. С. 213–244. № держреєстрації 0121U107701. URL: <https://repo.btu.kharkiv.ua/server/api/core/bitstreams/4d9ea1b4-3100-42e0-80f8-f53ff23f8ed5/content> ; Commission Delegated Regulation (EU) 2021/2139 of 4 June 2021 supplementing Regulation (EU) 2020/852 by establishing technical screening criteria. *Official Journal of the European Union*. 2021. L 442. P. 1–349. URL: https://eur-lex.europa.eu/eli/reg_del/2021/2139/oj ; Food and Agriculture Organization of the United Nations. The State of Food and Agriculture 2023: Revealing the true cost of food to transform agrifood systems. Rome : FAO, 2023. DOI: 10.4060/cc7724en

CONCLUSIONS

LCA, MCDM and system dynamics address distinct but complementary decision needs. Their selection should be governed by data quality, assessment purpose and regulatory context rather than methodological convention. The proposed framework makes this choice explicit, distinguishes attributional from consequential LCA, and identifies when standalone methods, complementary integration or transitional hybridisation are warranted.

Agri-food constraints – including Agri-PV allocation, bioenergy system boundaries, digestate treatment and spatial ecological effects – must enter method selection directly. A transitional hybrid is justified only where life-cycle evidence is required but representative inventories are insufficient. Adapted or proxy LCI datasets should then be used within a transparent proxy-based or streamlined LCA, with their geographical, temporal and technological representativeness, assumptions and uncertainty documented explicitly.

The framework remains conceptual and requires validation through Ukrainian case studies based on verified local inventories. Formalisation should define measurement scales and data-sufficiency thresholds for $I_1 - I_3$, rules for reconciling conflicting signals, and procedures for propagating proxy-data uncertainty into MCDM rankings. Further work should integrate dynamic and prospective LCA, SD, GIS and remote sensing with emerging European data infrastructure. The longer-term objective is a computational DPSIR–LCA–MCDM framework in which SD represents strategic feedback and scenario behaviour.

SUMMARY

The monograph chapter develops a structured decision framework for selecting methodological approaches to assessing the environmental safety of renewable energy projects in agri-food systems under transition-economy conditions. It focuses on life cycle assessment (LCA), multi-criteria decision-making (MCDM) and system dynamics (SD), while environmental safety is operationalised across climatic, ecosystem, resource and toxicological dimensions. *The purpose of the study* is to establish an explicit and auditable procedure for choosing an assessment approach according to data quality, assessment purpose and regulatory and institutional context, including a rule for resolving conflicting signals. *Methodology of the study* combines a targeted methodological review reported using the applicable PRISMA 2020 items and flow diagram, structured data coding, descriptive synthesis, comparative analysis and illustrative case application. The review covered peer-reviewed articles and reviews indexed in Scopus and the Web of Science Core Collection and published between 2019 and 2024. Of 512 records identified,

87 peer-reviewed articles and reviews were included in the final analytical sample. *The results* showed that 32 studies (36.8%) were classified in the LCA cluster, 30 (34.5%) in the MCDM cluster, and 25 (28.7%) in the system dynamics and integrated approaches cluster, while only 10 studies (11.5%) explicitly addressed transition-economy contexts. On this basis, a three-parameter, rule-based framework was developed, yielding five methodological outputs: LCA, MCDM, SD, complementary hybridisation and transitional hybridisation. Where the parameters conflict, priority is given to the approach required by the applicable regulatory or financing instrument. Where life-cycle evidence is required but a representative LCA is impracticable because verified national inventory data are unavailable or insufficient for the relevant processes, a transitional hybrid combining MCDM with LCIA indicators derived from adapted or proxy life cycle inventory datasets is recommended, with explicit documentation of assumptions and uncertainty. The framework is illustrated through hypothetical agrivoltaic and biogas projects in Ukraine; these cases demonstrate the selection logic but do not constitute empirical validation. *Practical implications.* The framework can support environmental policy, renewable-energy planning, green-finance appraisal and enterprise-level decision-making by converting implicit professional judgement into a documented and reviewable procedure. *Value/originality.* The originality of the study lies in structuring method selection as a sequential diagnostic process, treating the attributional–consequential LCA distinction as an independent choice dimension, incorporating agri-food-specific allocation and system-boundary problems directly into the decision logic, and deriving hybridisation from diagnosed assessment conditions rather than assuming it in advance. The framework remains conceptual and requires further formalisation and empirical validation using verified local data.

Bibliography

1. International Renewable Energy Agency. Renewable capacity statistics 2024. Abu Dhabi : IRENA, 2024. URL: <https://www.irena.org/Publications/2024/Mar/Renewable-capacity-statistics-2024>
2. International Energy Agency. Net Zero Roadmap: A Global Pathway to Keep the 1.5 °C Goal in Reach. 2023 Update. Paris : IEA, 2023. URL: <https://www.iea.org/reports/net-zero-roadmap-a-global-pathway-to-keep-the-15-c-goal-in-reach>
3. Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by P. R. Shukla et al. Cambridge ; New York : Cambridge University Press, 2022. DOI: 10.1017/9781009157926

4. Chatzipanagi A., Taylor N., Jaeger-Waldau A. Overview of the potential and challenges for agri-photovoltaics in the European Union. Luxembourg : Publications Office of the European Union, 2023. EUR 31482 EN. DOI: 10.2760/208702
5. Pohekar S. D., Ramachandran M. Application of multi-criteria decision making to sustainable energy planning : a review. *Renewable and Sustainable Energy Reviews*. 2004. Vol. 8. № 4. P. 365–381. DOI: 10.1016/j.rser.2003.12.007
6. Strantzali E., Aravossis K. Decision making in renewable energy investments : a review. *Renewable and Sustainable Energy Reviews*. 2016. Vol. 55. P. 885–898. DOI: 10.1016/j.rser.2015.11.021
7. Life cycle assessment and techno-economic analysis of sustainable bioenergy production : a review / A. I. Osman et al. *Environmental Chemistry Letters*. 2024. Vol. 22. № 3. P. 1115–1154. DOI: 10.1007/s10311-023-01694-z
8. Guinée J. B. (ed.). Handbook on life cycle assessment: operational guide to the ISO standards. Dordrecht : Kluwer Academic Publishers, 2002. DOI: 10.1007/0-306-48055-7
9. Laurent A., Olsen S. I., Hauschild M. Z. Limitations of carbon footprint as indicator for environmental sustainability and LCA-based screening tools. *Environmental Science & Technology*. 2012. Vol. 46. № 8. P. 4100–4108. DOI: 10.1021/es204163f
10. Lulovicova A., Bouissou S. Life cycle assessment as a prospective tool for sustainable agriculture and food planning at a local level. *Geography and Sustainability*. 2024. Vol. 5. № 2. P. 251–264. DOI: 10.1016/j.geosus.2024.01.008
11. Пузік В. К., Криштоп Є. А. Системний підхід до екологічно збалансованого природокористування: концептуальні моделі, аналітичні рамки та індикатори. *Методологічні підходи та практичне обґрунтування екологічно збалансованого природокористування і шляхи зниження антропогенного навантаження на природні і штучні екосистеми у сфері АПК : звіт про НДР (остаточний) / Державний біотехнологічний університет. Харків, 2025. Розд. VII. С. 213–244. № держреєстрації 0121U107701. URL: <https://repo.btu.kharkiv.ua/server/api/core/bitstreams/4d9ea1b4-3100-42e0-80f8-f53ff23f8ed5/content>*
12. Forrester J. W. Industrial dynamics. Cambridge, MA : MIT Press, 1961.
13. Armstrong A., Ostle N. J., Whitaker J. Solar park microclimate and vegetation management effects on grassland carbon cycling. *Environmental Research Letters*. 2016. Vol. 11. № 7. Article 074016. DOI: 10.1088/1748-9326/11/7/074016
14. Enhancing ecosystem services and biodiversity in agrivoltaics through habitat-enhancing strategies / A. Ludzuweit et al. *Renewable and*

Sustainable Energy Reviews. 2025. Vol. 212. Article 115380. DOI: 10.1016/j.rser.2025.115380

15. Long-term application of biogas digestate improves soil physical properties / M. Mayerová et al. *Soil and Tillage Research*. 2023. Vol. 231. Article 105715. DOI: 10.1016/j.still.2023.105715

16. Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching / T. A. Sogn et al. *International Journal of Recycling of Organic Waste in Agriculture*. 2018. Vol. 7. P. 49–58. DOI: 10.1007/s40093-017-0188-0

17. Digestate in replacement of synthetic fertilisers: a comparative 3-year field study of the crop performance and soil residual nitrates in West-Flanders / G. Reuland et al. *European Journal of Agronomy*. 2024. Vol. 161. Article 127380. DOI: 10.1016/j.eja.2024.127380

18. Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils / V. Moinard et al. *Applied Soil Ecology*. 2021. Vol. 168. Article 104149. DOI: 10.1016/j.apsoil.2021.104149

19. European Commission. Directorate-General for Environment. Guidance document on wind energy developments and EU nature legislation. Luxembourg : Publications Office of the European Union, 2020. DOI: 10.2779/095188

20. Harris Z. M., Spake R., Taylor G. Land use change to bioenergy: a meta-analysis of soil carbon and GHG emissions. *Biomass and Bioenergy*. 2015. Vol. 82. P. 27–39. DOI: 10.1016/j.biombioe.2015.05.008

21. A review of the impacts of Short Rotation Coppice cultivation on water issues / I. Dimitriou et al. *Landbauforschung*. 2009. Vol. 59. № 3. P. 197–206.

22. Dedicated biomass crops can enhance biodiversity in the arable landscape / A. J. Haughton et al. *GCB Bioenergy*. 2016. Vol. 8. № 6. P. 1071–1081. DOI: 10.1111/gcbb.12312

23. Landscape metrics and land-use patterns of energy crops in the agricultural landscape / X. Xu et al. *BioEnergy Research*. 2023. Vol. 16. P. 2178–2191. DOI: 10.1007/s12155-023-10584-9

24. Commission Delegated Regulation (EU) 2021/2139 of 4 June 2021 supplementing Regulation (EU) 2020/852 by establishing technical screening criteria. *Official Journal of the European Union*. 2021. L 442. P. 1–349. URL: https://eur-lex.europa.eu/eli/reg_del/2021/2139/oj

25. Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations. *Official Journal of the European Union*. 2021. L 471. P. 1–396. URL: <https://eur-lex.europa.eu/eli/reco/2021/2279/oj>

26. European Commission, Joint Research Centre. Environmental Footprint methods. URL: <https://eplca.jrc.ec.europa.eu/EnvironmentalFootprint.html>
27. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews / M. J. Page et al. *BMJ*. 2021. Vol. 372. Article n71. DOI: 10.1136/bmj.n71
28. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement / D. Moher et al. *PLoS Medicine*. 2009. Vol. 6. № 7. Article e1000097. DOI: 10.1371/journal.pmed.1000097
29. Tighnavard Balasbaneh A., Aldrovandi S., Sher W. A systematic review of implementing multi-criteria decision-making (MCDM) approaches for the circular economy and cost assessment. *Sustainability*. 2025. Vol. 17. № 11. Article 5007. DOI: 10.3390/su17115007
30. Mlybari E. A. Smart sustainability in construction: an integrated LCA–MCDM framework for climate-adaptive material selection in educational buildings. *Sustainability*. 2025. Vol. 17. № 21. Article 9650. DOI: 10.3390/su17219650
31. A critical review of life cycle assessment of renewable agricultural systems / M. Kiehadrouinezhad et al. *Sustainable Energy Technologies and Assessments*. 2025. Vol. 73. Article 104100. DOI: 10.1016/j.seta.2024.104100
32. Establishing the limitations of sustainable construction in developing countries: a systematic literature review using PRISMA / O. E. Ogunmakinde et al. *Smart and Sustainable Built Environment*. 2024. Vol. 13. № 3. P. 609–624. DOI: 10.1108/SASBE-10-2022-0223
33. Integrating life cycle assessment and multi criteria decision making for sustainable waste management: key issues and recommendations for future studies / A. E. Torkayesh et al. *Renewable and Sustainable Energy Reviews*. 2022. Vol. 168. Article 112819. DOI: 10.1016/j.rser.2022.112819
34. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change / ed. by O. Edenhofer et al. Cambridge ; New York : Cambridge University Press, 2014. DOI: 10.1017/CBO9781107415416
35. Dec K. Life cycle assessment of renewable energy sources – key issues. Bibliometric analysis of the literature. *Economics and Environment*. 2024. Vol. 89. № 2. Article 839. DOI: 10.34659/eis.2024.89.2.839
36. ISO 14040:2006. Environmental management – Life cycle assessment – Principles and framework. Geneva : International Organization for Standardization, 2006.
37. ISO 14044:2006. Environmental management – Life cycle assessment – Requirements and guidelines. Geneva : International Organization for Standardization, 2006.

38. International Reference Life Cycle Data System (ILCD) Handbook – General guide for Life Cycle Assessment – Detailed guidance / European Commission, Joint Research Centre, Institute for Environment and Sustainability. First edition. Luxembourg : Publications Office of the European Union, 2010. EUR 24708 EN. DOI: 10.2788/38479
39. Ekvall T., Weidema B. P. System boundaries and input data in consequential life cycle inventory analysis. *The International Journal of Life Cycle Assessment*. 2004. Vol. 9. № 3. P. 161–171. DOI: 10.1007/BF02994190
40. Saaty T. L. The analytic hierarchy process: planning, priority setting, resource allocation. New York : McGraw-Hill, 1980.
41. Hwang C.-L., Yoon K. Multiple attribute decision making: methods and applications. Berlin ; Heidelberg : Springer, 1981. DOI: 10.1007/978-3-642-48318-9
42. Next generation application of DPSIR for sustainable policy implementation / S. A. Carnohan et al. *Current Research in Environmental Sustainability*. 2023. Vol. 5. Article 100201. DOI: 10.1016/j.crsust.2022.100201
43. Sterman J. D. Business dynamics: systems thinking and modeling for a complex world. Boston : Irwin/McGraw-Hill, 2000.
44. Food and Agriculture Organization of the United Nations. The State of Food and Agriculture 2023: Revealing the true cost of food to transform agrifood systems. Rome : FAO, 2023. DOI: 10.4060/cc7724en

Information about the author:

Kryshchuk Yevhen,

Candidate of Agricultural Sciences,

Associate Professor at the Department of Ecology and Biotechnology
in Crop Production

State Biotechnological University

44, Alchevskykh Street, Kharkiv, Ukraine

ENVIRONMENTAL AND TECHNOLOGICAL SOLUTIONS FOR THE PROTECTION AND SUSTAINABLE USE OF WATER RESOURCES

Litvak Olga

DOI <https://doi.org/10.30525/978-9934-26-714-7-5>

INTRODUCTION

Water resources are a key component of the natural resource potential of territories, underpinning ecosystem functioning, economic development, and the quality of life of the population. Amid global transformations associated with climate change, increasing anthropogenic pressure, urbanisation, industrialisation, and unequal access to safe and high-quality water, the protection of water bodies is becoming particularly important. The deterioration of surface water and groundwater quality, depletion of water resources, disruption of natural hydrological regimes, and accumulation of pollutants in aquatic ecosystems pose risks not only to the environment but also to the socio-economic development of territories.

Anthropogenic pressure on water resources results from the discharge of insufficiently treated municipal and industrial wastewater, agricultural and stormwater runoff, excessive water abstraction, hydraulic modification of water bodies, and changes in the natural regime of catchments. Municipal wastewater is a source of organic, nutrient, and microbiological pollution, whereas industrial effluents may contain heavy metals, petroleum products, phenols, salts, dyes, and persistent organic compounds¹. Surface runoff from agricultural land and urbanized areas contributes to the input of nitrogen and phosphorus compounds, pesticides, suspended solids, petroleum products, and microplastics into water bodies. The combined effects of these factors lead to eutrophication, deterioration of oxygen conditions, accumulation of toxic substances in bottom sediments and biota, siltation, biodiversity loss, and depletion of water resources².

¹ Carey R. O., Hochmuth G. J., Martinez C. J., Boyer T. H., Dukes M. D., Toor G. S., Cisar J. L. Evaluating nutrient impacts in urban watersheds: challenges and research opportunities. *Environmental Pollution*. 2013. Vol. 173. P. 138–149. DOI: <https://doi.org/10.1016/j.envpol.2012.10.004>.

² Giri S., Qiu Z. Understanding the relationship of land use and water quality in twenty first century : a review. *Journal of Environmental Management*. 2016. Vol. 173. P. 41–48.

Particular attention should be paid to the growing water demand in agriculture, industry, energy production, and the municipal sector. Intensive water abstraction from natural sources, combined with droughts, uneven precipitation patterns, and rising temperatures, increases the risk of water scarcity and reduces the capacity of aquatic ecosystems for natural recovery³. Under such conditions, even relatively small amounts of pollutants may cause significant environmental impacts because of reduced water availability and diminished natural dilution of wastewater.

The relevance of this study is determined by the need to identify effective environmental and technological solutions aimed at reducing pollution of water bodies, improving wastewater treatment efficiency, and developing resource-efficient water-use models. Of particular importance is the transition from a predominantly reactive approach focused on mitigating the consequences of pollution to a preventive model that involves segregating contaminated streams at their points of generation, reducing wastewater volumes, introducing low-waste technologies, implementing water recirculation systems, and reusing treated water. This approach makes it possible to reduce both water abstraction from natural sources and the pressure on treatment facilities and aquatic ecosystems.

In the context of sustainable development, the protection of water bodies and the rational use of water resources are essential conditions for environmental safety, sustainable natural resource management, and enhancing the resilience of territories to contemporary environmental and socio-economic challenges. This issue is directly aligned with Sustainable Development Goal 6, “Clean Water and Sanitation”, which aims to ensure access to water, adequate wastewater treatment, and the sustainable management of water resources⁴. At the same time, the introduction of water-saving technologies, water reuse, and closed-loop production cycles contributes to Sustainable Development Goal 12, “Responsible Consumption and Production”. The link with Sustainable Development Goal 13, “Climate Action”, is determined by the need to adapt water management systems to droughts, floods, and uneven water availability. The protection of river basins also contributes to the achievement of Goals 14 and 15, as the condition of surface waters directly affects coastal, marine, and terrestrial ecosystems⁵.

³ Intergovernmental Panel on Climate Change. Climate change 2023: synthesis report. Geneva : IPCC, 2023. URL: <https://www.ipcc.ch/report/ar6/syr/>.

⁴ United Nations. Goal 6: Ensure access to water and sanitation for all. United Nations Sustainable Development Goals. URL: <https://www.un.org/sustainabledevelopment/water-and-sanitation/>.

⁵ UNESCO World Water Assessment Programme. The United Nations World Water Development Report 2024: Water for prosperity and peace. Paris : UNESCO, 2024. URL: <https://unesdoc.unesco.org/ark:/48223/pf0000388948>.

The aim of the study is to substantiate environmental and technological solutions for water treatment and priority areas of rational water use in the context of sustainable development. To achieve this aim, the main treatment methods and technological systems were compared in terms of their efficiency, application conditions, and water reuse potential, while approaches to the development of recirculating water-supply systems and improved water-use efficiency were also summarised.

1. Environmental and technological solutions for water treatment and pollution reduction in water bodies

Reducing pollution of water bodies requires the implementation of a set of environmental and technological solutions aimed at wastewater treatment, localisation of pollution sources and prevention of pollutant input into the aquatic environment. Under current conditions, the effectiveness of water protection activities is determined by the combination of technological modernisation of treatment facilities, local treatment of wastewater at the points of generation, the application of combined treatment methods, advanced treatment of water before discharge into water bodies, and the use of nature-based solutions to reduce surface runoff and restore the natural functions of aquatic ecosystems.

Traditional approaches to water treatment involve the sequential application of mechanical, physicochemical, chemical, biological and combined methods. Mechanical treatment is used to remove coarse impurities, suspended solids, sand, sludge and other insoluble components. Such processes include screening, sedimentation, filtration, grit removal and flotation. Despite its relative simplicity, mechanical treatment is an important initial stage, as it reduces the load on subsequent technological stages and improves the efficiency of further treatment⁶.

Physicochemical treatment methods are used to remove colloidal, finely dispersed, and dissolved pollutants. These include coagulation, flocculation, adsorption, ion exchange, membrane processes, electrochemical methods, oxidation, and other water treatment techniques. Such methods are particularly important for treating industrial wastewater, which may contain heavy metals, petroleum products, phenols, synthetic organic compounds, dyes, salts, and other persistent pollutants⁷. The advantage of physicochemical technologies is their capacity for the targeted removal of specific groups of pollutants; however,

⁶ Nachiyar C. V., Rakshi A. D., Sandhya S. N., Jebasta B. D., Nellore J. Developments in treatment technologies of dye-containing effluent: A review. *Case Studies in Chemical and Environmental Engineering*. 2023. Vol. 7. P. 100–139. DOI: 10.1016/j.csee.2023.100339.

⁷ Kato S., Kansha Y. Comprehensive review of industrial wastewater treatment techniques. *Environmental Science and Pollution Research*. 2024. Vol. 31. P. 51064–51097. DOI: 10.1007/s11356-024-34584-0.

their application requires the optimisation of reagent and energy consumption, as well as consideration of secondary waste generation.

For industrial wastewater, an important direction is local treatment directly at enterprises or production sites where specific pollutants are generated. This makes it possible to avoid mixing highly concentrated or toxic wastewater with the general wastewater flow, increase treatment efficiency and reduce the load on municipal wastewater treatment facilities⁸.

One of the widely used physicochemical methods for treating industrial wastewater is coagulation and flocculation, which are applied to remove colloidal, fine dispersed and partially dissolved pollutants. These processes are particularly effective when wastewater contains suspended particles, dyes, petroleum products, heavy metal compounds and other persistent pollutants that are difficult to remove using mechanical methods alone. The essence of the method lies in the introduction of coagulants into the water being treated, which promote destabilisation of the colloidal system, neutralisation of particle charges and the creation of conditions for their subsequent enlargement and sedimentation⁹. The sequence of coagulation and flocculation processes during industrial wastewater treatment is schematically presented in Figure 1.

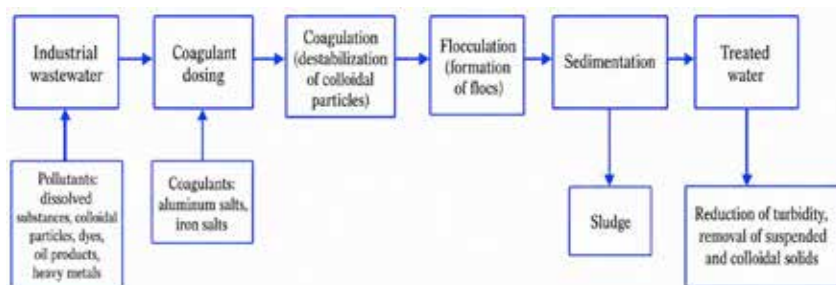


Fig. 1. Scheme of the coagulation and flocculation process during industrial wastewater treatment

Source: developed by the author

The processes of coagulation and flocculation include several main stages: the inflow of polluted wastewater, coagulant dosing, neutralisation of the charge

⁸ Sharma R., Verma N., Lugani Y., Kumar S., Asadnia M. Conventional and advanced techniques of wastewater monitoring and treatment. *Green Sustainable Process for Chemical and Environmental Engineering and Science. Elsevier*. 2021. P. 1–48. DOI: 10.1007/s11356-024-34584-0.

⁹ Sharghi E. A., Davarpanah L. Optimization of chemical coagulation–flocculation process of detergent manufacturing plant wastewater treatment for full-scale applications: a case study. *Desalination and Water Treatment*. 2022. Vol. 262. P. 38–53. DOI: <https://doi.org/10.5004/dwt.2022.28494>.

of colloidal particles, formation of flocs during flocculation, sedimentation and separation of sludge¹⁰. As a result, water turbidity decreases, the concentration of certain pollutants is reduced, and the water becomes more suitable for further treatment or reuse¹¹. The advantages of this method include the effective removal of suspended and colloidal particles, as well as a reduction in the content of some heavy metals and dyes; however, its application is associated with reagent consumption and sludge generation.

Membrane treatment is also a modern physicochemical method for the advanced removal of pollutants from water. The essence of the process is that polluted water, after preliminary treatment, is supplied under pressure to a membrane module, where a semi-permeable membrane separates the flow into two components: permeate, which is the treated water passing through the membrane, and concentrate, which is the stream containing retained pollutants¹². Depending on the type of membrane, the process can ensure the removal of suspended solids, microorganisms, organic pollutants, dyes, salts and other impurities.

Common membrane processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), which differ in their contaminant retention capacity and operating pressure requirements. Ultrafiltration is mainly used to remove suspended and colloidal particles, high-molecular-weight organic compounds, and microorganisms, whereas reverse osmosis provides more advanced removal of dissolved salts and low-molecular-weight contaminants.

One effective option for advanced treatment is the sequential application of ultrafiltration and reverse osmosis (UF/RO). In this treatment scheme, ultrafiltration serves as a membrane pretreatment stage, reducing the concentration of suspended and colloidal matter and lowering the risk of fouling of reverse osmosis membranes¹³. At the subsequent stage, reverse osmosis removes dissolved impurities and produces water suitable for reuse in technical and industrial processes (Figure 2).

¹⁰ Jachimowicz P., Cydzik-Kwiatkowska A. Coagulation and flocculation before primary clarification as efficient solutions for low-density microplastic removal from wastewater. *International Journal of Environmental Research and Public Health*. 2022. Vol. 19, no. 20. Art. 13013. DOI: 10.3390/ijerph192013013.

¹¹ Aloui F., Kchaou S., Sayadi S. Physicochemical treatments of anionic surfactants wastewater: effect on aerobic biodegradability. *Journal of Hazardous Materials*. 2009. Vol. 164, No. 1. P. 353–359. DOI: <https://doi.org/10.1016/j.jhazmat.2008.08.009>.

¹² Robinson S., Bérubé P. R. Membrane ageing in full-scale water treatment plants. *Water Research*. 2020. Vol. 169. Art. 115212. DOI: <https://doi.org/10.1016/j.watres.2019.115212>.

¹³ Fatima F., Du H., Kommalapati R. R. A Sequential Membrane Process of Ultrafiltration Forward Osmosis and Reverse Osmosis for Poultry Slaughterhouse Wastewater Treatment and Reuse. *Membranes (Basel)*. 2023. Vol.1 3, No. 3. Art. 296. DOI: <https://doi.org/10.3390/membranes13030296>.

Membrane treatment makes it possible to obtain high-quality water suitable for technical processes, cooling, recirculating systems, or reuse after further treatment. The advantages of membrane technologies include high selectivity and consistent treated-water quality, whereas their main limitations are energy consumption, membrane fouling, the need for periodic cleaning, and the subsequent management of the concentrate.

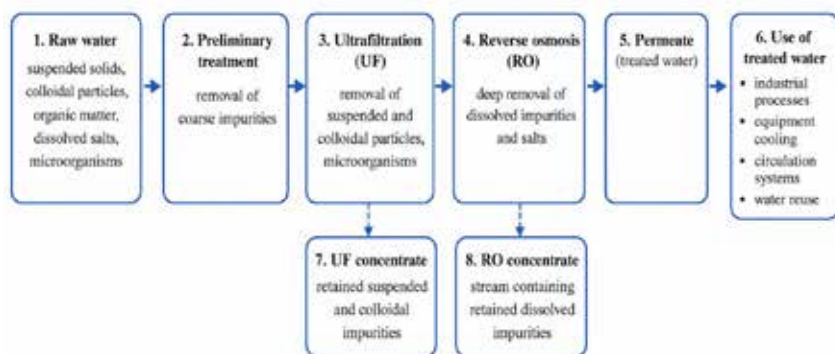


Fig. 2. Schematic diagram of membrane water treatment using ultrafiltration and reverse osmosis

Source: developed by the author

Biological treatment methods are based on the ability of microorganisms to degrade or transform organic pollutants. They are widely used for treating municipal and mixed wastewater dominated by biodegradable organic matter and nitrogen and phosphorus compounds. Such technologies include activated sludge tanks, biofilters, bioreactors, constructed wetlands, and other biological treatment systems¹⁴. The efficiency of biological methods depends on wastewater composition, temperature, oxygen concentration, the loading rate applied to activated sludge or biofilms, and the stability of the operating regime.

The intensification of biological treatment is of particular importance, including the use of combined aerobic-anaerobic processes, bioreactors with attached biomass, membrane bioreactors, nitrogen and phosphorus removal systems, and advanced treatment technologies. Such solutions make it possible to increase the removal of organic matter and nutrients, reduce the risk of

¹⁴ Dotro G., Langergraber G., Molle P., Nivala J., Puigagut J., Stein O., von Sperling M. Treatment wetlands. *Biological Wastewater Treatment Series*. Vol. 7. IWA Publishing, 2017. 172 p.

eutrophication of water bodies and improve the quality of treated water before its discharge or reuse.

A membrane bioreactor is one of the most effective modern technologies for intensifying biological wastewater treatment, as it combines the processes of biodegradation of pollutants by activated sludge and membrane separation of treated water. In such a system, wastewater enters an aeration tank, where activated sludge microorganisms degrade organic matter, participate in nitrification and denitrification processes, and also contribute to reducing the content of nitrogen and phosphorus compounds¹⁵. After biological treatment, the water passes through a membrane module, which retains suspended solids, microorganisms and activated sludge particles, ensuring the production of high-quality permeate (Figure 3).

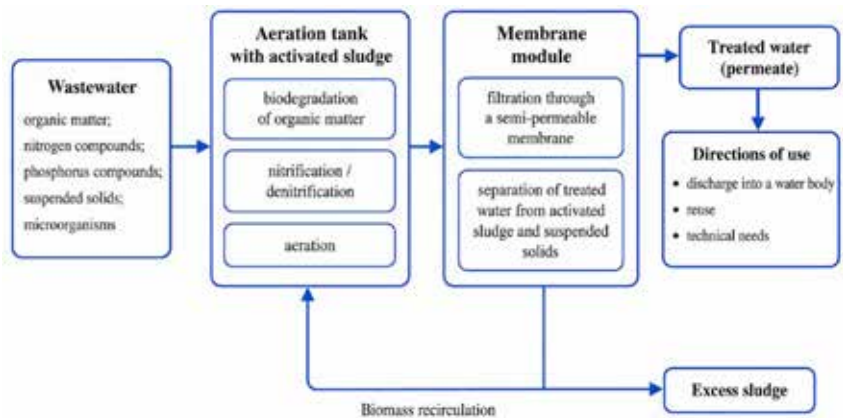


Fig. 3. Scheme of wastewater treatment in a membrane bioreactor

Source: developed by the author

The advantage of a membrane bioreactor lies in the combination of biological treatment with physical barrier separation, which makes it possible to improve the quality of treated water, reduce the content of organic matter and nutrients, and ensure the possibility of water reuse for technical needs or its safe discharge into a water body. The efficiency of such a system depends on the composition of wastewater, dissolved oxygen concentration, temperature, the load on activated sludge, the condition of membranes and the stability of the technological regime¹⁶.

¹⁵ Мокієнко А. В. Актуальні водні проблеми. Інноваційні водні технології. Острог, 2024. 381 с.

¹⁶ Melin T., Jefferson B., Bixio D., Thoeye C., De Wilde W., De Koning J., van der Graaf J., Wintgens T. Membrane bioreactor technology for wastewater treatment and reuse. *Desalination*. 2006. Vol. 187, No. 1–3. P. 271–282. DOI: <https://doi.org/10.1016/j.desal.2005.04.086>.

A separate important direction for reducing pollution of water bodies is the treatment of stormwater runoff from urbanised areas. Stormwater formed during precipitation or snowmelt washes pollutants from roads, industrial sites, car parks, building roofs and other impervious surfaces. It may contain petroleum products, heavy metals, suspended solids, microplastics, residues of de-icing agents, organic impurities and other pollutants. In the absence of proper treatment, such runoff directly enters rivers, water bodies or stormwater drainage systems, increasing chemical and mechanical pollution of the aquatic environment.

Both engineering and nature-based solutions can be used for stormwater treatment. Engineering structures include grit chambers, oil separators, sedimentation tanks, filtration units, local treatment facilities and storage reservoirs. They ensure the retention of sand, suspended solids, petroleum products and other impurities before water is discharged into natural water bodies or drainage systems. At the same time, engineering solutions alone are often insufficient for the comprehensive regulation of surface runoff, especially under conditions of intensive urbanisation, expansion of impervious surfaces and increasing frequency of extreme precipitation events.

In this regard, nature-based solutions are becoming particularly important, as they combine the functions of treatment, retention, infiltration and natural runoff regulation. Such solutions include rain gardens, constructed wetlands, infiltration basins, green ditches, riparian buffer strips, phytoremediation areas and vegetated filtration systems¹⁷. Their action is based on the use of natural processes: filtration through the soil-vegetation layer, sedimentation of suspended solids, uptake of nutrients by plants, microbiological transformation of organic matter, sorption of pollutants and slowing of surface runoff (Table 1, Figure 4).

A key advantage of nature-based solutions is their multifunctionality. They not only contribute to the treatment of stormwater and surface runoff but also improve local hydrological conditions, reduce the risk of localised flooding, support biodiversity, enhance green infrastructure in urban areas, and increase the resilience of built-up territories to climate change. When combined with conventional engineering structures, such solutions make it possible to develop a more flexible and environmentally balanced system for surface runoff management.

¹⁷ International Union for Conservation of Nature. Global Standard for Nature-based Solutions: a user-friendly framework for the verification, design and scaling up of NbS. 1st ed. Gland, Switzerland : IUCN, 2020. 30 p.

Table 1

**Main nature-based solutions for water treatment
and surface runoff regulation**

Nature-based solution	Main purpose	Main environmental effect
Rain gardens	Retention and partial treatment of stormwater runoff	Reduction of surface runoff volume; retention of suspended solids and nutrients
Infiltration basins	Accumulation and infiltration of rainwater into the soil	Reduction of peak runoff, groundwater recharge and lower risk of flooding
Constructed wetlands	Biological advanced treatment of water and retention of pollutants	Reduction in the content of organic matter, nitrogen, phosphorus and suspended particles
Bioplato systems	Advanced treatment of wastewater or stormwater involving higher aquatic plants	Reduction of nutrient load and improvement of water quality
Riparian buffer strips	Interception of pollutants at the boundary between the catchment and the water body	Reduction of the input of soil particles, nutrients and agrochemicals into water bodies
Phytoremediation areas	Uptake and transformation of pollutants by plants	Reduction in the concentration of selected pollutants and ecological stabilisation of the area
Green ditches and biochannels	Slowing, retention and preliminary treatment of surface runoff	Reduction of runoff velocity, sedimentation of suspended solids and partial filtration of pollutants
Vegetated filtration systems	Filtration and advanced treatment of rainwater and stormwater	Improvement of water quality and reduction of the load on stormwater drainage systems

The informed selection of a wastewater treatment method requires consideration of its technological implementation and subsequent operation. Important factors include application conditions, typical limitations, the generation of by-products and secondary streams, and the potential for treated water reuse. A comparative assessment of the treatment methods and technological systems considered according to these criteria is presented in Table 2.

A quantitative comparison was performed for the removal of chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), suspended solids, total nitrogen, and total phosphorus by mechanical, physicochemical, biological, and membrane treatment methods, membrane bioreactors, and nature-based systems. These indicators were selected because they are widely reported for different treatment technologies, whereas data on heavy metals, specific organic compounds, and microbiological contaminants are less consistent. For each

method, a representative value close to the midpoint of the reported efficiency range was selected. The numerical values used in the comparison were derived from the studies cited in the source notes to Figures 5 and 6. The values are indicative, as influent composition and operating conditions varied among the source studies, and should not be interpreted as directly comparable under identical conditions.

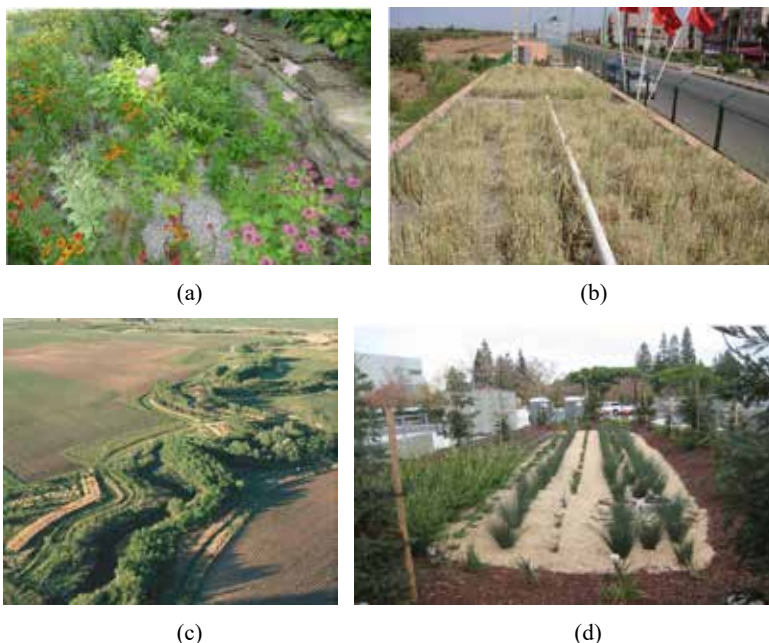


Fig. 4. Examples of nature-based solutions: (a) rain garden¹⁸; (b) constructed wetland¹⁹; (c) riparian buffer strip²⁰; (d) stormwater bioretention area²¹

¹⁸ Steakley J. Rain garden (2014): photograph. Wikimedia Commons, 2014. URL: [https://commons.wikimedia.org/wiki/File:Rain_garden_\(2014\).JPG](https://commons.wikimedia.org/wiki/File:Rain_garden_(2014).JPG).

¹⁹ SuSanA Secretariat. Constructed wetland (5547195530): photograph / photo by H. Hoffmann. Wikimedia Commons, 2009. URL: [https://commons.wikimedia.org/wiki/File:Constructed_wetland_\(5547195530\).jpg](https://commons.wikimedia.org/wiki/File:Constructed_wetland_(5547195530).jpg).

²⁰ United States Department of Agriculture. Riparian buffer on Bear Creek in Story County, Iowa: photograph. Wikimedia Commons. URL: https://commons.wikimedia.org/wiki/File:Riparian_buffer_on_Bear_Creek_in_Story_County,_Iowa.JPG.

²¹ Guzzardo Partnership. 2012-12-04 Stormwater Bio-Treatment Area: photograph. Wikimedia Commons, 2012. URL: https://commons.wikimedia.org/wiki/File:2012-12-04_Stormwater_Bio-Treatment_Area.jpg.

Table 2

Comparative characteristics of the operational features of the main water treatment methods and technological systems

Key application conditions and limitations	By-products or secondary streams	Treated water reuse potential
Mechanical treatment		
Involves the use of screens, grit chambers, sedimentation tanks, or filters and the periodic removal of retained impurities. It does not remove dissolved organic matter, salts, or nutrient compounds.	Sludge, grit, and retained solid waste	When used alone, it generally does not provide water quality suitable for reuse.
Physicochemical treatment		
Requires the selection and dosing of reagents, pH adjustment, appropriate contact time, and controlled mixing conditions. Its efficiency depends on wastewater composition, and the resulting sludge requires further management.	Chemical sludge, spent sorbents, and regeneration solutions	Reuse is possible after additional treatment in accordance with the requirements of the intended application.
Combined UF/RO membrane system		
Requires water pretreatment, operating pressure, membrane fouling control, and periodic membrane cleaning. The concentrate must be treated or properly discharged.	UF retentate, RO concentrate, and membrane cleaning water	High potential for reuse for technical and selected industrial purposes, provided that the required water quality standards are met.
Activated sludge biological treatment		
Requires stable oxygen and temperature conditions, maintenance of the required activated sludge concentration, and an acceptable organic loading rate. It is sensitive to toxic substances and abrupt changes in wastewater composition.	Excess activated sludge	Reuse is generally possible after tertiary treatment and disinfection.
Membrane bioreactor (MBR)		
Requires stable biological operating conditions, aeration, transmembrane pressure control, and regular membrane cleaning. Its operation is more complex than that of conventional biological treatment systems.	Excess activated sludge and membrane cleaning solutions	High potential for reuse in technical and industrial systems.
Nature-based solutions		
Require sufficient land area and consideration of the hydrological regime, vegetation selection, and climatic conditions. Their efficiency may vary depending on the season and hydraulic loading.	Accumulated sediment and plant biomass	Depends on the type of system, the quality of the treated water, and the requirements of its intended use.

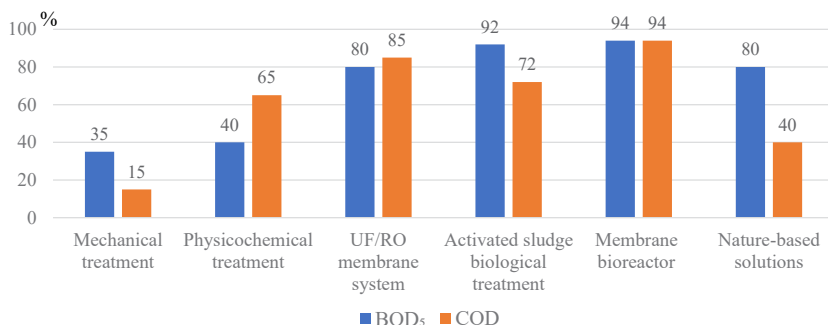


Fig. 5. Comparative efficiency of organic pollution removal by BOD₅ and COD using different water treatment methods, %

Source: compiled by the author based on data from the cited studies^{22, 23, 24, 25, 26}

The comparison results reveal substantial differences among the methods in both the removal efficiency of individual pollutant groups and the conditions required for their technological implementation. The diagram in Figure 5 shows that the highest efficiency of organic pollution removal is achieved by membrane bioreactors (MBRs) and conventional activated sludge treatment, owing to the direct involvement of microorganisms in the degradation of organic compounds. Membrane treatment (UF/RO) also demonstrates high performance, although it is less effective than biological methods in terms of BOD₅ removal because membranes function primarily as physical barriers and are less efficient in removing dissolved, readily oxidizable compounds. Notably, nature-based solutions, despite their relatively moderate COD removal efficiency, provide a sufficiently high level of BOD₅ reduction due to the biological activity of microorganisms and vegetation within these systems. Mechanical and physicochemical treatment remain the least effective methods

²² Oakley S. Preliminary treatment and primary settling. Global Water Pathogen Project / eds. J. B. Rose, B. Jiménez-Cisneros. East Lansing, MI : Michigan State University; UNESCO, 2018. 21 p.

²³ Carnevale Miino M. et al. Treatment of a Real Brewery Wastewater with Coagulation and Flocculation: Impact on Organic Substance and Nutrient Concentrations. *Applied Sciences*. 2025. Vol. 15(6). Art. 2999. DOI: <https://doi.org/10.3390/app15062999>.

²⁴ Kitanou S. et al. A comparative examination of MBR and SBR performance for municipal wastewater treatment. *Water Practice & Technology*. 2021. Vol. 16, No. 2. P. 582–591. DOI: <https://doi.org/10.2166/wpt.2021.016>.

²⁵ Ghonimy M. et al. Improving Wastewater Quality Using Ultrafiltration Technology for Sustainable Irrigation Reuse. *Water*. 2025. Vol. 17(6). Art. 870. DOI: <https://doi.org/10.3390/w17060870>.

²⁶ Gizińska-Górna M. et al. Reliability and Efficiency of Pollutant Removal in Four-Stage Constructed Wetland of SSVF-SSHF-SSHF-SSVF Type. *Water*. 2020. Vol. 12(11). Art. 3153. DOI: <https://doi.org/10.3390/w12113153/>.

for organic pollution removal, confirming their primary role as preliminary stages of wastewater treatment.

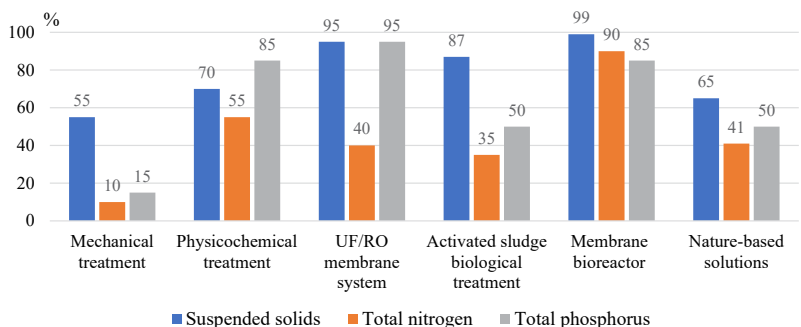


Fig. 6. Comparative removal efficiency of suspended solids, total nitrogen, and total phosphorus using different water treatment methods, %

Source: compiled by the author based on data from the cited studies^{27, 28, 29, 30, 31}

For the three indicators – suspended solids, total nitrogen, and total phosphorus (Figure 6) – the membrane bioreactor demonstrates the highest overall efficiency, combining biological nutrient removal with the membrane’s high retention capacity for suspended solids. Membrane treatment (UF/RO) also provides high removal efficiencies for suspended solids and phosphorus, whereas its nitrogen removal efficiency remains moderate. Nature-based solutions demonstrate moderate removal of nitrogen and phosphorus, while their effectiveness depends considerably on system design, vegetation, hydraulic loading, and operating conditions. Physicochemical treatment and membrane bioreactors show similarly high phosphorus removal efficiencies,

²⁷ Kitanou S. et al. A comparative examination of MBR and SBR performance for municipal wastewater treatment. *Water Practice & Technology*. 2021. Vol. 16, No. 2. P. 582–591. DOI: <https://doi.org/10.2166/wpt.2021.016>.

²⁸ Ghonimy M. et al. Improving Wastewater Quality Using Ultrafiltration Technology for Sustainable Irrigation Reuse. *Water*. 2025. Vol. 17(6). Art. 870. DOI: <https://doi.org/10.3390/w17060870>.

²⁹ Diallo H. M. et al. Evaluation of the potential ultrafiltration to improve the quality of secondary effluents in tertiary treatment and reuse. *Physics and Chemistry of the Earth, Parts A/B/C*. 2024. Vol. 135. Art. 103672. DOI: <https://doi.org/10.1016/j.pce.2024.103672>.

³⁰ Denisova V. et al. Comparison of phosphorus removal efficiency of conventional activated sludge system and sequencing batch reactors in a wastewater treatment plant. *Agronomy Research*. 2020. Vol. 18. P. 771–780. DOI: <https://doi.org/10.1515/AR.20.049>.

³¹ Gizińska-Górna M. et al. Reliability and Efficiency of Pollutant Removal in Four-Stage Constructed Wetland of SSVF-SSHF-SSHF-SSVF Type. *Water*. 2020. Vol. 12(11). Art. 3153. DOI: <https://doi.org/10.3390/w12113153/>.

although they rely on different removal mechanisms. Mechanical treatment has the lowest performance for all three indicators because it does not provide targeted nutrient removal.

Thus, the quantitative and qualitative comparison does not support identifying any single method as universally superior. High treatment efficiency should be assessed together with technological requirements, by-product generation, water reuse potential, and operational limitations. The most appropriate approach is to develop combined treatment schemes in which mechanical treatment is used for preliminary water preparation, biological and physicochemical processes remove the main pollutants, membrane technologies provide advanced treatment, and nature-based solutions reduce nutrient loads and contribute to the ecological stabilisation of the aquatic environment.

The effective integration of engineering treatment methods, on-site wastewater treatment, and nature-based elements makes it possible not only to reduce pollutant concentrations but also to prevent further degradation of aquatic ecosystems, decrease wastewater discharge volumes, and promote more sustainable water-use models.

2. Rational water use within the sustainable development framework

Rational water use is one of the key prerequisites for sustainable development, as water resources support the functioning of natural ecosystems, human well-being, and the development of industry, agriculture, energy production, and municipal services. Under conditions of increasing water scarcity and deteriorating water quality, the transition from the traditional model of intensive water consumption to a resource-efficient model of water management is becoming particularly important³².

The need to adopt rational water-use models is confirmed by the sectoral structure and regional distribution of global water abstraction. According to the *FAO AQUASTAT Water Data Snapshot 2025*, in 2022 agriculture accounted for 71.5% of global water abstraction, industry for 15.3%, and the municipal sector for 13.2%. The highest abstraction volumes were recorded in Southern Asia, Europe and Northern America, and Eastern Asia, whereas the lowest were observed in Oceania and Sub-Saharan Africa (Figure 7). Trends for 2015–2022 indicate increasing water abstraction in several regions exposed to a heightened risk of water scarcity, reflecting the uneven implementation of resource-efficient water-use models³³.

³² Jamali B., Bach P. M., Deletic A. Rainwater harvesting for urban flood management: An integrated modelling framework. *Water Research*. 2020. Vol. 171. Art.115372. DOI: <https://doi.org/10.3390/w12113153>/<https://doi.org/10.1016/j.watres.2019.115372>.

³³ Hernández Lagana M., Mejías Moreno P. *AQUASTAT Water Data Snapshot 2025*. Rome : FAO, 2025. 48 p. DOI: <https://doi.org/10.4060/cd7054en>.

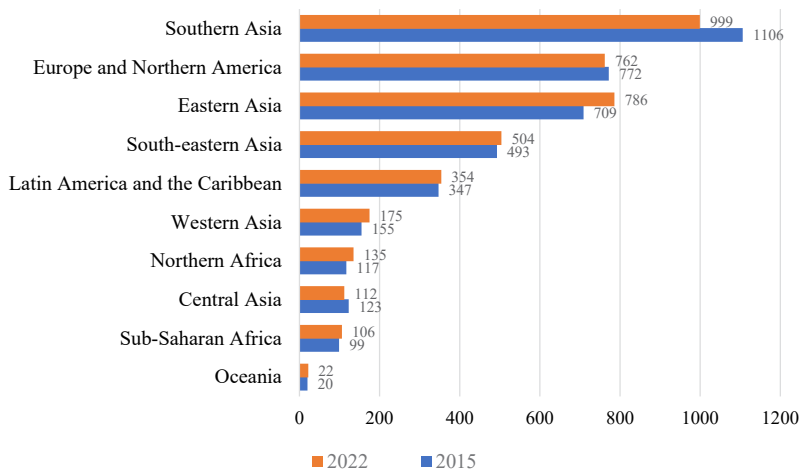


Fig. 7. Total water abstraction by world region in 2015 and 2022, billion m³

Source: compiled by the author based on FAO AQUASTAT data³⁴

The availability of renewable water resources per capita has declined by 7% over the past decade, from 5.719 to 5.326 m³. North Africa, Southern Asia, and Western Asia have the lowest freshwater availability per capita, while Kuwait, the United Arab Emirates, Qatar, and Yemen are among the countries with the lowest total volumes of renewable water resources³⁵.

The analysis of global water-use trends is particularly relevant when compared with Ukraine's national indicators. According to the *FAO AQUASTAT Water Data Snapshot 2025*, Ukraine exhibits a water-use profile that differs substantially from the global average, while partially corresponding to the characteristics of the region to which it is assigned in the AQUASTAT classification – Europe and Northern America.

Unlike the global structure of water abstraction, in which agriculture accounts for the largest share, industry is the main water user in Ukraine, representing 44.8% of total water abstraction, while agriculture accounts for only 21.1% and the municipal sector for 34.0% (Figure 8(a)). This distribution is broadly similar to the overall water-use structure of Europe and Northern

³⁴ Jamali B., Bach P. M., Deletic A. Rainwater harvesting for urban flood management: An integrated modelling framework. *Water Research*. 2020. Vol. 171. Art. 115372. DOI: <https://doi.org/10.3390/w12113153>/<https://doi.org/10.1016/j.watres.2019.115372>.

³⁵ UN sounds alarm over rising demands on water resources as scarcity increases. UN News, 2025. URL: <https://news.un.org/en/story/2025/12/1166582>.

America, but differs in the notably higher share of municipal water use, which may reflect the specific characteristics of the national economy and municipal infrastructure.

Water stress reflects the relationship between the volume of water abstraction and the available renewable water resources: the higher its level, the greater the pressure on water resources³⁶. Renewable water resources in Ukraine amount to 4,270 m³ per capita, which is below the global average; however, the country’s water stress level remains relatively low at 6.3%³⁷. This is considerably lower than both the global average and the value for Europe and Northern America (Figure 8(b)), indicating a comparatively favourable balance between water abstraction and available renewable water resources. At the same time, the predominance of the industrial and municipal sectors in the structure of water abstraction makes the implementation of resource-efficient technologies in these sectors a priority.

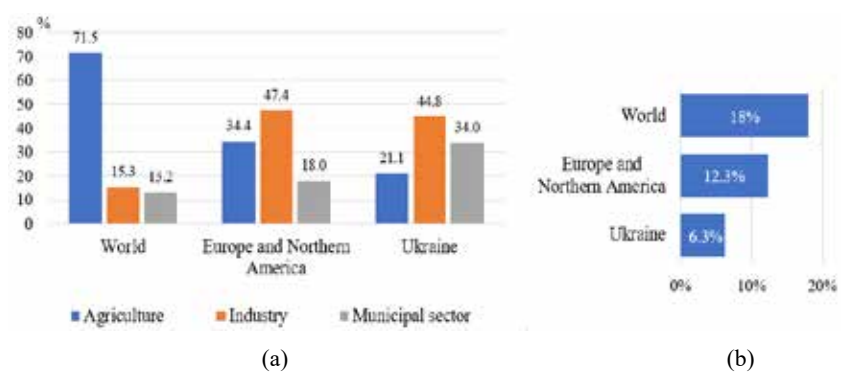


Fig. 8. Comparison of Ukraine’s water-use indicators with global and regional values in 2022: (a) structure of water abstraction by economic sectors, %; (b) water stress level, %

Source: compiled by the author based on United Nations data for (a) and UN-Water data for (b) ^{38, 39}

³⁶ United Nations. The Sustainable Development Goals Extended Report 2025. Clean Water and Sanitation. URL: https://unstats.un.org/sdgs/report/2025/extended-report/Extended-Report-2025_Goal-6.pdf.

³⁷ Progress on Level of Water Stress. UN-Water. URL: <https://sdg6data.org/index.php/en/indicator/6.4.2>.

³⁸ UN sounds alarm over rising demands on water resources as scarcity increases. UN News, 2025. URL: <https://news.un.org/en/story/2025/12/1166582>.

³⁹ United Nations. The Sustainable Development Goals Extended Report 2025. Clean Water and Sanitation. URL: https://unstats.un.org/sdgs/report/2025/extended-report/Extended-Report-2025_Goal-6.pdf.

Rational water use involves not only reducing water abstraction from natural sources but also improving water-use efficiency at all stages of the water management cycle. These stages include water abstraction, treatment, transportation, use in industrial or domestic processes, wastewater treatment, water reuse, and safe return to the natural environment. The main areas of rational water use, the mechanisms for their implementation, and the expected outcomes for sustainable development are summarised in Figure 9.

One of the key areas of rational water use is reducing water losses during transportation and distribution. Deteriorated water-supply networks, frequent pipeline failures, and insufficient monitoring of consumption volumes lead to the inefficient use of water resources. Therefore, the modernisation of water infrastructure, the introduction of metering systems, automated leak detection, network zoning, and digital water-supply management are essential conditions for improving the reliability of water provision.

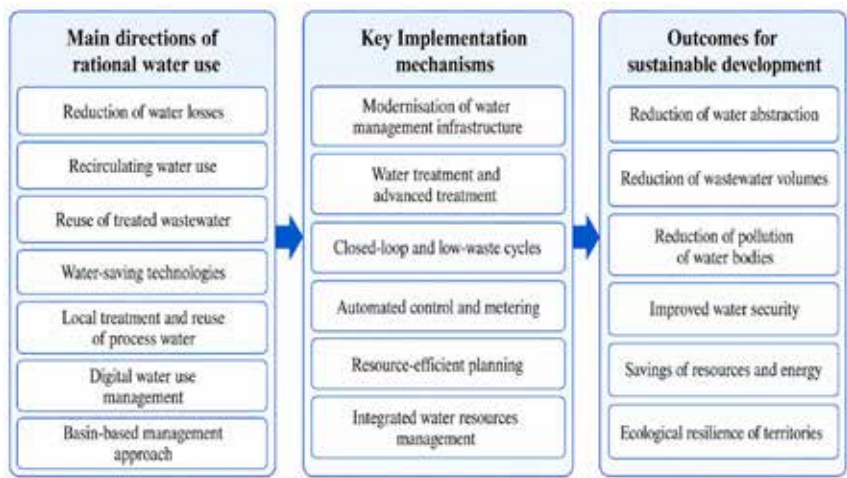


Fig. 9. Main areas of rational water use within the sustainable development framework

Source: developed by the author

An essential component of rational water use is the introduction of water-saving technologies in the municipal, industrial, and agricultural sectors. In the municipal sector, this involves the use of water-efficient equipment, consumption metering and monitoring systems, and the modernisation of water-supply and wastewater infrastructure. In industry, appropriate measures include

optimising technological processes, reducing the water intensity of production, and introducing closed-loop systems. In agriculture, effective solutions include drip irrigation, precision farming, soil conservation technologies, regulation of irrigation rates, and the use of treated wastewater, provided that sanitary and environmental requirements are met⁴⁰.

Rational water use is closely linked to the principles of the circular economy, within which water is regarded not as a single-use resource but as an element of a closed-loop system. This involves its treatment and reuse in industrial or economic processes, the recovery of valuable components, and the reduction of waste generation. Such an approach contributes to reducing the water footprint of products, decreasing polluted wastewater discharges, and improving the overall environmental efficiency of production.

In industry, the practical implementation of these principles is most commonly associated with recirculating and closed-loop water-supply systems. These systems involve the use of water in technological processes, followed by treatment, cooling, or advanced treatment and its return to the production cycle. Local treatment of individual process water streams plays a particularly important role, as it reduces the load on centralized wastewater treatment facilities and limits the discharge of excess wastewater⁴¹. The schematic diagram of recirculating water use and water reuse within an industrial water-supply system is presented in Figure 10.

A rational industrial water-use system includes the abstraction and treatment of fresh water, its use in technological processes, local treatment or cooling, advanced treatment, and the return of part of the water to the production cycle. Only the portion of wastewater that cannot be reused after treatment is discharged into a water body.

The reuse of treated wastewater is a promising approach to improving resource efficiency. With an appropriate level of treatment, such water can be used for non-potable purposes, including irrigation of green spaces, cleaning of indoor and outdoor areas, equipment cooling, firefighting, agricultural irrigation, and replenishment of non-potable storage reservoirs. This is particularly important for regions experiencing water scarcity and high pressure on natural water sources⁴².

⁴⁰ Офіс сталих рішень. Повторне використання води: Кращий європейський досвід. URL: <https://ukraine-oss.com/povtorne-vykorystannya-vody-krashhyj-yevropejskyj-dosvid>.

⁴¹ Sharma K., Nagabhooshanam N., Dasi S., Guntaj J., Srinivas B., Kaur K., Rajaram A. Design and implementation of closed-loop water reuse systems in urban and industrial settings for maximizing resource recovery and minimizing waste. *Desalination and Water Treatment*. 2024. Vol. 320. Art.100850. DOI: <https://doi.org/10.1016/j.dwt.2024.100850>.

⁴² Ramin E., Faria L., Gargalo C. L., Ramin P., Flores-Alsina X., Andersen M. M., Gernaey K. V. Water innovation in industrial symbiosis : A global review. *Journal of Environmental Management*. 2024. Vol. 349. Art. 119578. DOI: <https://doi.org/10.1016/j.jenvman.2023.119578>.

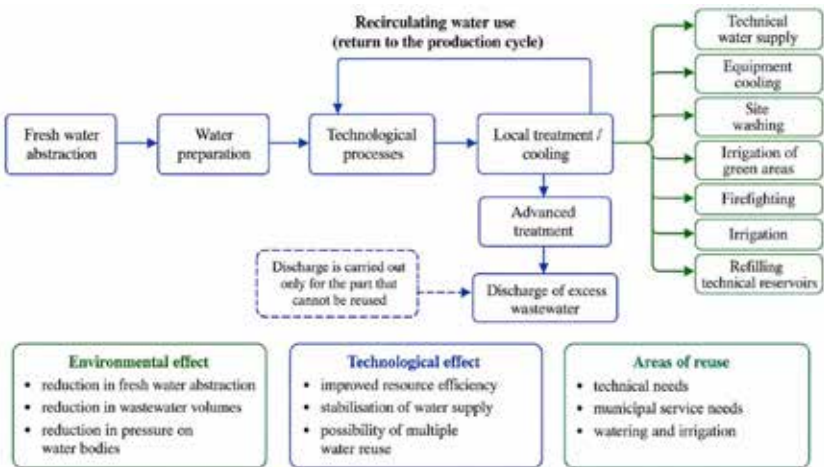


Fig. 10. Schematic diagram of recirculating water use and water reuse within an industrial water-supply system

Source: developed by the author

Despite the considerable potential for treated wastewater reuse, actual global rates remain far below the available potential. According to a study conducted by Utrecht University and the United Nations University Institute for Water, Environment and Health (UNU-INWEH), of the total volume of wastewater generated worldwide each year – 359.4 billion m³, based on 2015 data – only 63% is collected through centralized sewerage systems, 52% undergoes treatment, and just 11% of the total volume is reused (Figure 11)⁴³. According to UN-Water estimates, the potential volume of wastewater available for reuse worldwide is approximately 320 billion m³ per year, which is more than ten times the combined capacity of all existing desalination plants globally⁴⁴. More recent data indicate that, as of 2022, safe treatment covered 56–58% of domestic wastewater and only 38% of industrial wastewater⁴⁵. This confirms that treated wastewater reuse remains an underused opportunity for improving the resource efficiency of water use.

⁴³ Jones E. R., van Vliet M. T. H., Qadir M., Bierkens M. F. P. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth System Science Data*. 2021. Vol. 13, No. 2. P. 237–254. DOI: <https://doi.org/10.5194/essd-13-237-2021>.

⁴⁴ Progress on Wastewater Treatment (SDG indicator 6.3.1). *UN-Water*, 2024. URL: <https://sdg6data.org/en/indicator/6.3.1>.

⁴⁵ Water Quality and Wastewater. *UN-Water*, 2024. URL: <https://www.unwater.org/water-facts/water-quality-and-wastewater>.

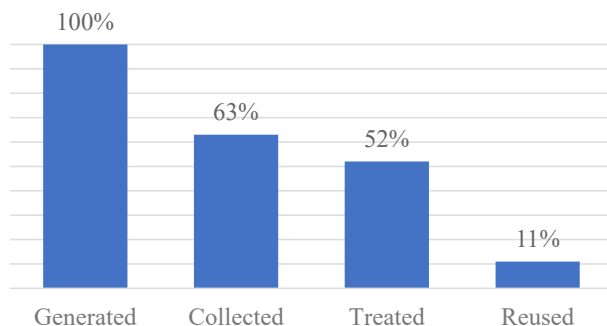


Fig. 11. Global wastewater flows, % of total wastewater generated (359.4 billion m³/year; 2015 data)

Source: compiled by the author based on data from Jones et al.⁴⁶

Bridging the gap between the volume of wastewater treated and the amount actually reused largely depends on the implementation of specific water-saving technologies at the level of individual economic sectors. To summarize the quantitative performance indicators of such approaches, a table was developed based on an analysis of scientific and industry sources. It presents the main water-saving technologies applicable across different sectors of the economy and the expected water savings confirmed by the relevant studies (Table 3). The indicators provided enable a well-founded comparison of the potential of different technological solutions and help identify priority areas for their implementation according to the sector-specific characteristics of water use.

The data summarised in Table 3 indicate that the greatest water-saving potential is associated with technological solutions in the industrial sector, particularly closed-loop cooling systems, which can reduce water use by up to 70%, and recirculating water-supply systems, which can provide savings of up to 50–60%. This is explained by the possibility of repeatedly using the same volume of water within the technological cycle without substantial losses through evaporation or discharge.

In agriculture, which remains the largest consumer of water resources globally, the introduction of drip irrigation and precision farming technologies can provide water savings ranging from 15% to 70%, depending on the crop, soil conditions, and the initial irrigation method. This confirms the considerable untapped potential for improving water-use efficiency in the agricultural sector.

⁴⁶ Ramin E., Faria L., Gargalo C. L., Ramin P., Flores-Alsina X., Andersen M. M., Gernaey K. V. Water innovation in industrial symbiosis: A global review. *Journal of Environmental Management*. 2024. Vol. 349. Art. 119578. DOI: <https://doi.org/10.1016/j.jenvman.2023.119578>.

Table 3

Water-saving technologies by economic sector and their expected water-saving effects

Technology	Sector of application	Reported or expected water savings, %
Drip irrigation	Agriculture	30–70% compared with conventional sprinkler or surface irrigation ¹
Precision agriculture (variable rate irrigation, VRI)	Agriculture	15–50% compared with standard uniform irrigation ²
Water-efficient plumbing fixtures (aerators, faucets, dual-flush toilets)	Municipal sector	20–30%, depending on the type of fixture ³
Recirculating and closed-loop water-supply systems in industrial processes	Industry	Up to 50–60% of the enterprise's total water consumption ⁴
Closed-loop cooling systems	Industry, digital infrastructure	Up to 70% compared with open evaporative cooling systems ⁵
Reuse of treated wastewater for technical and irrigation purposes	Municipal sector, agriculture	Depends on the extent to which primary water abstraction is replaced; assessed individually for each facility

Technologies used in the municipal sector, particularly water-efficient plumbing fixtures, demonstrate a more moderate but consistent water-saving effect of 20–30%. These savings can be achieved with relatively low capital investment and short payback periods, making such solutions an accessible means of improving water-use efficiency in households and public buildings. At the same time, the effect of treated wastewater reuse cannot be expressed as a universal quantitative value, as it depends on the characteristics of each facility, including its water consumption, the technical feasibility of replacing primary water abstraction, and the required level of advanced treatment. Therefore, a separate technical and economic assessment is required in each specific case. Overall, the data confirm that the greatest effect can be achieved through the integrated rather than isolated implementation of the technologies considered across the industrial, municipal, and agricultural sectors.

The practical implementation of rational water-use principles can be observed in the activities of international companies across various economic sectors, where water-use efficiency is becoming an integral component of environmental policy and production modernisation. In the food industry, Nestlé improves water-use efficiency at its manufacturing sites, monitors water abstraction and discharge quality, and introduces water reuse and recirculation. As a result, between 2010 and 2024, water abstraction per tonne of product was

reduced by 20%, contributing to lower freshwater consumption and reduced pressure on aquatic ecosystems⁴⁷.

In the chemical industry, BASF provides a notable example by applying on-site treatment of industrial wastewater, membrane bioreactors, and water quality monitoring systems. As of 2024, the company had achieved 65% of its target for implementing sustainable water management at production sites located in water-stressed areas. By 2030, it plans to ensure full coverage of such sites, which account for approximately 90% of the company's total water abstraction⁴⁸. This example demonstrates the importance of combining conventional treatment technologies with modern high-efficiency advanced treatment methods.

In the beverage industry, Coca-Cola companies implement water treatment, recirculation, and reuse during equipment cleaning, container preparation, and other technological operations. Between 2015 and 2024, the Water Use Ratio was reduced from 1.98 to 1.78 litres of water per litre of beverage produced, corresponding to an approximately 10% improvement in water-use efficiency⁴⁹. These measures help reduce specific water consumption per unit of product and decrease wastewater generation.

Water-use efficiency is also becoming increasingly important for the digital infrastructure sector, particularly in data centre operations. Microsoft is introducing technologies aimed at reducing freshwater use and improving the efficiency of cooling systems. From the early 2000s to 2023, the water-use intensity of its data centres decreased by more than 80%⁵⁰. In fiscal year 2025, Microsoft replenished more than 14 million m³ of water globally, exceeding its total water withdrawals for the first time and marking an important milestone towards becoming water positive by 2030⁵¹.

The examples presented confirm that, in contemporary practice, rational water use is implemented through a combination of technological, organizational, digital, and managerial solutions. At the same time, the effectiveness of such measures increases when they are introduced within the framework of integrated water resources management, which involves coordinating the interests of different water users, considering the ecological needs of aquatic ecosystems,

⁴⁷ Nestlé's Sustainability Strategy. The Sustainable Innovation, 2026. URL: <https://thesustainableinnovation.com/nestle-sustainability/>.

⁴⁸ BASF. E3 water: BASF report 2024. URL: <https://report.basf.com/2024/en/combined-managements-report/consolidated-sustainability-statement/environment/e3-water.html>.

⁴⁹ The Coca-Cola Company. 2024 Environmental Update. URL: <https://www.coca-colacompany.com/content/dam/corporate/us/en/reports/2024-environmental-update/2024-environmental-update.pdf>.

⁵⁰ Sustainable by design: Transforming datacenter water efficiency. Microsoft Cloud Blog, 2024. URL: <https://www.microsoft.com/en-us/microsoft-cloud/blog/2024/07/25/sustainable-by-design-transforming-datacenter-water-efficiency/>.

⁵¹ Microsoft. 2026 Environmental Sustainability Report. 2026. URL: <https://surl.li/zwvnvzh>.

applying the river-basin management principle, and integrating the quantitative and qualitative aspects of water use. Within this approach, decisions concerning water abstraction, wastewater discharge, wastewater treatment, the protection of water bodies, and the development of water infrastructure should be made with regard to the condition of the entire river basin rather than individual administrative or industrial areas.

Within the sustainable development framework, rational water use performs not only an environmental but also a socio-economic function. It contributes to ensuring access to high-quality water, reducing risks to public health, improving the reliability of water supply, and strengthening the environmental safety of territories. In the long term, the rational use of water resources is a prerequisite for adaptation to climate change, the prevention of water scarcity, and the maintenance of the resilience of natural and economic systems.

CONCLUSIONS

The study has demonstrated that the protection of water bodies and rational water use are essential components of sustainable development, as water resources support the functioning of natural ecosystems, industrial processes, food security, and quality of life. Under conditions of climate change, increasing anthropogenic pressure, and growing risks of water scarcity, water protection measures should be regarded as part of an integrated environmental safety system.

It has been established that effective water treatment requires an integrated approach combining mechanical, physicochemical, biological, membrane, and nature-based methods with on-site industrial wastewater and stormwater treatment. The comparison showed that no single method can be regarded as universally superior. Membrane bioreactors provide the highest overall removal efficiency for organic matter, suspended solids, and nutrients, whereas nature-based solutions are particularly important for regulating surface runoff and providing complementary removal of nutrients and suspended solids. The most appropriate approach is to develop combined treatment schemes that take into account wastewater composition, the nature of pollution, operational limitations, and the potential for treated water reuse.

The analysis of water-use indicators showed that agriculture accounts for the largest share of water abstraction globally, whereas the industrial and municipal sectors predominate in Ukraine. Despite Ukraine's relatively low level of water stress, the specific structure of water abstraction, losses in water-supply networks, and insufficient levels of treated wastewater reuse necessitate the targeted implementation of resource-efficient solutions.

It has been substantiated that rational water use should be regarded as a component of a resource-efficient and circular development model. Its priority areas include reducing water losses, modernizing water infrastructure, introducing water-saving technologies, implementing recirculating water use, applying on-site treatment of process streams, reusing treated wastewater, and adopting a river-basin approach to water resources management. The implementation of these measures contributes to reducing water abstraction and wastewater generation, decreasing pressure on water bodies, and enhancing the water security of territories.

The review of current practices confirmed the feasibility of shifting from the linear “abstraction – use – discharge” model to recirculating and closed-loop water-supply systems. Water recirculation, treated wastewater reuse, modernisation of treatment systems, and monitoring of specific water consumption make it possible to improve resource efficiency both in traditionally water-intensive industries and in emerging economic sectors.

An important prerequisite for the further development of sustainable water use is the comprehensive assessment of the effectiveness of combined water treatment technologies, nature-based solutions, and water reuse and recirculation systems, taking into account the environmental, technological, and economic feasibility of their implementation. A well-founded combination of these approaches creates the conditions for reducing anthropogenic pressure on aquatic ecosystems, improving resource efficiency, and ensuring the long-term water security of territories.

SUMMARY

The article substantiates environmental and technological solutions for water treatment and identifies priority areas of rational water use in the context of sustainable development. Mechanical, physicochemical, biological, membrane-based, and nature-based methods of wastewater treatment are considered. These methods are comparatively assessed in terms of their efficiency in removing organic matter, suspended solids, and nutrients; application conditions; by-product generation; and the potential for treated water reuse. It is established that the choice of treatment method should take into account wastewater composition, the nature of pollution, application conditions, and treated-water quality requirements, while the highest efficiency can be achieved by combining complementary technologies in integrated treatment schemes. Current trends in global and national water use, the sectoral structure of water abstraction, and water stress levels are analysed. The need to reduce water losses, modernize water infrastructure, introduce water-saving technologies, and implement recirculating and closed-loop water-supply systems is substantiated. The role

of treated wastewater reuse and the transition from a linear water-consumption model to a resource-efficient circular model is highlighted. It is concluded that integrating treatment technologies, nature-based approaches, and rational water use provides a foundation for reducing anthropogenic pressure on water bodies and ensuring long-term water security.

Bibliography

1. Carey R. O., Hochmuth G. J., Martinez C. J., Boyer T. H., Dukes M. D., Toor G. S., Cisar J. L. Evaluating nutrient impacts in urban watersheds: challenges and research opportunities. *Environmental Pollution*. 2013. Vol. 173. P. 138–149. DOI: <https://doi.org/10.1016/j.envpol.2012.10.004>.
2. Giri S., Qiu Z. Understanding the relationship of land use and water quality in twenty first century: a review. *Journal of Environmental Management*. 2016. Vol. 173. P. 41–48.
3. Intergovernmental Panel on Climate Change. *Climate change 2023: synthesis report*. Geneva : IPCC, 2023. URL: <https://www.ipcc.ch/report/ar6/syr/>.
4. United Nations. Goal 6: Ensure access to water and sanitation for all. *United Nations Sustainable Development Goals*. URL: <https://www.un.org/sustainabledevelopment/water-and-sanitation/>.
5. UNESCO World Water Assessment Programme. *The United Nations World Water Development Report 2024: Water for prosperity and peace*. Paris : UNESCO, 2024. URL: <https://unesdoc.unesco.org/ark:/48223/pf0000388948>.
6. Nachiyar C. V., Rakshi A. D., Sandhya S. N., Jebasta B. D., Nellore J. Developments in treatment technologies of dye-containing effluent: A review. *Case Studies in Chemical and Environmental Engineering*. 2023. Vol. 7. P. 100–139. DOI: 10.1016/j.csee.2023.100339.
7. Kato S., Kansha Y. Comprehensive review of industrial wastewater treatment techniques. *Environmental Science and Pollution Research*. 2024. Vol. 31. P. 51064–51097. DOI: 10.1007/s11356-024-34584-0.
8. Sharma R., Verma N., Lugani Y., Kumar S., Asadnia M. Conventional and advanced techniques of wastewater monitoring and treatment. *Green Sustainable Process for Chemical and Environmental Engineering and Science* / eds. Inamuddin R. Boddula A. M. Asiri. Elsevier, 2021. P. 1–48. DOI: <https://doi.org/10.1016/B978-0-12-821883-9.00009-6>.
9. Sharghi E. A., Davarpanah L. Optimization of chemical coagulation–flocculation process of detergent manufacturing plant wastewater treatment for full-scale applications: a case study. *Desalination and Water Treatment*. 2022. Vol. 262. P. 38–53. DOI: <https://doi.org/10.5004/dwt.2022.28494>.

10. Jachimowicz P., Cydzik-Kwiatkowska A. Coagulation and flocculation before primary clarification as efficient solutions for low-density microplastic removal from wastewater. *International Journal of Environmental Research and Public Health*. 2022. Vol. 19, No. 20. Art. 13013. DOI: 10.3390/ijerph192013013.
11. Aloui F., Kchaou S., Sayadi S. Physicochemical treatments of anionic surfactants wastewater: effect on aerobic biodegradability. *Journal of Hazardous Materials*. 2009. Vol. 164, No. 1. P. 353–359. DOI: <https://doi.org/10.1016/j.jhazmat.2008.08.009>.
12. Robinson S., Bérubé P. R. Membrane ageing in full-scale water treatment plants. *Water Research*. 2020. Vol. 169. Art. 115212. DOI: <https://doi.org/10.1016/j.watres.2019.115212>.
13. Fatima F., Du H., Kommalapati R. R. A Sequential Membrane Process of Ultrafiltration Forward Osmosis and Reverse Osmosis for Poultry Slaughterhouse Wastewater Treatment and Reuse. *Membranes (Basel)*. 2023. Vol. 13, No. 3. 296. DOI: <https://doi.org/10.3390/membranes13030296>.
14. Dotro G., Langergraber G., Molle P., Nivala J., Puigagut J., Stein O., von Sperling M. *Treatment wetlands*. Biological Wastewater Treatment Series. Vol. 7. IWA Publishing, 2017. 172 p.
15. Мокієнко А. В. Актуальні водні проблеми. *Інноваційні водні технології*. Острозь, 2024. 381 с.
16. Melin T., Jefferson B., Bixio D., Thoeye C., De Wilde W., De Koning J., van der Graaf J., Wintgens T. Membrane bioreactor technology for wastewater treatment and reuse. *Desalination*. 2006. Vol. 187, No. 1–3. P. 271–282. DOI: <https://doi.org/10.1016/j.desal.2005.04.086>.
17. International Union for Conservation of Nature. *Global Standard for Nature-based Solutions: a user-friendly framework for the verification, design and scaling up of NbS*. 1st ed. Gland, Switzerland : IUCN, 2020. 30 p.
18. Steakley J. Rain garden (2014): photograph. Wikimedia Commons, 2014. URL: [https://commons.wikimedia.org/wiki/File:Rain_garden_\(2014\).JPG](https://commons.wikimedia.org/wiki/File:Rain_garden_(2014).JPG). License: CC BY-SA 4.0.
19. SuSanA Secretariat. Constructed wetland (5547195530) photograph / photo by H. Hoffmann. Wikimedia Commons, 2009. URL: [https://commons.wikimedia.org/wiki/File:Constructed_wetland_\(5547195530\).jpg](https://commons.wikimedia.org/wiki/File:Constructed_wetland_(5547195530).jpg). License: CC BY 2.0.
20. United States Department of Agriculture. Riparian buffer on Bear Creek in Story County, Iowa: photograph. Wikimedia Commons. URL: https://commons.wikimedia.org/wiki/File:Riparian_buffer_on_Bear_Creek_in_Story_County_Iowa.JPG. Public domain.

21. Guzzardo Partnership. 2012-12-04 Stormwater Bio-Treatment Area: photograph. Wikimedia Commons, 2012. URL: https://commons.wikimedia.org/wiki/File:2012-12-04_Stormwater_Bio-Treatment_Area.jpg. License: CC BY-SA 4.0.
22. Oakley S. Preliminary treatment and primary settling. *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project)* / eds. J. B. Rose, B. Jiménez-Cisneros; part 4 eds. J. R. Mihelcic, M. E. Verbyla. East Lansing, MI: Michigan State University; UNESCO, 2018. Part 4: *Management of Risk from Excreta and Wastewater*. Section: *Sanitation System Technologies, Pathogen Reduction in Sewered System Technologies*. 21 p.
23. Carnevale Miino M., Torretta V., Repková M., Hlavínek P., Telek J. Treatment of a Real Brewery Wastewater with Coagulation and Flocculation: Impact on Organic Substance and Nutrient Concentrations. *Applied Sciences*. 2025. Vol. 15(6). Art. 2999. DOI: <https://doi.org/10.3390/app15062999>.
24. Kitanou S., Ayyoub H., Tourir J., Zdeg A., Benabdallah S., Taky M., Elmidaoui A. A comparative examination of MBR and SBR performance for municipal wastewater treatment. *Water Practice & Technology*. 2021. Vol. 16, No. 2. P. 582–591. DOI: <https://doi.org/10.2166/wpt.2021.016>.
25. Ghonimy M., Alharbi A., Saad S. A. H., Hussein N. S. Improving Wastewater Quality Using Ultrafiltration Technology for Sustainable Irrigation Reuse. *Water*. 2025. Vol. 17(6). 870. DOI: <https://doi.org/10.3390/w17060870>.
26. Diallo H. M., Ayyoub H., Elazhar F., Tahaikt M., Elmidaoui A., Taky M. Evaluation of the potential ultrafiltration to improve the quality of secondary effluents in tertiary treatment and reuse. *Physics and Chemistry of the Earth, Parts A/B/C*. 2024. Vol. 135. Art. 103672. DOI: <https://doi.org/10.1016/j.pce.2024.103672>.
27. Denisova V., Tihomirova K., Neilands J., Gruskevica K., Mezule L., Juhna T. Comparison of phosphorus removal efficiency of conventional activated sludge system and sequencing batch reactors in a wastewater treatment plant. *Agronomy Research*. 2020. Vol. 18. P. 771–780. DOI: <https://doi.org/10.1515/AR.20.049>.
28. Gizińska-Górna M., Jóźwiakowski K., Marzec M. Reliability and Efficiency of Pollutant Removal in Four-Stage Constructed Wetland of SSVF-SSHF-SSHF-SSVF Type. *Water*. 2020. Vol. 12(11). 3153. DOI: <https://doi.org/10.3390/w12113153/>.
29. Jamali B., Bach P. M., Deletic A. Rainwater harvesting for urban flood management: An integrated modelling framework. *Water Research*. 2020. Vol. 171. 115372. DOI: <https://doi.org/10.1016/j.watres.2019.115372>.

30. Hernández Lagana M., Mejias Moreno P. AQUASTAT water data snapshot 2025. Rome : FAO, 2025. DOI: <https://doi.org/10.4060/cd7054en>.
31. UN sounds alarm over rising demands on water resources as scarcity increases. *UN News*, 2025. URL: <https://news.un.org/en/story/2025/12/1166582>.
32. United Nations. The Sustainable Development Goals Extended Report 2025. Clean Water and Sanitation. URL: https://unstats.un.org/sdgs/report/2025/extended-report/Extended-Report-2025_Goal-6.pdf.
33. Progress on Level of Water Stress. UN-Water. URL: <https://sdg6data.org/index.php/en/indicator/6.4.2>.
34. Офіс сталих рішень. Повторне використання води: Кращий європейський досвід. URL: <https://ukraine-oss.com/povtorne-vykorystannya-vody-krashhyj-yevropejskyj-dosvid>.
35. Sharma K., Nagabhooshanam N., Dasi S., Guntaj J., Srinivas B., Kaur K., Rajaram A. Design and implementation of closed-loop water reuse systems in urban and industrial settings for maximizing resource recovery and minimizing waste. *Desalination and Water Treatment*. 2024. Vol. 320. Art. 100850. DOI: <https://doi.org/10.1016/j.dwt.2024.100850>.
36. Ramin E., Faria L., Gargalo C. L., Ramin P., Flores-Alsina X., Andersen M. M., Gernaey K. V. Water innovation in industrial symbiosis: A global review. *Journal of Environmental Management*. 2024. Vol. 349. Art. 119578. DOI: <https://doi.org/10.1016/j.jenvman.2023.119578>.
37. Jones E. R., van Vliet M. T. H., Qadir M., Bierkens M. F. P. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth System Science Data*. 2021. Vol. 13, No. 2. P. 237–254. DOI: <https://doi.org/10.5194/essd-13-237-2021>.
38. Progress on Wastewater Treatment (SDG indicator 6.3.1). *UN-Water*, 2024. URL: <https://sdg6data.org/en/indicator/6.3.1>.
39. Water Quality and Wastewater. *UN-Water*, 2024. URL: <https://www.unwater.org/water-facts/water-quality-and-wastewater>.
40. Shamshery P., Wang R. Q., Tran D. V., Winter V. A. G. Modelling the future of irrigation: a parametric description of pressure compensating drip irrigation emitter performance. *PLoS ONE*. 2017. Vol. 12, No. 4. Art. e0175241. DOI: <https://doi.org/10.1371/journal.pone.0175241>.
41. Optimizing Agricultural Water Use and Management. *USDA Agricultural Research Service*. URL: <https://www.ars.usda.gov/Optimizing-Agricultural-Water-Use-and-Management/>.
42. Schein J., Bickmore T., Sovocool K. Assessing Water Use in WaterSense-Labeled Homes and Quantifying the Savings. *Journal AWWA*. 2022. Vol. 114(5). P. 10–19. DOI: <https://doi.org/10.1002/awwa.1899>.

43. Aguilar-Vásquez E. A., Rojas-Flores S., González-Delgado Á. D. Effect of water regeneration and integration on technical indicators of PVC manufacturing using process system engineering. *Polymers*. 2025. Vol. 17, No. 17. Art. 2418. DOI: <https://doi.org/10.3390/polym17172418>.
44. Khatib L., Pham A., Ahmed K., Frenkel V. S. Data centers and water: challenges and solutions for sustainable cooling. *Journal AWWA*. 2025. Vol. 117. P. 48–53. DOI: <https://doi.org/10.1002/awwa.2504>.
45. Nestlé's Sustainability Strategy. *The Sustainable Innovation*, 2026. URL: <https://thesustainableinnovation.com/nestle-sustainability/>.
46. BASF. *E3 water: BASF report 2024*. URL: <https://report.basf.com/2024/en/combined-managements-report/consolidated-sustainability-statement/environment/e3-water.html>.
47. The Coca-Cola Company. Environmental Update. 2024. URL: <https://www.coca-colacompany.com/content/dam/corporate/us/en/reports/2024-environmental-update/2024-environmental-update.pdf>.
48. Sustainable by design: Transforming datacenter water efficiency. Microsoft Cloud Blog, 2024. URL: <https://www.microsoft.com/en-us/microsoft-cloud/blog/2024/07/25/sustainable-by-design-transforming-datacenter-water-efficiency/>.
49. Microsoft. 2026 Environmental Sustainability Report. 2026. URL: <https://surl.li/zwnvzh>.

Information about the author:

Litvak Olga,

Candidate of Economic Sciences, Associate Professor,
Associate Professor at the Department of Ecology and Environmental
Protection Technologies
Admiral Makarov National University of Shipbuilding
9, Heroiv Ukrainy Avenue, Mykolaiv, Ukraine

REVIEW OF CURRENT RESEARCH ON THE EFFECTS OF HEAVY METALS ON TISSUE REGENERATION PROCESSES: ECOLOGICAL AND MEDICAL-BIOLOGICAL ASPECTS

Lukonin Oleksandr, Smyrnov Viktor

DOI <https://doi.org/10.30525/978-9934-26-714-7-6>

INTRODUCTION

Rapid industrialization, urbanization, and intensification of agricultural production are accompanied by unprecedented anthropogenic pressure on the environment. Among the numerous environmental pollutants, heavy metals occupy a special place due to their high toxicity, resistance to degradation, ability for long-term migration in biosphere components, and bioaccumulation in living organisms^{1, 2}. Priority ecotoxics in this group include lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr), and nickel (Ni), which enter the environment primarily through the activities of metallurgical, galvanic, chemical enterprises, thermal power generation, motor transport, as well as through the use of mineral fertilizers and plant protection agents^{3,4}.

The problem of heavy metal contamination becomes particularly relevant for Ukraine – a country with significant industrial potential, a large share of heavy industry, and high anthropogenic pressure on water bodies. In a number of regions, particularly in Dnipropetrovsk, Donetsk, Zaporizhzhia, and Mykolaiv regions, concentrations of heavy metals in surface waters, bottom sediments, and soils exceed permissible levels many times over, posing a real threat to

¹ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529

² Войцїцький В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування*. 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007

³ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P.100529. DOI: 10.1016/j.cobme.2024.100529

⁴ Хижняк С. В., Войцїцький В. М., Данчук В. М., Мідик С. В., Лапоша О. А., Ушкалов В. О. Шляхи міграції стійких пестицидів трофічними ланцюгами наземних і водних екосистем. *Біоресурси і природокористування*. 2018. Т. 10. № № 1–2. С. 36–43.

public health. Aquatic ecosystems are particularly vulnerable to heavy metal contamination, as they serve as final reservoirs for most pollutants entering through atmospheric precipitation, industrial and domestic wastewater, and surface runoff from agricultural lands⁵.

It has been established that metals can induce powerful oxidative stress, damage nucleic acids, cause mitochondrial dysfunction, disrupt epigenetic regulation of gene expression, and activate apoptotic and ferroptotic signaling cascades. Of particular concern is the effect of heavy metals on reparative tissue regeneration processes, as impaired wound healing leads to chronic wounds, reduced patient quality of life, increased frequency of postoperative complications, and economic losses for the healthcare system. Numerous experimental studies on animals and cell cultures demonstrate that chronic heavy metal exposure is accompanied by inhibition of fibroblast proliferation, impaired collagen synthesis, reduced angiogenesis, and slowed keratinocyte migration in the wound defect⁶. Clinical observations confirm these data, indicating an increased incidence of trophic ulcers, diabetic foot, and slow-healing postoperative wounds in patients living in areas of technogenic pollution⁷.

Despite significant progress in studying individual aspects of the problem, comprehensive studies that combine ecological monitoring of aquatic ecosystems with in-depth investigation of molecular-cellular mechanisms and clinical manifestations of regeneration disorders in people living in areas with varying anthropogenic loads are critically insufficient. This is particularly true for the Mykolaiv region, where, due to the activities of industrial enterprises (including metallurgical plants, shipyards, and chemical industry enterprises), significant contamination of water bodies with heavy metals is observed, yet systematic medical-biological studies of the impact of this contamination on tissue regeneration are practically absent⁸.

This necessitates systematic and critical generalization of available scientific data to form a holistic understanding of the impact of heavy metals on tissue regeneration processes, identify research gaps, and develop a conceptual model that integrates ecological, molecular-biological, and clinical approaches.

⁵ Repair and regeneration: ferroptosis in the process of remodeling and fibrosis in impaired organs. *Cell Death Discov.* 2024. № 10. P. 424. DOI: 10.1038/s41420-024-02181-2

⁶ Peters K., Staehle S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

⁷ Repair and regeneration: ferroptosis in the process of remodeling and fibrosis in impaired organs. *Cell Death Discov.* 2024. № 10. P. 424. DOI: 10.1038/s41420-024-02181-2

⁸ Войцickий В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування.* 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007

Such a model can serve as a theoretical basis for planning further empirical studies aimed at assessing public health risks associated with environmental heavy metal contamination and developing effective strategies for the prevention and correction of regeneration disorders.

The aim of this work is to systematize and generalize current scientific data on the sources, migration, and bioaccumulation of heavy metals in aquatic ecosystems, the molecular and cellular mechanisms of their toxic effects with an emphasis on disorders of reparative tissue regeneration, and the analysis of clinical consequences of metal bioaccumulation for the development of a conceptual research model.

1. Ecological aspects of heavy metal contamination of aquatic ecosystems and their bioaccumulation

1.1. Sources of input and migration of heavy metals in aquatic ecosystems

The main anthropogenic sources of heavy metal input into aquatic ecosystems are industrial discharges (enterprises of metallurgical, machine-building, galvanic, chemical, and wood-processing industries), atmospheric deposition (products of fuel combustion, motor vehicle emissions, technological emissions from industrial enterprises), agricultural runoff (washout from fields of mineral fertilizers, pesticides, ameliorants), as well as domestic wastewater (containing detergents, cosmetics, pharmaceuticals containing metals). In addition, surface runoff from urbanized areas, where metals accumulate in soils, road dust, and atmospheric emissions, is a significant source of heavy metals⁹.

Once in the aquatic environment, heavy metals undergo complex physicochemical transformations that determine their migration capacity, bioavailability, and ecological hazard. Metals can be present in water in dissolved ionic form, as part of complex compounds (with carbonates, sulfates, chlorides, organic ligands), sorbed on suspended particles (clay minerals, iron and manganese hydroxides, organic matter), or precipitated in bottom sediments. The distribution of metals between these forms depends on numerous factors: pH and redox potential of the aqueous medium, temperature, salinity, presence of complexing agents (humic and fulvic acids), hydrodynamic conditions (flow velocity, mixing), and biological activity. The most bioavailable and toxic form is the free ionic form of metals, while sorbed and complexed forms are less accessible to aquatic organisms.

The migration capacity of heavy metals in aquatic ecosystems varies significantly. Thus, cadmium and zinc are characterized by high mobility and the ability for long-term transport in dissolved state, while lead and copper

⁹ Амонс С. Е., Яковець Л. А. Вплив важких металів на основні фізіологічні процеси зернових та зернобобових культур. *Сільське господарство та лісівництво*. 2023. № 30. С. 211–227. DOI: 10.37128/2707-5826-2023-3-16

tend to sorb onto suspended particles and precipitate into bottom sediments. Chromium in its toxic hexavalent form (Cr^{6+}) is highly mobile, while the less toxic trivalent chromium (Cr^{3+}) rapidly hydrolyzes and precipitates. Nickel occupies an intermediate position, demonstrating moderate mobility and the ability to form complexes with organic substances¹⁰.

1.2. Comparative characteristics of heavy metal concentrations in water bodies of Ukraine and the world

Analysis of literature data indicates significant regional differences in levels of heavy metal contamination of aquatic ecosystems. The highest concentrations of Pb, Cd, Cu, Zn, Cr, and Ni in surface waters and bottom sediments are observed in industrially developed regions of the world: Southeast Asia (China, India, Vietnam), Latin America (Mexico, Peru, Chile), Eastern Europe (Poland, Czech Republic, Ukraine), and certain areas of Africa (South Africa, Zambia). Table 1 presents summarized data on heavy metal concentrations in water bodies of various regions of the world.

Table 1

**Average concentrations of heavy metals (mg/L) in surface waters
of different regions of the world**

Region	Pb	Cd	Cu	Zn	Cr	Ni
Western Europe	3–15	0.3–2	5–30	20–100	2–15	5–25
Eastern Europe	10–80	1–15	10–80	50–400	10–60	10–50
Southeast Asia	20–150	2–30	15–120	80–600	15–100	15–80
North America	2–10	0.2–1.5	3–25	15–80	1–10	3–20
Ukraine (overall)	15–60	1–10	10–70	40–300	8–50	8–40
Mykolaiv region	25–90	3–18	20–120	60–450	15–80	12–60
MPC Ukraine*	30	5	10	100	50	20

Summarized from data^{11, 12, 13}.

**MPC – maximum permissible concentration for water bodies of domestic drinking water use (according to Ukrainian regulatory documents).*

¹⁰ Черниченко І. О., Литвиченко О. М., Бабій В. Ф., Кондратенко О. Є., Главачек Д. О. До питання фонового рівня забруднення ґрунтів важкими металами для визначення впливу воєнних дій. *Довкілля та здоров'я*. 2025. № 1 (114). Р. 18–23. DOI: 10.32402/dovkil2025.01.018

¹¹ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng*. 2024. № 30. Р. 100529. DOI: 10.1016/j.cobme.2024.100529

¹² Войціцький В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування*. 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007

¹³ Жукова О. Г., Старжинський П. С., Прокопенко І. О. Забруднення водних екосистем важкими металами внаслідок військових дій та їх вплив на здоров'я людини. *Екологічна безпека держави 2025* : матеріали Науково-практичної конференції. Київ, 2025. С. 61–63. DOI: 10.18372/2786-8168.19.19971

As seen from Table 1, in water bodies of Ukraine, heavy metal concentrations significantly exceed levels characteristic of Western Europe and North America and approach those of Southeast Asia.

A particularly dangerous situation is observed in the Mykolaiv region, where exceedances of MPC for lead, cadmium, copper, zinc, chromium, and nickel range from 1.5 to 12 times. The high concentration of heavy metals in the water bodies of the Mykolaiv region is associated with the activities of large industrial enterprises, particularly the metallurgical plant “Naftokhimik”, shipbuilding yards, machine-building enterprises, and significant anthropogenic pressure on the Southern Bug River and its tributaries¹⁴.

1.3. Bioaccumulation and biomagnification of heavy metals in trophic chains

One of the most dangerous properties of heavy metals is their ability for bioaccumulation – selective accumulation in the tissues of living organisms at concentrations significantly exceeding their content in the environment¹⁵. Mechanisms of bioaccumulation include passive adsorption of metals on cell surface structures (especially algae and bacteria), active transport uptake through ion channels and transporters (for metals with physiological functions, e.g., Cu, Zn), as well as phagocytosis and pinocytosis of metal-containing particles. In aquatic organisms, metal bioaccumulation depends on a number of factors: species and physiological state of the organism, temperature, pH, salinity, metal concentration in water and food, exposure duration, presence of chelators and other competing ions¹⁶.

Of greatest ecological and medical concern is biomagnification – the increase in heavy metal concentrations with each successive trophic level. Studies show that bioaccumulation factors (ratio of metal concentration in organism tissues to its concentration in water) for different metals and species range from tens to hundreds of thousands¹⁷. In particular, for cadmium, the bioaccumulation factor in algae can reach 10^4 – 10^5 , in mollusks – 10^3 – 10^4 , in fish – 10^2 – 10^3 . This

¹⁴ Войціцький В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування*. 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007

¹⁵ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng*. 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529

¹⁶ Черниченко І. О., Литвиченко О. М., Бабій В. Ф., Кондратенко О. Є., Главачек Д. О. До питання фонового рівня забруднення ґрунтів важкими металами для визначення впливу воєнних дій. *Довкілля та здоров'я*. 2025. № 1 (114). P. 18–23. DOI: 10.32402/dovkil2025.01.018

¹⁷ Хижняк С. В., Войціцький В. М., Данчук В. М., Мідик С. В., Лапоша О. А., Ушкалов В. О. Шляхи міграції стійких пестицидів трофічними ланцюгами наземних і водних екосистем. *Біоресурси і природокористування*. 2018. Т. 10. № № 1–2. С. 36–43.

means that even at relatively low metal concentrations in water, their content in fish tissues (especially predatory species at the top of the trophic pyramid) can reach toxic levels, posing a direct threat to the health of people consuming this fish¹⁸.

In Ukraine, the problem of heavy metal bioaccumulation in aquatic ecosystems is particularly acute. Studies conducted in the basins of the Dnipro, Dniester, Southern Bug, and Siverskyi Donets rivers indicate significant accumulation of Pb, Cd, Cu, Zn, Cr, and Ni in the tissues of indigenous fish species (carp, perch, pike, zander, bream)¹⁹. It has been established that the content of lead in fish muscles from contaminated water bodies of the Mykolaiv region is 3–5 times higher, and cadmium – 2–8 times higher than permissible standards for food products. Consumption of such fish by the population leads to chronic intake of heavy metals into the body and their accumulation in critical organs (bones, kidneys, liver, hair, nails), which is confirmed by biomonitoring data²⁰.

1.4. Medical-ecological studies of heavy metal bioaccumulation in the population

Results of medical-ecological studies conducted in Ukraine and abroad convincingly demonstrate a clear correlation between the level of heavy metal bioaccumulation in the population and increased incidence of chronic diseases. Studies of populations living in areas of technogenic pollution reveal a significant increase in the content of Pb, Cd, Cu, Zn, Cr, and Ni in hair, blood, urine, and nails of residents compared to controls²¹. In Ukraine, the most indicative are studies conducted in the industrial regions of Donbas, Prydniprov'ia, and Pryazovia, where the content of heavy metals in biological substrates of the population is 2–10 times higher than background levels²².

Of particular interest are studies demonstrating a link between chronic heavy metal intake and tissue regeneration disorders. Thus, in patients with chronic

¹⁸ Жукова О. Г., Старжинський П. С., Прокопенко І. О. Забруднення водних екосистем важкими металами внаслідок військових дій та їх вплив на здоров'я людини. *Екологічна безпека держави 2025* : матеріали Науково-практичної конференції. Київ, 2025. С. 61–63. DOI: 10.18372/2786-8168.19.19971

¹⁹ Мосієнко Н. М. Вплив важких металів на екологічно безпечне споживання продукції рибництва і аквакультури. *Grail of Science*. 2022. № 17. Р. 204–206. DOI: 10.36074/grail-of-science.22.07.2022.036

²⁰ Jomova K., Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology*. 2023. № 498. Р. 153645. DOI: 10.1016/j.tox.2023.153645

²¹ Войціцький В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування*. 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007

²² Andrusyshyna I. M., Dmytrukha N. M. Biomonitoring of heavy metals in the population of Ukraine: consequences of the impact of the Chernobyl Accident and current challenges. *Ukrainian Journal of Medical Science*. 2026. Vol. 32 (1). Р. 105–119. DOI: 10.37621/ujms.32.1.2026.07

trophic ulcers of the lower extremities living in industrial areas, concentrations of Pb and Cd in blood and hair are significantly higher than in patients from favorable regions, and these indicators negatively correlate with the rate of ulcer healing²³. Similar data were obtained in the study of postoperative wounds: in patients with high body content of heavy metals, delayed epithelialization, increased frequency of suppuration, and formation of coarse scars are observed. Furthermore, chronic heavy metal intoxication is associated with an increased risk of cardiovascular diseases (ischemic heart disease, arterial hypertension), nephropathies, osteoporosis, neurodegenerative disorders (Alzheimer's disease, Parkinson's disease), which additionally complicates the wound process and hinders regeneration²⁴.

2. Molecular and cellular mechanisms of heavy metal effects on tissue regeneration processes

2.1. Induction of oxidative stress and its role in regeneration impairment

The primary and most universal mechanism of heavy metal toxicity in cells is the induction of oxidative stress, which consists of excessive generation of reactive oxygen species (ROS) and disruption of the prooxidant-antioxidant balance.

Heavy metals, especially variable-valence metals (iron, copper, chromium), can catalyze Fenton and Haber-Weiss reactions, resulting in the formation of highly reactive hydroxyl radicals (OH) that damage lipids, proteins, and DNA²⁵. In addition, metals (especially Pb and Cd) disrupt the functioning of the mitochondrial electron transport chain, leading to increased production of superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2)²⁶.

Chronic elevation of ROS levels in regenerating tissues causes a number of negative consequences. First, oxidative modification of lipid cell membranes disrupts their permeability and receptor function, which is particularly critical for actively proliferating cells (fibroblasts, keratinocytes, endothelial cells). Second, oxidative damage to proteins leads to their denaturation and aggregation, disrupting the function of enzymes, transport proteins, and signaling molecules. Third, DNA damage (formation of 8-oxoguanine, single- and double-strand breaks, crosslinks) activates repair systems, but under chronic oxidative stress,

²³ Черниченко І. О., Литвиченко О. М., Бабій В. Ф., Кондратенко О. Є., Главачек Д. О. До питання фонового рівня забруднення ґрунтів важкими металами для визначення впливу воєнних дій. Довкілля та здоров'я. 2025. № 1 (114). P. 18–23. DOI: 10.32402/dovkil2025.01.018

²⁴ Cosselman K. E., Navas-Acien A., Kaufman J. D. Environmental factors in cardiovascular disease. *Nat Rev Cardiol.* 2023. № 20 (7). P. 471–488.

²⁵ Stockwell B. R. Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell.* 2022. № 185 (14). P. 2401–2421.

²⁶ Jomova K., Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology.* 2023. № 498. P. 153645. DOI: 10.1016/j.tox.2023.153645

repair becomes inefficient, leading to accumulation of mutations and cellular senescence²⁷.

Particularly sensitive to oxidative stress caused by heavy metals are stem and progenitor cells, which play a key role in tissue regeneration. Studies show that mesenchymal stromal cells (MSCs), endothelial progenitor cells, and muscle satellite cells demonstrate high sensitivity to oxidative stress, manifested by decreased proliferative activity, impaired differentiation, activation of apoptosis, and premature senescence²⁸.

Since these cells provide continuous tissue renewal and reparative responses, their dysfunction under heavy metal exposure leads to depletion of tissue regenerative potential and chronicity of the pathological process.

2.2. DNA damage, epigenetic changes, and disruption of signaling pathways

Heavy metals cause direct and indirect (via ROS) DNA damage, activating the DNA damage response (DDR) signaling cascade, which includes phosphorylation of ATM (ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related) kinases, activation of effector Chk1/Chk2 kinases, and cell cycle arrest at G1/S and G2/M phases. When repair fails, proapoptotic signaling pathways (p53, caspase cascade) are activated, leading to elimination of damaged cells. In the context of tissue regeneration, excessive activation of apoptosis in proliferating cell pools leads to a deficiency of cellular material necessary for filling the wound defect and tissue remodeling.

In addition to DNA damage, heavy metals can induce epigenetic changes, including DNA hypo- and hypermethylation, histone modifications (acetylation, methylation, phosphorylation), and changes in microRNA expression¹. These epigenetic changes can lead to persistent alteration of gene expression regulating proliferation, differentiation, angiogenesis, and extracellular matrix synthesis, even after cessation of metal exposure. In particular, it has been established that chronic lead and cadmium exposure is accompanied by hypermethylation of promoters of tumor suppressor genes (p53, p16, p21), leading to their transcriptional repression and creating conditions for uncontrolled proliferation of fibroblasts and endothelial cells, which may contribute to fibrogenesis and disruption of normal tissue remodeling²⁹.

²⁷ Repair and regeneration: ferroptosis in the process of remodeling and fibrosis in impaired organs. *Cell Death Discov.* 2024. № 10. P. 424. DOI: 10.1038/s41420-024-02181-2

²⁸ Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

²⁹ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529

An important mechanism of regeneration impairment is the effect of heavy metals on key signaling pathways that regulate cell proliferation, differentiation, and migration. Studies show that metals can inhibit the Wnt/ β -catenin signaling pathway,

which plays a critical role in maintaining stemness and activating proliferation upon tissue damage³⁰. In addition, the TGF- β /Smad signaling pathway, which regulates fibroblast differentiation into myofibroblasts, collagen synthesis, and extracellular matrix production, is disrupted. The effect of metals on the PI3K/Akt/mTOR signaling pathway leads to disruption of cellular metabolism, reduced protein synthesis, and inhibition of angiogenesis. Disruption of the MAPK/ERK signaling pathway, which regulates keratinocyte proliferation and differentiation, slows epithelialization of the wound defect.

2.3. Mitochondrial dysfunction, apoptosis, and ferroptosis

Mitochondria are one of the primary targets of heavy metal toxicity. Metals accumulate in mitochondria, disrupting the functioning of the electron transport chain, inhibiting Krebs cycle enzymes and oxidative phosphorylation, and causing opening of the mitochondrial permeability transition pore (mPTP). This leads to uncoupling of oxidative phosphorylation, sharp reduction of ATP synthesis, increased ROS production, and release of proapoptotic factors (cytochrome c, AIF, Smac/DIABLO) from the intermembrane space into the cytosol³¹.

Mitochondrial dysfunction plays a key role in tissue regeneration impairment for several reasons. First, energy deficiency inhibits macromolecule synthesis (proteins, nucleic acids, lipids) necessary for cell proliferation and migration, as well as for extracellular matrix synthesis³². Second, mitochondrial ROS initiate chain reactions of oxidative damage spreading to other cellular compartments. Third, release of proapoptotic factors activates the caspase cascade, leading to massive apoptosis of cells in the wound bed, disrupting the normal course of inflammatory and proliferative phases of healing.

In recent years, particular attention has been drawn to the phenomenon of ferroptosis – an iron-dependent regulated form of cell death characterized by

³⁰ Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

³¹ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529

³² Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

accumulation of lipid peroxides, iron overload of cells, and oxidative membrane damage. Studies show that heavy metals, especially iron and copper, can actively induce ferroptosis through ROS generation, inhibition of the glutathione peroxidase 4 (GPX4) system, and accumulation of toxic lipid hydroperoxides. In the context of regeneration, ferroptosis can play a dual role. On one hand, it is necessary for elimination of damaged cells in the wound and initiation of the inflammatory response, which is critical for the onset of regeneration. On the other hand, excessive activation of ferroptosis in proliferating cells, endothelial cells, and fibroblasts leads to loss of cellular material, impaired angiogenesis, hypoxia, and chronic inflammation. Furthermore, ferroptosis is associated with fibroblast activation and excessive collagen deposition, contributing to fibrogenesis and disruption of normal tissue remodeling^{33, 34}.

2.4. Effect of heavy metals on key cellular processes of regeneration

The effects of heavy metals on key cellular processes of reparative regeneration have been studied in models of cell cultures (fibroblasts, keratinocytes, endothelial cells, MSCs) and experimental animals (rats, mice, guinea pigs)³⁵. Summarized data from these studies are presented in Table 2.

As seen from Table 2, heavy metals cause multifactorial disruption of almost all key processes that ensure normal wound healing. Particularly critical is the inhibition of angiogenesis and fibroblast proliferation, as this leads to hypoxia, nutrient deficiency, delayed clearance of the wound from necrotic masses, and formation of weak, immature scar tissue. At the same time, activation of fibrogenesis under metal influence can create a paradoxical situation where, despite excessive collagen deposition, the mechanical properties of the scar remain low due to its immaturity and disorganization of collagen fibers^{36, 37}.

Experimental studies in animals confirm that chronic heavy metal intake leads to significant slowing of healing of various types of wounds (mechanical, thermal, surgical), reduced mechanical strength of the scar, increased frequency of suppuration, and formation of coarse keloid scars. In animals with experimental diabetes mellitus additionally exposed to heavy metals,

³³ Andrusyshyna I. M., Dmytrukha N. M. Biomonitoring of heavy metals in the population of Ukraine: consequences of the impact of the Chernobyl Accident and current challenges. *Ukrainian Journal of Medical Science*. 2026. Vol. 32 (1). P. 105–119. DOI: 10.37621/ujms.32.1.2026.07

³⁴ Stockwell B. R. Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell*. 2022. № 185 (14). P. 2401–2421.

³⁵ Cosselman K. E., Navas-Acien A., Kaufman J. D. Environmental factors in cardiovascular disease. *Nat Rev Cardiol*. 2023. № 20 (7). P. 471–488.

³⁶ Cosselman K. E., Navas-Acien A., Kaufman J. D. Environmental factors in cardiovascular disease. *Nat Rev Cardiol*. 2023. № 20 (7). P. 471–488.

³⁷ Stockwell B. R. Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell*. 2022. № 185 (14). P. 2401–2421.

regeneration disorders were even more pronounced, indicating a synergistic negative effect of metals and metabolic disorders³⁸.

Table 2

Effects of heavy metals on key processes of tissue regeneration

Regeneration process	Effect of heavy metals	Mechanisms	Consequences
Fibroblast proliferation	Inhibition (up to 40–70% depending on concentration)	Oxidative stress, DNA damage, inhibition of signaling pathways (PI3K/Akt, MAPK/ERK)	Deficiency of extracellular matrix synthesis
Collagen synthesis	Reduction (up to 50–80%)	Oxidative modification of enzymes (prolyl hydroxylase, lysyl hydroxylase), disruption of collagen gene expression	Reduced mechanical strength of scar
Angiogenesis	Inhibition (up to 60–90%)	Oxidative stress, ferroptosis of endothelial cells, inhibition of VEGF/VEGFR signaling, reduced MMP-2, MMP-9 activity	Hypoxia, nutrient deficiency in wound
Keratinocyte migration	Slowing (up to 50–70%)	Disruption of cytoskeleton reorganization, decreased integrin and cadherin expression	Delayed wound epithelialization
MSC differentiation	Disruption	Altered expression of transcription factors (Runx2, PPAR γ , Sox9)	Deficiency of tissue regenerative potential
Fibrogenesis	Enhancement	TGF- β /Smad activation, excessive collagen deposition, activation of fibroblast ferroptosis	Formation of hypertrophic and keloid scars

Summarized from data³⁹.

3. Clinical consequences of heavy metal bioaccumulation and conceptual research model

3.1. Clinical observations and epidemiological data

Clinical observations in humans convincingly confirm the results of experimental studies, demonstrating a clear correlation between the level of heavy metal bioaccumulation and tissue regeneration impairment.

³⁸ Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

³⁹ Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

Epidemiological studies conducted in different countries (USA, China, Poland, Italy, Ukraine) demonstrate that populations living in areas of technogenic heavy metal contamination have significantly higher rates of chronic trophic ulcers, diabetic foot, slow-healing postoperative wounds, and postoperative complications (suppuration, wound dehiscence, formation of coarse scars).

In Ukraine, the most indicative are studies conducted in the Mykolaiv, Dnipropetrovsk, and Zaporizhzhia regions. Thus, in surgical patients in the city of Mykolaiv with high levels of Pb, Cd, Cu, Zn, Cr, and Ni in hair and blood, significant slowing of postoperative wound healing was observed (average healing time increased by 5–12 days), increased frequency of suppuration (by 2–4 times), and formation of hypertrophic scars (by 3–5 times) compared to the control group. Similar data were obtained in the study of patients with chronic trophic ulcers of the lower extremities: high concentration of heavy metals in the body was an independent risk factor for prolonged non-healing of ulcers, ineffectiveness of conservative therapy, and recurrence⁴⁰.

Clinical studies also confirm the link between heavy metal bioaccumulation and microcirculation disorders, which are important factors in regeneration. In patients with high blood levels of Pb, Cd, and Cr, decreased capillary blood flow, slowed venous outflow, increased blood viscosity, and endothelial dysfunction are observed, manifested by reduced vasodilation, increased endothelin-1 levels, and decreased nitric oxide (NO) levels⁴¹. These microcirculatory disorders create additional obstacles to the delivery of oxygen, nutrients, and immune system cells to the wound area, slowing healing and increasing the risk of infectious complications⁴².

3.2. Research gaps and justification for the need for a comprehensive approach

Despite a significant body of scientific data confirming the negative impact of heavy metals on tissue regeneration, there are substantial gaps that hinder the formation of a holistic picture and the development of effective prevention and treatment strategies. First, most studies focus on individual metals in isolated experimental systems, while in real conditions the body is exposed to a mixture of metals that can interact (synergistically, antagonistically, or additively).

⁴⁰ Хижняк С. В., Войцицький В. М., Данчук В. М., Мідик С. В., Лапоша О. А., Ушкалов В. О. Шляхи міграції стійких пестицидів трофічними ланцюгами наземних і водних екосистем *Біоресурси і природокористування*. 2018. Т. 10. № № 1–2. С. 36–43.

⁴¹ Cosselman K. E., Navas-Acien A., Kaufman J. D. Environmental factors in cardiovascular disease. *Nat Rev Cardiol*. 2023. № 20 (7). P. 471–488.

⁴² Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci*. 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

Second, the vast majority of work has been performed on cell cultures and experimental animals, while extrapolation of these results to humans requires caution due to species differences in metabolism, distribution, and sensitivity to metals⁴³.

Third, practically absent are comprehensive studies that combine ecological monitoring of aquatic ecosystems (metal concentrations in water, bottom sediments, soils) with biomonitoring of the population (metal content in hair, blood, nails, urine), in-depth study of molecular-cellular mechanisms of regeneration impairment (oxidative stress, mitochondrial dysfunction, ferroptosis, disruption of signaling pathways), and clinical observations (wound healing dynamics, complication rates, scar quality). This is particularly true for Ukraine, where despite high levels of environmental heavy metal contamination, systematic medical-ecological studies of the impact of this contamination on tissue regeneration are practically absent⁴⁴.

Fourth, insufficient attention has been paid to the study of populations living in areas with varying anthropogenic loads, particularly in the Mykolaiv region. The absence of such studies does not allow assessment of regional features of heavy metal impact on public health, identification of vulnerable risk groups, or development of targeted prevention and correction measures for regeneration disorders. Furthermore, there is a lack of studies evaluating the effectiveness of various strategies for correcting regeneration disorders (antioxidant therapy, chelation therapy, use of regenerative technologies) in patients chronically exposed to heavy metals⁴⁵.

3.3. Conceptual research model

Based on the generalization of analyzed scientific data and identified gaps, we have formulated a conceptual research model that describes the cascade of events from ecological load to clinical consequences of tissue regeneration impairment. The model includes four sequential levels that are logically interconnected (Fig. 1).

⁴³ Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529

⁴⁴ Хижняк С. В., Войціцький В. М., Данчук В. М., Мідик С. В., Лапоша О. А., Ушкалов В. О. Шляхи міграції стійких пестицидів трофічними ланцюгами наземних і водних екосистем. *Біоресурси і природокористування*. 2018. Т. 10. № № 1–2. С. 36–43.

⁴⁵ Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127

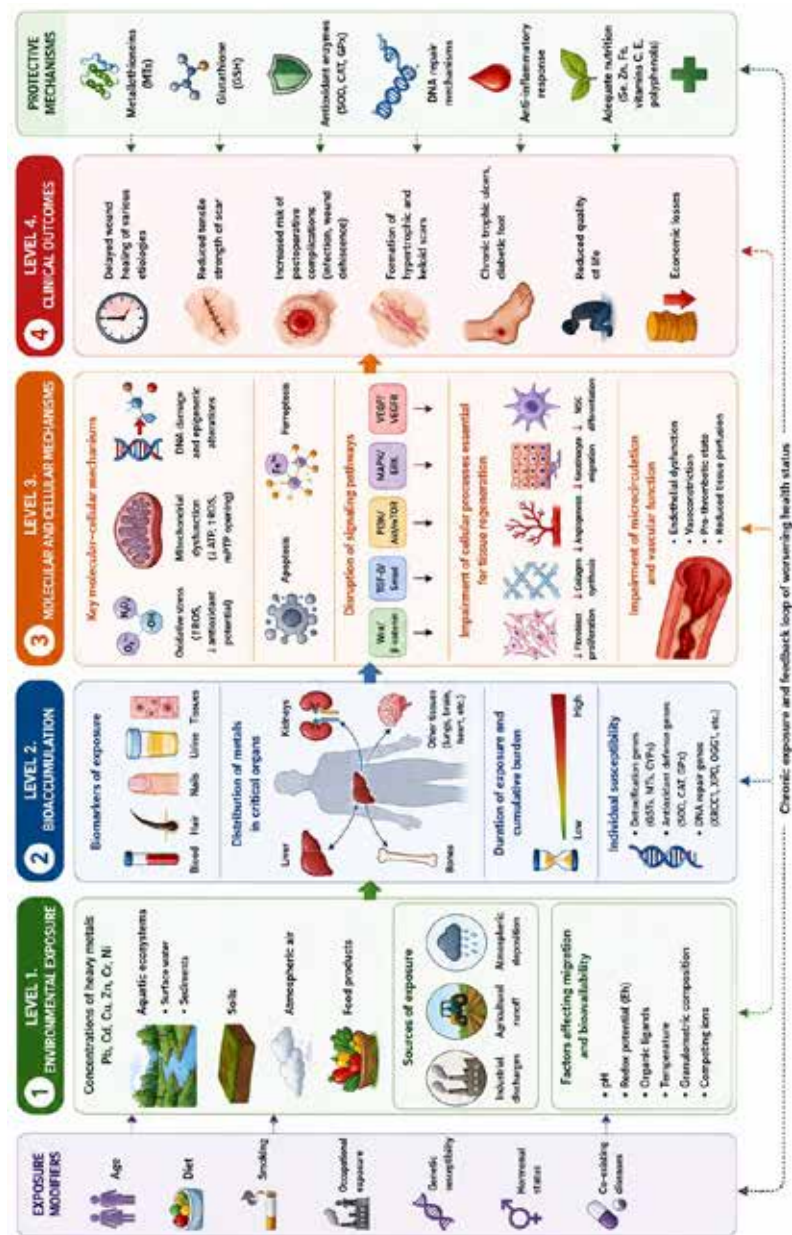


Fig. 1. Conceptual model of research on the effects of heavy metals on tissue regeneration

Level 1: Ecological load:

- concentrations of Pb, Cd, Cu, Zn, Cr, Ni in aquatic ecosystems (surface waters, bottom sediments);
- concentrations of metals in soils, atmospheric air, food products;
- sources of input (industrial discharges, agricultural runoff, atmospheric deposition);
- factors affecting migration and bioavailability (pH, redox potential, organic ligands).



Level 2: Bioaccumulation:

- metal content in hair, blood, nails, urine, tissues of the population;
- distribution of metals between critical organs (bones, kidneys, liver);
- duration of exposure, cumulative load;
- individual sensitivity (polymorphism of detoxification, antioxidant defense, DNA repair genes).



Level 3: Physiological effects at the molecular-cellular level:

- oxidative stress (ROS, decreased antioxidant potential);
- mitochondrial dysfunction (reduced ATP, ↑ROS, mPTP opening);
- DNA damage and epigenetic changes;
- apoptosis and ferroptosis of cells;
- disruption of signaling pathways (Wnt/β-catenin, TGF-β/Smad, PI3K/Akt/mTOR, MAPK/ERK, VEGF/VEGFR);
- inhibition of fibroblast proliferation, reduced collagen synthesis;
- impaired angiogenesis, keratinocyte migration, MSC differentiation;
- microcirculation disorders, endothelial dysfunction.



Level 4: Clinical consequences:

- delayed wound healing of various etiologies;
- reduced mechanical strength of the scar;
- increased frequency of postoperative complications (suppuration, wound dehiscence);
- formation of hypertrophic and keloid scars;
- chronicity of trophic ulcers, diabetic foot;
- reduced quality of life, economic losses.

The proposed model allows: a) logical linking of different research levels (ecological, biomonitoring, molecular-cellular, clinical); b) identification of critical links requiring priority investigation; c) justification of marker selection for each level; d) planning of empirical studies aimed at assessing the impact of specific environmental factors on tissue regeneration in specific

regions (particularly in the Mykolaiv region); e) development and evaluation of the effectiveness of strategies for prevention and correction of regeneration disorders caused by heavy metals.

The model has particular value for planning studies in the Mykolaiv region, where high levels of heavy metal contamination of aquatic ecosystems are observed, yet systematic medical-ecological works integrating all four levels are absent. Implementation of such studies will allow: 1) assessment of the real ecological load on the region's population; 2) determination of levels of metal bioaccumulation in different age and professional groups; 3) identification of molecular-cellular markers of regeneration impairment; 4) establishment of clinical correlates of these disorders; 5) development of scientifically grounded recommendations for the prevention and treatment of regeneration disorders under conditions of technogenic contamination.

CONCLUSIONS

1. The generalization of current scientific data indicates that aquatic ecosystems of Ukraine, particularly in the Mykolaiv region, are subject to significant anthropogenic pressure from heavy metals (Pb, Cd, Cu, Zn, Cr, Ni), the concentrations of which in surface waters and bottom sediments exceed maximum permissible levels many times over. Due to their ability for bioaccumulation and biomagnification, these metals enter the human body through drinking water and food products (fish, seafood), posing a threat of chronic intoxication.

2. The molecular and cellular mechanisms of heavy metal toxicity leading to impairment of reparative tissue regeneration include induction of oxidative stress, DNA damage, mitochondrial dysfunction, activation of apoptosis and ferroptosis, disruption of signaling pathways regulating cell proliferation, differentiation and migration, as well as epigenetic changes. These mechanisms manifest as inhibition of fibroblast proliferation, reduced collagen synthesis, impaired angiogenesis, slowed keratinocyte migration, as confirmed by the results of experimental studies on animals and cell cultures.

3. Clinical observations and epidemiological studies confirm the correlation between the level of heavy metal bioaccumulation in the population and increased incidence of chronic diseases, delayed wound healing, increased frequency of postoperative complications, and formation of coarse scars. Particularly indicative are data from industrial regions of Ukraine, especially the Mykolaiv region, where high levels of environmental heavy metal contamination are associated with unsatisfactory tissue regeneration indicators in surgical patients.

4. Significant research gaps have been identified: the absence of comprehensive studies that combine ecological monitoring of aquatic

ecosystems with biomonitoring of the population, in-depth study of molecular-cellular mechanisms of regeneration impairment, and clinical observations, particularly in the Mykolaiv region. Insufficient attention has been paid to individual sensitivity to metals related to genetic polymorphism, as well as the effectiveness of strategies for correcting regeneration disorders.

5. A conceptual research model has been developed that integrates four levels – ecological load → bioaccumulation → physiological effects (molecular-cellular disorders) → clinical consequences – which can serve as a theoretical basis for planning empirical studies aimed at assessing public health risks associated with environmental heavy metal contamination and developing scientifically grounded recommendations for the prevention and correction of regeneration disorders under conditions of anthropogenic pressure.

SUMMARY

This article systematizes and summarizes current scientific data on the sources of heavy metal (Pb, Cd, Cu, Zn, Cr, Ni) input into aquatic ecosystems, their migration, transformation, and bioaccumulation in trophic chains. A comparative characterization of metal concentrations in various water bodies of Ukraine and the world is presented, and regional features of contamination are identified. The molecular mechanisms of heavy metal toxicity are analyzed, including the induction of oxidative stress, DNA damage, mitochondrial dysfunction, apoptosis, ferroptosis, and disruption of signaling pathways that regulate cell proliferation, differentiation, and migration. Special attention is paid to the effects of metals on reparative tissue regeneration – fibroblast proliferation, collagen synthesis, angiogenesis, keratinocyte migration, and scar tissue formation. A comparative analysis of the results of experimental studies on animals (rats, mice, guinea pigs) and cell cultures, as well as clinical observations in humans, is presented. The results of medical-ecological studies conducted in Ukraine and abroad are summarized, demonstrating a correlation between the level of heavy metal bioaccumulation in the population and an increased incidence of chronic diseases, delayed wound healing, and increased frequency of postoperative complications. Significant research gaps are identified, particularly the absence of comprehensive studies that combine ecological monitoring of aquatic ecosystems with clinical observations of tissue regeneration in people living in areas with varying anthropogenic loads (particularly in the Mykolaiv region). A conceptual research model is formulated that integrates ecological load, bioaccumulation, physiological effects, and clinical consequences, which can serve as a basis for designing empirical studies.

Bibliography

1. Environmental exposure of arsenic impairs the cardiometabolic profile, skeletal muscle health, and neurological function. *Curr Opin Biomed Eng.* 2024. № 30. P. 100529. DOI: 10.1016/j.cobme.2024.100529
2. Войцицький В. М., Хижняк С. В., Данчук В. В., Мідик С. В., Кеппл О. Ю., Ушкалов В. О. Надходження і міграція важких металів наземними та водними екосистемами. *Біоресурси і природокористування.* 2019. Т. 11. № № 1–2. С. 59–68. DOI: 10.31548/bio2019.01.007
3. Хижняк С. В., Войцицький В. М., Данчук В. М., Мідик С. В., Лапоша О. А., Ушкалов В. О. Шляхи міграції стійких пестицидів трофічними ланцюгами наземних і водних екосистем. *Біоресурси і природокористування.* 2018. Т. 10. № № 1–2. С. 36–43.
4. Jomova K., Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology.* 2023. № 498. P. 153645. DOI: 10.1016/j.tox.2023.153645
5. Repair and regeneration: ferroptosis in the process of remodeling and fibrosis in impaired organs. *Cell Death Discov.* 2024. № 10. P. 424. DOI: 10.1038/s41420-024-02181-2
6. Peters K., Staehlke S., Rebl H., et al. Impact of Metal Ions on Cellular Functions: A Focus on Mesenchymal Stem/Stromal Cell Differentiation. *Int J Mol Sci.* 2024. № 25 (18). P. 10127. DOI: 10.3390/ijms251810127
7. Амонс С. Е., Яковець Л. А. Вплив важких металів на основні фізіологічні процеси зернових та зернобобових культур. *Сільське господарство та лісівництво.* 2023. № 30. С. 211–227. DOI: 10.37128/2707-5826-2023-3-16
8. Черниченко І. О., Литвиченко О. М., Бабій В. Ф., Кондратенко О. Є., Главачек Д. О. До питання фонового рівня забруднення ґрунтів важкими металами для визначення впливу воєнних дій. *Довкілля та здоров'я.* 2025. № 1 (114). P. 18–23. DOI: 10.32402/dovkil2025.01.018
9. Жукова О. Г., Старжинський П. С., Прокопенко І. О. Забруднення водних екосистем важкими металами внаслідок військових дій та їх вплив на здоров'я людини. *Екологічна безпека держави 2025 : матеріали Науково-практичної конференції.* Київ, 2025. С. 61–63. DOI: 10.18372/2786-8168.19.19971
10. Мосієнко Н. Вплив важких металів на екологічно безпечне споживання продукції рибництва і аквакультури. *Grail of Science.* 2022. № 17. P. 204–206. DOI: 10.36074/grail-of-science.22.07.2022.036
11. Andrusyshyna I. M., Dmytrukha N. M. Biomonitoring of heavy metals in the population of Ukraine: consequences of the impact of the Chernobyl

Accident and current challenges. *Ukrainian Journal of Medical Science*. 2026. Vol. 32 (1). P. 105–119. DOI: 10.37621/ujms.32.1.2026.07

12. Cosselman K. E., Navas-Acien A., Kaufman J. D. Environmental factors in cardiovascular disease. *Nat Rev Cardiol*. 2023. № 20 (7). P. 471–488.

13. Manceau A., Marcus M. A., Tamura N. Quantitative speciation of heavy metals in soils and sediments. *Environ Sci Technol*. 2022. № 56 (22). P. 15418–15430.

Information about the authors:

Lukonin Oleksandr,

Postgraduate Student at the Department of Ecology
Petro Mohyla Black Sea National University
10, 68 Desantnykyv Street, Mykolaiv, Ukraine

Smyrnov Viktor,

Candidate of Geological Sciences, Associate Professor,
Associate Professor at the Department of Ecology
Petro Mohyla Black Sea National University
10, 68 Desantnykyv Street, Mykolaiv, Ukraine

МЕТОДИЧНІ ЗАСАДИ РИЗИК-ОРІЄНТОВАНОГО ОЦІНЮВАННЯ РЕКРЕАЦІЙНИХ ВОД ТА ЇХ АПРОБАЦІЯ НА ДІЛЯНКАХ МИКОЛАЇВСЬКОЇ ОБЛАСТІ

Магась Наталія

DOI <https://doi.org/10.30525/978-9934-26-714-7-7>

ВСТУП

Рекреаційні водні об'єкти є водночас компонентами природного середовища, територіями відпочинку та потенційними шляхами впливу біологічних і хімічних чинників на здоров'я населення. Їхній стан визначається водністю, температурним режимом, інтенсивністю водообміну, надходженням поверхневого й каналізаційного стоку, процесами перемішування, природною мінералізацією та характером господарського освоєння берегової смуги. Тому оцінювання рекреаційної води не може обмежуватися одиничним порівнянням лабораторного результату з нормативним значенням: воно має враховувати просторово-часову мінливість, тип водного об'єкта, повторюваність небезпечних епізодів і можливість оперативного реагування. Такий підхід відповідає сучасному розумінню якості рекреаційного водного середовища й переходу від суто нормативного контролю до управління ризиками¹.

Рекомендації Всесвітньої організації охорони здоров'я передбачають поєднання лабораторного моніторингу індикаторів фекального забруднення із профілюванням пляжів, санітарним обстеженням водозбору, оцінюванням короточасних епізодів забруднення та своєчасним інформуванням населення. Центральним є принцип, за яким якість води для купання має оцінюватися не лише за окремою пробєю, а за послідовністю спостережень і відомостями про джерела небезпеки².

¹ Хільчевський В. К. Оцінювання якості рекреаційного водного середовища: світові тенденції, рекомендації ВООЗ, директива ЄС щодо води для купання. *Гідрологія, гідрохімія і гідроекологія*. 2021. № 4 (62). С. 6–17. DOI: 10.17721/2306-5680.2021.4.1

² Guidelines on Recreational Water Quality / World Health Organization. Volume 1 : Coastal and Fresh Waters. Geneva : WHO, 2021. URL: <https://www.who.int/publications/i/item/9789240031302>

Європейська система управління водою для купання також ґрунтується на серіях спостережень за *Escherichia coli* та кишковими ентерококами, класифікації ділянок, підготовці профілів води для купання та відкритому інформуванні користувачів. Для України цей підхід є важливим орієнтиром гармонізації моніторингу, особливо для міських пляжів і лиманно-гирлових акваторій, де якість води може швидко змінюватися під впливом погодних і гідродинамічних подій³.

У 2024 році в Україні затверджено окремі гігієнічні нормативи якості води для купання, які посилюють значення *E. coli* та кишкових ентерококів і передбачають оцінювання за довшими рядами спостережень. Для наукового аналізу це означає необхідність відокремлювати оперативний статус конкретної проби від довгострокової класифікації рекреаційної ділянки⁴.

Для Миколаївської області проблема має додаткову специфіку. Дослідження охоплює пляж «Райдуга» на річці Південний Буг у м. Первомайськ, пляж «Стрілка» на річці Інгул та пляж «Чайка» на Бузькому лимані в м. Миколаїв. Досліджувані рекреаційні ділянки належать до різних гідрохімічних типів: річкова ділянка в Первомайську репрезентує прісноводні умови Південного Бугу, тоді як міські ділянки Миколаєва перебувають під впливом гирлово-лиманних процесів та солонуватих вод. За цих умов пряме ранжування ділянок лише за сухим залишком, хлоридами або сульфатами може змішувати природну мінералізацію з антропогенним навантаженням. Сучасні дослідження рекреаційних вод України наголошують на необхідності врахування природного типу водного об'єкта й цільового виду водокористування⁵.

Метою дослідження є обґрунтування методичних засад ризик-орієнтованого оцінювання рекреаційних вод та їх апробація на прикладі ділянок «Райдуга», «Стрілка» та «Чайка» у Миколаївській області. Для досягнення мети систематизовано архівні фізико-хімічні й мікробіологічні матеріали за 2018, 2021 і 2024 роки, опрацьовано офіційні повідомлення за 2025–2026 роки, виконано описове зіставлення ділянок і повторюваності несприятливих епізодів, а також оцінено доказову спроможність наявних даних для характеристики небезпеки та кількісного визначення ризику.

³ Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality. URL: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006L0007>

⁴ Про затвердження Гігієнічних нормативів якості води для купання : наказ Міністерства охорони здоров'я України від 3 грудня 2024 року № 2005. URL: <https://zakon.rada.gov.ua/go/z1958-24>

⁵ Хільчевський В. К., Забокрицька М. Р. Водні об'єкти України: якість води та рекреація. *Конструктивна географія та раціональне використання природних ресурсів*. 2024. Спеціальний випуск. С. 38–43. DOI: 10.17721/2786-4561.2024.4.special-5/19

1. Теоретико-методичні засади та організація дослідження

1.1. Рекреаційні води як об'єкт еколого-гігієнічного оцінювання

Рекреаційне водокористування охоплює купання, плавання, перебування на мілководді, веслування, серфінг, аматорське рибальство та відпочинок поблизу води. Ці види діяльності суттєво різняться за тривалістю та характером контакту з водним середовищем. Найвищу потенційну експозицію формують сценарії із зануренням голови та випадковим проковтуванням води; меншу – короткочасний контакт шкіри й слизових оболонок; найнижчу – перебування на березі без прямого контакту. Відповідно, однакова концентрація забруднювача не означає однаковий ризик для всіх категорій рекреантів.

Основні небезпеки рекреаційного водного середовища доцільно поділяти на мікробіологічні, хімічні, органолептичні, фізичні й інфраструктурні. Мікробіологічна група пов'язана передусім із фекальним забрудненням та можливим надходженням патогенних мікроорганізмів. Хімічні небезпеки охоплюють речовини, здатні спричиняти гострі або хронічні ефекти за відповідного шляху й дози надходження. Органолептичні показники характеризують умови рекреації та можуть сигналізувати про небажані процеси, але не завжди безпосередньо відображають ризик для здоров'я. Фізичні й інфраструктурні небезпеки включають течії, стан дна, уламки, аварійні споруди, відсутність санітарної інфраструктури й адміністративні обмеження.

Експозиція та сприйнятливість населення є неоднорідними. Для дітей характерні триваліше перебування на мілководді, частіший контакт рук із ротом і більша ймовірність випадкового проковтування води. Підвищеної обережності потребують також люди похилого віку, вагітні, особи із хронічними захворюваннями, ушкодженнями шкіри або ослабленою імунною системою. Належність до вразливої групи не змінює лабораторного результату, проте має враховуватися під час формування рекомендацій щодо безпечності купання.

Окремого контролю потребує ціанобактеріальне «цвітіння». Відповідність води за *E. coli* та кишковими ентерококами не усуває небезпеки ціанотоксинів. Видимі скупчення, поверхнева плівка, різка зміна кольору або неприємний запах мають розглядатися як підстава для цільового обстеження і тимчасового попереджувального інформування населення до отримання повного комплексу лабораторних результатів.

Фекальне забруднення може надходити з неочищеними або неналежно очищеними господарсько-побутовими стічними водами, унаслідок аварій каналізаційних мереж, змиву з урбанізованих територій після опадів, діяльності тваринницьких об'єктів, присутності птахів і тварин

і безпосереднього навантаження від відвідувачів. Потенційними джерелами хімічного навантаження є промислові й комунальні випуски, зливовий стік, сільськогосподарський дренаж, судноплавство, аварійні розливи та забруднені донні відклади. Водночас природні процеси – водообмін із лиманом, згінно-нагінні явища, випаровування, розмивання берегів і скаламучування донних відкладів – також можуть істотно змінювати мінералізацію, прозорість і вміст завислих речовин.

1.2. Нормативна основа та принципи ризик-орієнтованого оцінювання

Повна характеристика рекреаційної водойми потребує поєднання гігієнічного, екологічного та ризик-орієнтованого підходів. Гігієнічне оцінювання визначає безпечність контакту людини з водою; екологічне характеризує стан водної екосистеми та відхилення від природних умов; ризик-орієнтований підхід установлює, які небезпеки, джерела та сценарії потребують першочергового контролю. Екологічний клас масиву поверхневих вод не можна визначати лише за кількома фізико-хімічними показниками, а відповідність окремої мікробіологічної проби не означає доброго екологічного стану всієї водойми. Біоіндикація може інтегрувати довготривалі зміни, однак не замінює оперативних санітарно-мікробіологічних вимірювань.

E. coli та кишкові ентерококи є індикаторами фекального забруднення, а не прямими вимірюваннями всіх можливих патогенів. Підвищена чисельність індикаторів означає зростання імовірності присутності патогенних мікроорганізмів, але без патогеноспецифічних даних, експозиційної моделі та залежності доза – відповідь не дає змоги визначити точну інфекційну дозу або індивідуальну ймовірність захворювання.

Чинні Гігієнічні нормативи якості води для купання передбачають класифікацію за *E. coli* та кишковими ентерококами з урахуванням типу води. Довгострокове оцінювання здійснюють після завершення купального сезону за даними поточного та трьох попередніх сезонів. Набір має містити щонайменше 16 проб, упродовж кожного сезону – не менше чотирьох проб, включно із пробю перед його початком, а інтервал між відборами не має перевищувати одного місяця. Для класифікації використовують 90-й і 95-й процентилі логарифмованих мікробіологічних даних, за якими воду відносять до поганої, достатньої, доброї або відмінної якості. Тому оперативний статус однієї проби та довгостроковий клас рекреаційної ділянки є різними результатами оцінювання.

Архівні повідомлення за окремі дати 2018, 2021 та 2024 років переважно містять дані про лактозопозитивні кишкові палички (далі – ЛКП),

коліфаги та нерівномірні серії відбору, а не повний чотирисезонний ряд абсолютних концентрацій *E. coli* та кишкових ентерококів. ЛКП є історичним санітарно-мікробіологічним індикатором; наведені для них числові значення не тотожні концентраціям *E. coli* за чинними гігієнічними нормативами. Ці матеріали придатні для виявлення епізодів небезпеки, просторово-часового зіставлення і формування пріоритетів моніторингу, але не для ретроспективного присвоєння сучасного формального класу якості води для купання.

Нормативне порівняння залишається необхідною складовою частиною оцінювання, проте воно має застосовуватися з урахуванням призначення конкретного нормативу. Гігієнічні нормативи для господарсько-побутових потреб не завжди можуть автоматично переноситися на природні солонуваті води. Зокрема, підвищені значення сухого залишку, хлоридів і сульфатів у лиманно-гирловій системі можуть бути природними гідрохімічними характеристиками. Водночас вони є важливими для типізації води, просторового порівняння та виявлення різких відхилень від фонового стану. Порівняльні критерії для водних об'єктів інших видів водокористування застосовано з урахуванням вимог чинних гігієнічних нормативів⁶.

Ризик-орієнтоване оцінювання повинно розрізняти лабораторний факт, характеристику небезпеки, оцінку експозиції та кількісну характеристику ризику. Лабораторний факт фіксує вимірювану концентрацію або невідповідність. Характеристика небезпеки визначає санітарне чи екологічне значення результату. Кількісний ризик потребує моделі, яка містить обсяг контакту, частоту експозиції, дозо-реакційну залежність і невизначеність. За відсутності цих складників точні відсотки ймовірності інфікування не є науково обґрунтованими. Українські дослідження нормативного оцінювання рекреаційних вод також підкреслюють необхідність розмежування різних систем нормування та цілей оцінювання⁷.

1.3. Район дослідження, матеріали та методи

Дослідження охоплює три рекреаційні ділянки Миколаївської області. Пляж «Райдуга» розташований у межах міста Первомайськ на річці Південний Буг і характеризує річкову рекреаційну систему. Пляж «Стрілка»

⁶ Про затвердження Гігієнічних нормативів якості води водних об'єктів для задоволення питних, господарсько-побутових та інших потреб населення : наказ Міністерства охорони здоров'я України від 2 травня 2022 р. № 721. URL: <https://zakon.rada.gov.ua/go/z0524-22>

⁷ Хільчевський В. К., Забокрицька М. Р. Особливості нормативного оцінювання якості води водних об'єктів для рекреаційних цілей в Україні. *Гідрологія, гідрохімія і гідроекологія*. 2022. № 1 (63). С. 40–53. DOI: 10.17721/2306-5680.2022.1.4

у Миколаєві розташований на річці Інгул у зоні гирлового впливу. пляж «Чайка» належить до акваторії Бузького лиману й відображає умови солонуватих лиманних вод. Поєднання цих об'єктів дає змогу зіставити прісноводний і лиманно-гирловий гідрохімічні типи та визначити, які відмінності є системними, а які можуть відображати локальні епізоди навантаження.

Просторове розташування досліджуваних рекреаційних ділянок наведено на рисунку 1.

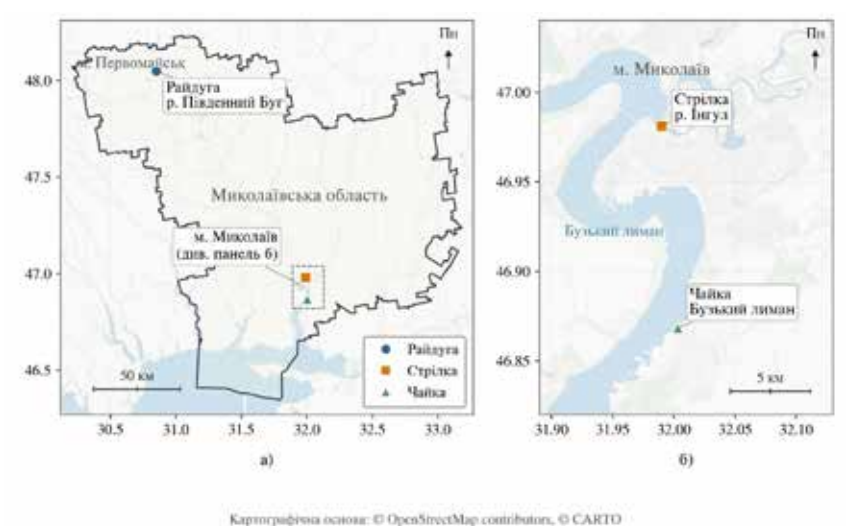


Рис. 1. Розташування досліджуваних рекреаційних ділянок «Райдуга», «Стрілка» та «Чайка» у Миколаївській області: а – територія області з позначенням пунктів спостереження; б – збільшений фрагмент території в межах міста Миколаєва

Розподіл фізико-хімічних проб за ділянками та роками узагальнено в таблиці 1.

Таблиця 1

Розподіл фізико-хімічних проб за ділянками й роками

Рекреаційна ділянка	Водний об'єкт	2018 р.	2021 р.	2024 р.	Разом
Пляж «Райдуга»	р. Південний Буг, м. Первомайськ	12	12	10	34
Пляж «Стрілка»	р. Інгул, м. Миколаїв	4	4	4	12
Пляж «Чайка»	Бузький лиман, м. Миколаїв	4	4	4	12
Разом	–	20	20	18	58

Дослідження має ретроспективний спостережний дизайн і охоплює всі доступні записи, що відповідали трьом обраним ділянкам і визначеним періодам; апіорний розрахунок обсягу вибірки не проводили. Під час формування аналітичного масиву уніфіковано назви ділянок, дати, десяткові роздільники й одиниці вимірювання, перевірено повтори записів і логічну відповідність діапазонів. Спостереження не вилучали лише через велике або мале значення і не замінювали статистично оціненими величинами. Первинні лабораторні протоколи не для всіх років містили відомості про прилади, межі виявлення, невизначеність і внутрішній контроль якості, тому результати розглянуто як вторинні моніторингові дані, а міжрічні порівняння інтерпретовано з відповідним застереженням.

У пробах води визначали температуру, рН, прозорість, каламутність, запах, кольоровість, завислі речовини, амоній, нітрити, нітрати, розчинений кисень, хімічне споживання кисню (далі – ХСК), біохімічне споживання кисню за п'ять діб (далі – БСК₅), сухий залишок, сульфати, хлориди, фосфати, залізо та нафтопродукти. Для описового узагальнення за кожною ділянкою обчислено медіанні, мінімальні та максимальні значення основних показників. Перевищення ХСК визначали щодо порога 30 мг О₂/дм³. Нижчий поріг 15 мг О₂/дм³, застосований у частині наданих раніше розрахунків, не використовували, оскільки він не відповідав прийнятому в цьому дослідженні критерію та штучно збільшував розраховану кратність перевищення.

Завислі речовини аналізували за фактичними концентраціями без розрахунку кратності перевищення: значення 0,75 мг/дм³ характеризує допустимий приріст щодо природного фонового рівня, а не універсальну абсолютну межу. За відсутності синхронної фонові проби визначення кратності було б методично некоректним. Сухий залишок, сульфати та хлориди інтерпретували з урахуванням природної мінералізації та водообміну лиманно-гирлової системи. Загальні засади такої інтерпретації відповідають підходам до рекреаційного оцінювання водних об'єктів України⁸.

Мікробіологічний блок включав архівні повідомлення про ЛКП, коліфаги та загальний статус відповідності проб за окремі дати 2018, 2021 і 2024 років. Для 2025–2026 років створено матрицю датованих статусів «відповідає / не відповідає» за офіційними повідомленнями. Одиницею статусного аналізу було одне повідомлення для конкретної ділянки на конкретну дату; статус переносили з офіційного джерела без

⁸ Хільчевський В. К., Гребін В. В. Водні об'єкти України та рекреаційне оцінювання якості води : навчальний посібник. Київ : ДІА, 2022. 240 с.

ретроспективної перекласифікації. Повідомлення, у яких наведено лише кратність перевищення без абсолютної концентрації та використаного нормативу, аналізували описово. Статусні дані не об'єднували з кількісним рядом попередніх років, оскільки більшість новіших повідомлень не містила абсолютних концентрацій.

1.4. Аналітичний підхід та обмеження оцінювання ризику

Фізико-хімічний масив використано для описового зіставлення трьох ділянок за медіанами та діапазонами основних показників. Окрему увагу приділено рН, розчиненому кисню, ХСК, сухому залишку, хлоридам і завислим речовинам. Значення ХСК порівнювали з порогом 30 мг О₂/дм³. Порогове значення використовували лише для скринінгової інтерпретації окремих спостережень, а не для кількісного визначення імовірності ефекту для здоров'я.

Для завершеного статусного ряду 2025 року обчислювали частки повідомлень із невідповідністю та 95% довірчі інтервали Вілсона; попарні відмінності між ділянками перевіряли двобічним точним критерієм Фішера з поправкою Холма. Для статистичних перевірок приймали критичний рівень значущості $\alpha = 0,05$. Розрахунки виконано у Python 3.12 із використанням SciPy 1.17.0; рисунок побудовано засобами Matplotlib 3.10.8. Відомості 2026 року аналізували лише як оперативні, оскільки купальний сезон не завершений. Архівні кількісні результати за ЛКП не об'єднували зі статусними рядами 2025–2026 років і не переоцінювали за сучасними критеріями для *E. coli* та кишкових ентерококів.

Основними обмеженнями є нерівномірна щільність відбору, мала кількість проб на ділянках «Стрілка» та «Чайка», неповнота відомостей про методики й контроль якості в частині первинних протоколів, а також відсутність абсолютних концентрацій у низці офіційних повідомлень. Тому наведені узагальнення призначені для типізації ділянок, виявлення повторюваних небезпек і визначення пріоритетів моніторингу, але не для формування незмінного рейтингу пляжів.

Наявні методики кількісної мікробіологічної оцінки ризику, зокрема чотириетапний підхід QMRA, рекомендований Усесвітньою організацією охорони здоров'я, у цьому дослідженні не застосовували для розрахунку точного інфекційного ризику. Наявний архівний масив не містить патогеноспецифічних концентрацій, розподілів обсягу випадково проковтнутої води та частоти контактів, характеристик уразливих груп населення і валідованих дозо-реакційних коефіцієнтів. Тому попередні експоненційні розрахунки, в яких концентрації індикаторних ЛКП фактично привірювали до отриманої дози патогену, не відтворювали.

Відсутні значення ентерококів або коліфагів не замінювали нулями; результати обмежили частотою і кратністю перевищень, повторюваністю епізодів, описовим зіставленням фізико-хімічних характеристик і статистичним аналізом завершеного статусного ряду 2025 року⁹.

2. Узагальнена фізико-хімічна характеристика рекреаційних ділянок

2.1. Описова характеристика основних показників

Фізико-хімічний масив охоплює 58 проб: 34 з ділянки «Райдуга» та по 12 із ділянок «Стрілка» і «Чайка». Вода всіх трьох об'єктів переважно мала слабколузну реакцію. У наявних разових вимірюваннях концентрація розчиненого кисню не знижувалася нижче 4 мг О₂/дм³, однак відсутність безперервних добових спостережень не дає змоги виключити короткочасні мінімуми в теплий період. Окремі максимальні значення 16,95–17,59 мг О₂/дм³, зафіксовані за температури води 7–8 °С, можуть відображати денне фотосинтетичне перенасичення; через відсутність відомостей про точний час відбору їх інтерпретовано обережно.

Описове зіставлення засвідчує чітку відмінність прісноводної ділянки «Райдуга» від двох миколаївських акваторій за сухим залишком, хлоридами, ХСК і завислими речовинами. Узагальнені медіани та повні діапазони спостережених значень наведено в таблиці 2.

Таблиця 2

Описові фізико-хімічні характеристики рекреаційних ділянок

Показник	«Райдуга», n = 34	«Стрілка», n = 12	«Чайка», n = 12
pH	8,13 [7,87–8,45]	8,33 [8,20–8,52]	8,39 [7,98–8,52]
Розчинений кисень, мг О ₂ /дм ³	8,55 [4,98–15,38]	9,78 [6,27–16,95]	10,99 [6,05–17,59]
ХСК, мг О ₂ /дм ³	25,96 [16,00–36,00]	36,83 [20,50–65,91]	34,47 [20,39–64,34]
Сухий залишок, мг/дм ³	618 [340–697]	5 474 [720–7 124]	6 020 [1 194–10 067]
Хлориди, мг/дм ³	55,30 [29,70–66,65]	2 383,50 [124,70–3 362,49]	2 879,77 [368,70–4 759,43]
Завислі речовини, мг/дм ³	2,80 [0–22,80]	43,85 [0–182,40]	42,00 [1,60–150,00]

Примітка. Наведено медіану [мінімум – максимум]. Показники характеризують наявний ретроспективний масив і не є довгостроковим нормативним класом води для купання.

Медіанні значення ХСК на «Стрілці» та «Чайці» були вищими, ніж на «Райдузі», що визначає пріоритет контролю органічного й іншого

⁹ Quantitative Microbial Risk Assessment: Application for Water Safety Management / World Health Organization. Geneva: WHO, 2016. 204 p. URL: <https://www.who.int/publications/i/item/9789241565370>

окиснюваного навантаження в лиманно-гирлових акваторіях. Водночас ХСК є інтегральною фізико-хімічною характеристикою і не може безпосередньо ототожнюватися з мікробіологічною небезпекою або індивідуальним ризиком захворювання.

2.2. Інтерпретація гідрохімічних відмінностей

Річкова ділянка «Райдуга» характеризувалася значно нижчими значеннями сухого залишку й хлоридів, тоді як «Стрілка» та «Чайка» відображали вплив лиманно-гирлового водообміну. Тому підвищену мінералізацію миколаївських акваторій не можна автоматично трактувати як техногенне забруднення без даних про солоність, електропровідність, водообмін і природний фон.

Підвищені й мінливі концентрації завислих речовин можуть бути пов'язані з вітровим хвилюванням, скаламучуванням донних відкладів, течією, рівнем води та локальним поверхневим стоком. Значення 0,75 мг/дм³ характеризує допустимий приріст щодо фоновому рівня, тому без синхронної фонові проби розрахунок кратності перевищення є методично некоректним.

Одержані відмінності обґрунтовують диференційовану програму спостережень: для «Райдуги» пріоритетними є контроль епізодів органічного й мікробіологічного навантаження, а для «Стрілки» та «Чайки» – додаткові визначення електропровідності, солоності, основних іонів, завислих речовин і гідродинамічних умов.

2.3. Обмеження описової оцінки

Нерівномірність вибірки обмежує пряме міжділянкове та міжрічне порівняння: для «Райдуги» переважали щомісячні спостереження, тоді як для «Стрілки» та «Чайки» наявно по чотири сезонні проби на рік. Окрім того, не всі архівні протоколи містять інформацію про межі виявлення, невизначеність вимірювань і внутрішній лабораторний контроль.

Узагальнені значення варто розглядати як описову апробацію ризик-орієнтованого підходу, а не як підставу для незмінного ранжування рекреаційних ділянок. Виявлені відмінності визначають напрями додаткового контролю, але потребують підтвердження рівномірними синхронними рядами спостережень.

Особливо обережної інтерпретації потребують інтегральні показники. Підвищене ХСК сигналізує про наявність окиснюваних речовин, але не визначає їхній склад і токсикологічне значення; завислі речовини без фонові проби не дають змоги відокремити природне скаламучування від локального антропогенного внеску.

Для подальшої верифікації необхідні одночасні вимірювання фізико-хімічних, мікробіологічних, метеорологічних і гідрологічних характеристик із фіксацією точного часу відбору, опадів, вітру, рівня води та стану берегової інфраструктури.

3. Мікробіологічна безпека, характеристика ризику та пріоритети управління

3.1. Мікробіологічні епізоди 2018, 2021 і 2024 років

Архівні матеріали свідчать про повторювані, але нерівномірні в часі епізоди мікробіологічного забруднення. У 2018 році на ділянці «Райдуга» повідомлялося про значення ЛКП 240 000 та підвищений вміст колифагів. На ділянці «Стрілка» фіксували 24 000 і 70 000 ЛКП, на ділянці «Чайка» – 6 200 і 24 000. У 2021 році одна із серій відповідала нормативам на ділянках «Райдуга» і «Стрілка», однак 3 вересня на ділянці «Райдуга» знову зафіксовано 240 000 і 24 000 ЛКП.

Ключові мікробіологічні результати архівного періоду узагальнено в таблиці 3.

Таблиця 3

Ключові мікробіологічні результати архівного періоду

Дата	Ділянка	Оприлюднений результат	Статус
26 липня 2018 р.	«Райдуга»	ЛКП 240 000; колифаги 250	невідповідність
26 липня 2018 р.	«Стрілка»	ЛКП 24 000 та 70 000	невідповідність
26 липня 2018 р.	«Чайка»	ЛКП 6 200 та 24 000	невідповідність
27 серпня 2021 р.	«Райдуга» / «Стрілка»	відповідали	відповідність
3 вересня 2021 р.	«Райдуга»	ЛКП 240 000 та 24 000	невідповідність
26 липня 2024 р.	«Стрілка»	ЛКП 9 800	невідповідність
26 липня 2024 р.	«Чайка»	ЛКП 24 000 та 20 000	невідповідність
16 серпня 2024 р.	«Стрілка» / «Чайка»	ЛКП 24 000	невідповідність
30 серпня 2024 р.*	«Райдуга»	ЛКП 70 000	невідповідність
30 серпня 2024 р.*	«Стрілка»	ЛКП 20 000	невідповідність
30 серпня 2024 р.*	«Чайка»	ЛКП 24 000	невідповідність

*Примітки. ЛКП – історичний санітарно-мікробіологічний показник; його значення не тотожні концентраціям E. coli за чинними нормативами; * – дані за 30 серпня 2024 р. наведено за абсолютними значеннями офіційного повідомлення; оприлюднені кратності з ними не узгоджуються.*

У 2024 році спостерігалася швидка зміна ситуації. Станом на 26 липня «Райдуга» відповідала нормативам, тоді як «Стрілка» та «Чайка» мали

підвищені значення ЛКП. Станом на 16 серпня «Райдуга» знову відповідала нормативам, а на двох миколаївських пляжах фіксували 24 000 ЛКП. Наприкінці серпня невідповідність охоплювала всі три ділянки. Офіційні матеріали Центру громадського здоров'я підтверджують короточасну мінливість статусів упродовж купального сезону¹⁰.

Для 30 серпня 2024 року офіційне повідомлення Миколаївського обласного центру контролю та профілактики хвороб містить кратності, які не повністю узгоджуються з абсолютними значеннями вихідної таблиці. До отримання первинних протоколів у кількісному аналізі використано абсолютні значення, а розбіжність джерел розглянуто як елемент невизначеності¹¹.

3.2. Оперативний моніторинг у 2025–2026 роках

У 2025 році на всіх трьох ділянках траплялися короточасні епізоди невідповідності. На ділянці «Чайка» вони зафіксовані 27 червня, 18 липня та 1 серпня; 18 липня кишкова паличка перевищувала нормативне значення в 1,3 раза. На ділянці «Стрілка» невідповідність зареєстровано 4 липня. На ділянці «Райдуга» відхилення відзначено 25 липня та 1 серпня. Починаючи щонайменше з 8 серпня, усі три об'єкти в доступних повідомленнях відповідали нормативам. Офіційні підсумки сезону підтверджують необхідність датованого, а не узагальненого опису якості води¹².

Частка повідомлень із невідповідністю у 2025 році становила 18,2% для «Райдуги» (2 з 11), 9,1% для «Стрілки» (1 з 11) і 27,3% для «Чайки» (3 з 11). Попарні точні критерії Фішера з поправкою Холма не підтвердили відмінностей між ділянками (для всіх трьох порівнянь $p_{\text{Holm}} = 1,000$). Через малу кількість спостережень і широкі довірчі інтервали науково коректним є висновок про наявність короточасних епізодів на всіх трьох об'єктах, а не про стійкий рейтинг пляжів за мікробіологічною небезпекою. Частки невідповідних повідомлень та 95% довірчі інтервали наведено на рисунку 2.

¹⁰ Якість води у водоймах і річках у межах пляжів станом на 16.08.2024 / Центр громадського здоров'я МОЗ України. URL: <https://phc.org.ua/news/yakist-vodi-u-vodoymakh-i-richkakh-u-mezhakh-plyazhiv-monitoring-vodnikh-obektiv-5>

¹¹ Миколаївський обласний центр контролю та профілактики хвороб. *Результати моніторингових досліджень якості води на пляжах Миколаївщини*. 30 серпня 2024 року. URL: <https://mk.cdc.gov.ua/news/rezultaty-monitoringovyh-doslidzen-yakosti-vody-na-plyazhah-mykolayivshyny/>

¹² Якість води на пляжах України станом на 29 серпня 2025 року / Центр громадського здоров'я МОЗ України. URL: <https://phc.org.ua/news/yakist-vodi-na-plyazhakh-ukraini-stanom-na-29-serpnya-de-bezpechno-kupatisya>

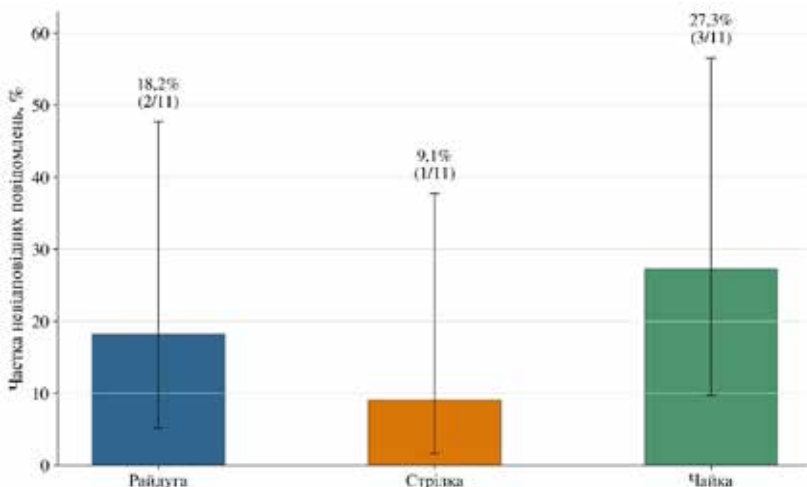


**Рис. 2. Частка офіційних повідомлень із мікробіологічною невідповідністю на рекреаційних ділянках у 2025 році.
Вертикальні лінії – 95% довірчі інтервали Вілсона**

Джерело: побудовано автором за результатами опрацювання офіційних повідомлень і статистичних розрахунків

У доступних повідомленнях за 25 травня, 1 і 29 червня, 9 та 16 липня 2026 року проби з «Райдуги», «Стрілки» та «Чайки» відповідали мікробіологічним нормативам. Отже, для кожної ділянки зафіксовано 0 невідповідностей із 5 наявних серій. Цей результат є позитивним оперативним сигналом, але не доводить покращення порівняно із 2025 роком, оскільки сезон не завершений, вибірка мала, а верхня межа 95% довірчого інтервалу за нульової кількості подій залишається широкою. Стан даних відповідає офіційній інформації на 16 липня 2026 року¹³.

У пробі від 25 травня 2026 року для «Стрілки» було окремо повідомлено про перевищення ХСК у 12 разів. Цей результат не включено до описового фізико-хімічного узагальнення, оскільки джерело не наводить абсолютної концентрації та нормативної межі, використаної для розрахунку кратності. Він має розглядатися як сигнал для запиту первинного протоколу й повторного відбору, а не як остаточне кількісне значення.

¹³ Центр громадського здоров'я МОЗ України. Якість води на пляжах України станом на 16 липня 2026 року. URL: <https://phc.org.ua/news/yakist-vodi-na-plyazhakh-ukraini-stanom-na-16-lipnya-de-bezpechno-kupatisya>

3.3. Характеристика ризику та методичне узагальнення

Наявні матеріали підтримують два рівні висновків: лабораторно підтверджений факт і характеристику небезпеки. До першого належать виміряні концентрації, статуси відповідності та датовані епізоди. До другого – висновки про повторювані випадки фекального забруднення, відмінності гідрохімічного типу й потребу в посиленому контролі ХСК та завислих речовин. Точний індивідуальний ризик інфікування за цими даними розрахувати неможливо. Рекомендовану трирівневу схему оцінювання рекреаційної безпеки подано в таблиці 4.

Таблиця 4

Рекомендована трирівнева схема оцінювання рекреаційної безпеки

Рівень	Зміст	Необхідні дані
Регуляторний	Відповідність конкретної проби та довгострокова класифікація води для купання.	Абсолютні концентрації <i>E. coli</i> й ентерококів, дати, нормативні критерії.
Скринінговий	Профіль небезпек, повторюваність епізодів, пріоритети моніторингу.	Наявний фізико-хімічний і статусний мікробіологічний масив.
Кількісний	Імовірність інфікування або іншого ефекту для заданого сценарію.	Патогеноспецифічні дані, експозиція, доза – відповідь, невизначеність.

Методичним результатом дослідження є узгодження обсягу висновків із доказовою спроможністю вихідних даних. Регуляторна відповідність окремої проби, скринінговий профіль повторюваних небезпек і кількісна оцінка індивідуального ризику розглядаються як різні рівні, для яких потрібні різні масиви спостережень.

Апробація схеми на матеріалах Миколаївської області показала, що архівні фізико-хімічні й статусні мікробіологічні дані придатні для типізації ділянок, виявлення несприятливих епізодів і планування моніторингу. Водночас вони не дають підстав прирівнювати природну солонуватість до техногенного забруднення або подавати невалідізовані розрахунки як точну ймовірність інфікування.

Практичне значення одержаних результатів полягає у визначенні пріоритетів моніторингу. Для «Стрілки» та «Чайки» першочерговими є встановлення джерел органічної речовини, контроль ХСК і БСК₅, визначення електропровідності, солоності та режиму завислих речовин. Для всіх трьох ділянок необхідні регулярні визначення *E. coli* й кишкових ентерококів, оперативний повторний відбір після невідповідності та реєстрація погодних і гідрологічних умов.

4. Обговорення

4.1. Методичні обмеження та перспективи ризик-орієнтованої оцінки

Отримані результати потребують чіткого розмежування понять «небезпека» і «ризик». Виявлення перевищення нормативу, повторюваної мікробіологічної невідповідності або підвищеної частоти несприятливих епізодів засвідчує потенційну епідеміологічну небезпеку рекреаційної ділянки. Водночас ці показники не визначають індивідуальну ймовірність інфікування чи захворювання конкретного відвідувача. Така ймовірність залежить не лише від якості води, а й від тривалості контакту, випадково проковтнутого об'єму, частоти купання, віку, стану здоров'я та властивостей конкретного патогену. Тому частки повідомлень із невідповідністю і довірчі інтервали в цьому дослідженні варто трактувати як показники повторюваності небезпечних ситуацій, а не як частку осіб, які можуть захворіти.

Найсуттєвішим інформаційним обмеженням є неоднорідність мікробіологічних рядів. Архівні повідомлення за окремі дати 2018, 2021 та 2024 років містять переважно ЛКП, коліфаги й окремі абсолютні значення, тоді як матеріали 2025–2026 років часто подають лише інтегральний статус відповідності або кратність перевищення. ЛКП можуть використовуватися для історичного зіставлення та виявлення фекального забруднення, але вони не є еквівалентом концентрації конкретного патогену й не повністю відповідають сучасній системі довгострокової класифікації за *E. coli* та кишковими ентерококами. Відсутність рівномірного чотирирічного ряду не дозволяє ретроспективно присвоювати ділянкам формальні класи якості води для купання.

Експоненційна залежність виду $P = 1 - \exp(-rd)$ є одним із можливих типів дозо-реакційних моделей, однак її застосування є коректним лише тоді, коли d означає фактично отриману дозу визначеного життєздатного патогену, а коефіцієнт r валідований для цього патогену й досліджуваного наслідку. Безпосереднє підставлення кількості індикаторних бактерій у фіксованому об'ємі проби означало б неявне припущення про обсяг проковтування та про те, що кожна індикаторна клітина є інфекційним збудником. Такі припущення наявними матеріалами не підтверджуються. Відповідно до посібника WHO, повна QMRA має охоплювати ідентифікацію небезпеки, оцінку експозиції, дозо-реакційний аналіз, характеристику ризику та явне представлення невизначеності¹⁴.

¹⁴ World Health Organization. Quantitative Microbial Risk Assessment: Application for Water Safety Management... 2016.

Окремого врахування потребують пропущені й неповні дані. Невизначений показник не можна прирівнювати до нульового, оскільки це створює систематичне заниження небезпеки. Так само нуль невідповідностей у п'яти доступних серіях 2026 року не означає нульового ризику: за малої вибірки верхня межа 95% довірчого інтервалу залишається широкою, а купальний сезон не завершений. Із цієї причини статусний ряд 2026 року розглянуто як оперативний сигнал, але не включено до міжрічного ранжування і не використано як доказ стійкого покращення.

Подібне розмежування необхідне для фізико-хімічних показників. Перевищення порогового значення ХСК або іншого показника визначає пріоритет контролю та пошуку джерел навантаження, але саме собою не дорівнює імовірності токсичного ефекту. Для повної оцінки хімічного ризику потрібні концентрації окремих речовин, сценарії випадкового ковтання та дермального контакту, частота й тривалість експозиції, маса тіла, біодоступність і токсикологічні референтні величини. Для лиманно-гирлових вод додатково необхідно відокремлювати природну мінералізацію від антропогенного внеску, щоб не трактувати сухий залишок, хлориди й сульфати як автоматичні докази небезпечного хімічного забруднення.

Описові результати також мають обмежені межі узагальнення. Нерівномірна щільність відбору, мала кількість проб на «Стрілці» та «Чайці», відсутність частини первинних протоколів і широкі інтервали для мікробіологічних часток не дозволяють сформуванню незмінний рейтинг пляжів. Найбільш надійними є висновки про відмінність гідрохімічних типів, повторювані короткочасні епізоди та необхідність подієвого моніторингу.

Запропонована трирівнева система відповідає фактичній доказовій силі даних. Перший рівень – регуляторна оцінка – установлює відповідність конкретної проби або довгостроковий клас за повного нормативного ряду. Другий рівень – скринінговий профіль небезпек – узагальнює частоту та кратність перевищень, повторюваність епізодів, потенційні джерела та гідрометеорологічний контекст. Саме ці два рівні реалізовано в цьому дослідженні. Третій рівень – кількісна оцінка ризику – має вводитися лише після формування патогеноспецифічного й експозиційного масиву та не повинен підмінятися механічним перерахунком індикаторних показників у відсоток захворювання.

Перспективна QMRA для рекреаційних вод Миколаївської області повинна бути сценарною і ймовірнісною. Доцільно передбачити окремі сценарії для дітей і дорослих, звичайного й інтенсивного купання, фонових умов і періодів після опадів або аварійних скидів. Поряд з *E. coli* та кишковими ентерококами необхідні визначення пріоритетних

референтних патогенів або обґрунтовані співвідношення між індикаторами й патогенами. Розподіли концентрацій, обсягу проковтування та частоти контактів варто включати до моделювання Монте-Карло, а результати подавати з інтервалами невизначеності й аналізом чутливості. Такий дизайн дасть змогу перейти від характеристики потенційної небезпеки до науково обґрунтованої оцінки ризику для здоров'я населення.

4.2. Практичні рекомендації щодо ризик-орієнтованого моніторингу

Кожна рекреаційна ділянка повинна мати паспорт із точними координатами створу, описом водозбору, схемою водообміну, переліком потенційних джерел забруднення та історією короткочасних епізодів. Базова програма має включати нормативно визначену кількість проб протягом купального сезону й додатковий відбір після сильних опадів, аварійних скидів, різких змін рівня або температури води. Разом із лабораторними показниками необхідно реєструвати опади, вітер, температуру, рівень води, прозорість і електропровідність.

Мікробіологічний блок варто будувати навколо *E. coli* та кишкових ентерококів як ключових індикаторів води для купання. ЛКП і колифаги можуть зберігатися як додаткові показники для зіставлення з історичним рядом, але не мають замінювати сучасні критерії. У разі невідповідності необхідно повторити відбір у скорочений строк, обстежити можливі джерела фекального забруднення, стан зливової та господарсько-побутової каналізації та погодні умови. Відновлення безпечного статусу має підтверджуватися повторною серією проб.

Для лиманно-гирлових ділянок треба включити електропровідність або солоність та основні іони, щоб відокремлювати природний гідрохімічний фон від антропогенного внеску. Для завислих речовин необхідний синхронний фоновий відбір. Подієвий аналіз доцільно проводити із часовими лагами 1–7 діб між опадами, вітром, рівнем води та зміною ХСК, завислих речовин і мікробіологічного статусу.

Повідомлення для населення має містити дату й час відбору, точну назву ділянки, визначені показники, абсолютні значення і одиниці вимірювання, нормативний критерій, статус відповідності та чітку рекомендацію щодо купання. Використання лише кратності без абсолютної концентрації ускладнює наукову перевірку та порівняння між роками. Лабораторна відповідність води водночас не має автоматично ототожнюватися із загальним дозволом на купання, оскільки управлінське рішення може враховувати воєнні, гідрометеорологічні й інші безпекові обмеження.

4.3. Перспективи подальших досліджень

Найближчим пріоритетом є формування стандартизованого ряду абсолютних концентрацій *E. coli* та кишкових ентерококів із нормативною сезонною частотою відбору. Для лиманно-гірлових акваторій його варто доповнити електропровідністю або солоністю, основними іонами та синхронними фоновими пробами завислих речовин. Просторове розширення мережі доцільно здійснювати за гідрохімічними типами вод: додаткова річкова ділянка Південного Бугу, лиманно-морська ділянка в районі Очакова й одна з ділянок Березанського або Тилігульського лиману можуть бути включені лише за зіставних методик і частоти відбору.

Після накопичення повних датованих рядів доцільно виконати подієвий аналіз із часовими лагами 1–7 діб між опадами, вітром, рівнем води та змінами ХСК, завислих речовин і мікробіологічного статусу. Перехід до QMRA для дітей і дорослих потребуватиме патогеноспецифічних концентрацій, експозиційних розподілів і дозо-реакційних моделей; кілька повних сезонів також створять основу для розроблення та незалежної перевірки моделі раннього попередження.

ВИСНОВКИ

1. Ризик-орієнтоване оцінювання рекреаційних вод має поєднувати нормативну перевірку конкретних проб, характеристику повторюваних небезпек, відомості про джерела забруднення та умови експозиції населення.

2. Описове зіставлення 58 фізико-хімічних проб підтвердило відмінність прісноводної ділянки «Райдуга» від лиманно-гірлових ділянок «Стрілка» та «Чайка». Високу мінералізацію миколаївських акваторій не можна автоматично трактувати як техногенне забруднення без даних про солоність, водообмін і природний фон.

3. Підвищені значення ХСК і завислих речовин на миколаївських ділянках визначають пріоритети додаткового контролю, однак інтегральні фізико-хімічні показники самі собою не характеризують індивідуальний ризик для здоров'я та потребують уточнення джерел і гідродинамічного контексту.

4. Мікробіологічні невідповідності мали короточасний епізодичний характер. У 2025 році їх зафіксовано на всіх трьох об'єктах, а в п'яти доступних серіях 2026 року до 16 липня невідповідностей не зареєстровано; незавершеність сезону не дозволяє робити висновок про довгострокове покращення.

5. Архівні значення ЛКП придатні для виявлення історичних епізодів фекального забруднення, але не є тотожними концентраціям *E. coli* за

чинними нормативами і не забезпечують ретроспективного присвоєння сучасного класу води для купання.

6. Отримані результати характеризують потенційну епідеміологічну небезпеку та повторюваність несприятливих ситуацій, але не індивідуальну ймовірність інфікування або захворювання. Повна QMRA потребує патогеноспецифічних концентрацій, експозиційних параметрів і валідованих дозо-реакційних моделей.

7. Запропонована трирівнева схема розмежовує регуляторну відповідність, скринінговий профіль небезпек і кількісну оцінку ризику та може бути використана для планування моніторингу, повторних відборів і оперативного інформування населення.

АНОТАЦІЯ

Обґрунтовано методичні засади ризик-орієнтованого оцінювання рекреаційних вод і апробовано їх на ділянках «Райдуга», «Стрілка» та «Чайка» у Миколаївській області. Фізико-хімічний масив охоплює 58 проб за 2018, 2021 і 2024 роки, а мікробіологічний блок – архівні результати за окремі дати 2018, 2021 та 2024 років, завершений ряд офіційних повідомлень за 2025 рік і п'ять оперативних серій 2026 року до 16 липня. Описове зіставлення показало відмінність прісноводної річкової та лиманно-гирлових ділянок за мінералізацією, ХСК і завислими речовинами, що потребує врахування природного типу водного об'єкта. Мікробіологічні невідповідності характеризувалися короткочасною внутрішньосезонною мінливістю; відсутність невідповідностей у доступних серіях 2026 року не є достатньою підставою для висновку про довгострокове покращення. Показано, що архівні значення ЛКП і статусні повідомлення придатні для скринінгової характеристики небезпеки, але не забезпечують точного розрахунку індивідуальної ймовірності захворювання. Запропоновано трирівневу схему оцінювання, яка розмежовує регуляторну відповідність, скринінговий профіль небезпек і повну кількісну оцінку ризику за наявності патогеноспецифічних і експозиційних даних. Результати обґрунтовують диференційовані пріоритети моніторингу й оперативного інформування населення.

Література

1. Хільчевський В. К. Оцінювання якості рекреаційного водного середовища: світові тенденції, рекомендації ВООЗ, директива ЄС щодо води для купання. *Гідрологія, гідрохімія і гідроекологія*. 2021. № 4 (62). С. 6–17. DOI: 10.17721/2306-5680.2021.4.1

2. Guidelines on Recreational Water Quality / World Health Organization. Volume 1 : Coastal and Fresh Waters. Geneva : WHO, 2021. URL: <https://www.who.int/publications/i/item/9789240031302> (дата звернення: 20.07.2026).

3. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality. URL: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006L0007> (дата звернення: 20.07.2026).

4. Про затвердження Гігієнічних нормативів якості води для купання : наказ Міністерства охорони здоров'я України від 3 грудня 2024 року № 2005. URL: <https://zakon.rada.gov.ua/go/z1958-24> (дата звернення: 20.07.2026).

5. Хільчевський В. К., Забокрицька М. Р. Водні об'єкти України: якість води та рекреація. *Конструктивна географія та раціональне використання природних ресурсів*. 2024. Спеціальний випуск. С. 38–43. DOI: 10.17721/2786-4561.2024.4.special-5/19

6. Про затвердження Гігієнічних нормативів якості води водних об'єктів для задоволення питних, господарсько-побутових та інших потреб населення : наказ Міністерства охорони здоров'я України від 2 травня 2022 року № 721. URL: <https://zakon.rada.gov.ua/go/z0524-22> (дата звернення: 20.07.2026).

7. Хільчевський В. К., Забокрицька М. Р. Особливості нормативного оцінювання якості води водних об'єктів для рекреаційних цілей в Україні. *Гідрологія, гідрохімія і гідроекологія*. 2022. № 1 (63). С. 40–53. DOI: 10.17721/2306-5680.2022.1.4

8. Хільчевський В. К., Гребінь В. В. Водні об'єкти України та рекреаційне оцінювання якості води : навчальний посібник. Київ : ДІА, 2022. 240 с.

9. World Health Organization. Quantitative Microbial Risk Assessment: Application for Water Safety Management. Geneva : WHO, 2016. 204 p. URL: <https://www.who.int/publications/i/item/9789241565370> (дата звернення: 20.07.2026).

10. Якість води у водоймах і річках у межах пляжів станом на 16 серпня 2024 року / Центр громадського здоров'я МОЗ України. URL: <https://phc.org.ua/news/yakist-vodi-u-vodoymakh-i-richkakh-u-mezhakh-plyazhiv-monitoring-vodnikh-obektiv-5> (дата звернення: 20.07.2026).

11. Результати моніторингових досліджень якості води на пляжах Миколаївщини / Миколаївський обласний центр контролю та профілактики хвороб. 30 серпня 2024 року. URL: <https://mk.cdc.gov.ua/news/rezultaty-monitoringovyh-doslidzen-yakosti-vody-na-plyazhah-mykolayivshhyny/> (дата звернення: 20.07.2026).

12. Якість води на пляжах України станом на 29 серпня 2025 року / Центр громадського здоров'я МОЗ України. URL: <https://phc.org.ua/news/yakist-vodi-na-plyazhakh-ukraini-stanom-na-29-serpnya-de-bezpechno-kupatisya> (дата звернення: 20.07.2026).

13. Якість води на пляжах України станом на 16 липня 2026 року / Центр громадського здоров'я МОЗ України. URL: <https://phc.org.ua/news/yakist-vodi-na-plyazhakh-ukraini-stanom-na-16-lipnya-de-bezpechno-kupatisya> (дата звернення: 20.07.2026).

Information about the author:

Magas Nataliia,

Candidate of Technical Sciences, Associate Professor,
Associate Professor at the Department of Ecology
and Environmental Technologies
Admiral Makarov National University of Shipbuilding
9, Heroes of Ukraine Avenue, Mykolaiv, Ukraine

CURRENT APPROACHES IN MAINTENANCE GUT HEALTH IN FARMING ANIMALS. PART 1. THE ISOTONIC PROTEIN BLEND INHIBITS PORCINE EPIDEMIC DIARRHEA VIRUS EXCRETION AND AMELIORATES INTESTINAL BARRIER

Masiuk Dmytro, Nedzvetsky Victor, Kokariiev Andrii
DOI <https://doi.org/10.30525/978-9934-26-714-7-8>

INTRODUCTION

Modern requirements for limiting the antibiotic use in animal husbandry are becoming widespread in most countries^{1, 2, 3}. In this regard, research into feed additives as antibiotic substitutes or targeted aids is gaining considerable interest^{4, 5, 6}. The main objective of evaluating antibiotic alternatives for sustainable production is to assess their effectiveness to support intestinal health, immune response, and productivity⁷. A special place among the alternatives is occupied by a feed mixture^{8, 9, 10}. In addition to protein, such

¹ Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health / B. Catry et al. *International Journal of Antimicrobial Agents*. 2015. Vol. 46, No. 3. P. 297–306.

² Tasho R. P., Cho J. Y. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment*. 2016. Vol. 563–564. P. 366–376.

³ McEwen S. A., Collignon P. J. Antimicrobial resistance: one health perspective. *Microbiology Spectrum*. 2018. Vol. 6, No. 2.

⁴ Multidrug-resistant bacteria and alternative methods to control them: an overview / R. Vivas et al. *Microbial Drug Resistance*. 2019. Vol. 25, No. 6. P. 890–908.

⁵ Potential of aromatic plant-derived essential oils for the control of foodborne bacteria and antibiotic resistance in animal production: a review / L. Zhang et al. *Antibiotics (Basel)*. 2022. Vol. 11, No. 11. Art. 1673.

⁶ Apple Polyphenols Improve Intestinal Antioxidant Capacity and Barrier Function by Activating the Nrf2/Keap1 Signaling Pathway in a Pig Model / T. Huang et al. *Journal of Agricultural and Food Chemistry*. 2022. Vol. 70, No. 24. P. 7576–7585.

⁷ Protective effect of berberine against LPS-induced injury in the intestine: a review / F. Izadparast et al. *Cell Cycle*. 2022. Vol. 21, No. 22. P. 2365–2378.

⁸ Yefimov V., Kostushkevych K., Rakytiarskyi V. Effect of feeding treated peat as a supplement on the parameters of cellular immunity, antioxidant status and performance of piglets in early post-weaning period. *Human & Veterinary Medicine*. 2016. Vol. 8, No. 3. P. 133–136.

⁹ Multidrug-resistant bacteria and alternative methods to control them: an overview / R. Vivas et al. *Microbial Drug Resistance*. 2019. Vol. 25, No. 6. P. 890–908.

¹⁰ Effects of supplemental myo-inositol on growth performance and apparent total tract digestibility of weanling piglets fed reduced protein high-phytate diets and intestinal epithelial cell proliferation and function / T. Z. Ogunribido et al. *Journal of Animal Science*. 2022. Vol. 100, No. 7. Art. skac187.

a mixture contains a large number of nucleotides, B vitamins, amino acids and polysaccharides^{11, 12}. Being an important component, nucleotides are particularly important in improving growth performance, regulating immune function and healing the animals' gastrointestinal tract. However, protein mixtures from different manufacturers have an extremely high divergence in respect with biological activity^{13, 14, 15}. That is why different protein mixtures' molecular and cellular effects require detailed study in order to determine their effectiveness and prospects for use as an alternative to antibiotics in animal husbandry.

The benefits for using protein mixtures in poultry production have already been described in recent studies. However, the potential of using protein mixtures in pig production, in particular to maintain piglet health, remains unclear. One of the protein mixtures' protective mechanisms may be the intestinal microbiota modulation and improvement of intestinal resistance to external damage¹⁶. The intestinal barrier function is primarily provided by intercellular adhesion proteins of the intestinal epithelial layers^{17, 18}. The content of such proteins causes the barrier to strengthen, or conversely, their loss leads to growth of intestinal permeability for the number of enteropathogens^{19, 20, 21}. The weakening of the intestinal barrier function leads to microorganisms and toxins entering the integumental system cells²². Similar invasion initiates cellular damage and increases the infectious diseases risk.

¹¹ Effects of dietary addition of heat-killed *Mycobacterium phlei* on growth performance, immune status and anti-oxidative capacity in early weaned piglets / J. F. Zhong et al. *Archives of Animal Nutrition*. 2016. Vol. 70, No. 4. P. 249–262.

¹² Chlorogenic acid improves intestinal morphology by enhancing intestinal stem-cell activity / Y. Qin et al. *Journal of the Science of Food and Agriculture*. 2023. Vol. 103, No. 7. P. 3287–3294.

¹³ Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks / J. Zhao et al. *Poultry Science*. 2010. Vol. 89, No. 10. P. 2147–2156.

¹⁴ Lee M. A. A global comparison of the nutritive values of forage plants grown in contrasting environments. *Journal of Plant Research*. 2018. Vol. 131, No. 4. P. 641–654.

¹⁵ Petry A. L., Patience J. F. Xylanase supplementation in corn-based swine diets: a review with emphasis on potential mechanisms of action. *Journal of Animal Science*. 2020. Vol. 98, No. 11. Art. skaa318.

¹⁶ Weaning stress and intestinal health of piglets : A review / X. Tang et al. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1042778.

¹⁷ Changes in progenitors and differentiated epithelial cells of neonatal piglets / L. Yin et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 265–276.

¹⁸ Intestinal single-cell atlas reveals novel lymphocytes in pigs with similarities to human cells / J. E. Wiarda et al. *Life Science Alliance*. 2022. Vol. 5, No. 10. Art. e202201442.

¹⁹ MicroRNA-29a mediates the impairment of intestinal epithelial integrity induced by intrauterine growth restriction in pig / Y. Zhu et al. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. 2017. Vol. 312, No. 5. P. 434–442.

²⁰ Protecting intestinal epithelial cells against deoxynivalenol and *E. coli* damage by recombinant porcine IL-22 / Y. Li et al. *Veterinary Microbiology*. 2019. Vol. 231. P. 154–159.

²¹ Combined effects of cadmium and ochratoxin A on intestinal barrier dysfunction in human Caco-2 cells and pig small intestinal epithelial cells / S. H. Kim et al. *Mycotoxin Research*. 2022. Vol. 38, No. 4. P. 253–264.

²² Combined Soluble Fiber-Mediated Intestinal Microbiota Improve Insulin Sensitivity of Obese Mice / C. Xu et al. *Nutrients*. 2020. Vol. 12, No. 2. Art. 351.

Small piglets are particularly susceptible to infectious diseases. The intestinal barrier is a critical target for many enteroviruses. One of the most contagious and widespread in the world and in Ukraine is Porcine Epidemic Diarrhea Virus (PEDV)^{23, 24}. There are a lot of results which evidence that PEDV replicates in the absorptive epithelial cells of the small intestine and induces apoptosis and necrosis, epithelial cell detachment and involvement of villi stroma cores^{25, 26, 27}. This leads to a decrease in the ratio of villi height to crypt depth and thereby damages the intestinal barrier^{28, 29}. However, at the present time the molecular mechanisms of intestinal barrier disruption induced by coronaviruses remain unclear. In this regard, the search for products that would contribute to the strengthening and rapid intestinal barrier recovery under the pathogenic effect of enteroviruses is currently an important research area^{30, 31}.

Therefore, our aim was to find out the role of adhesive proteins in maintaining intestinal barrier in piglets affected with coronavirus enteric infection.

1. Materials and methods to assess the intestinal disturbances in PEDV infected animals

A model experiment was conducted with using the PEDV infected suckling piglets to demonstrate the state of the intestinal barrier via measuring molecular markers level. Two groups of piglets (control and experimental) were formed with two weeks aged 30 animals, accordingly 15 animals in each group.

The highly contagious entero-tropic epidemic diarrhea virus (PEDV) was used to initiate the damaging of the intestinal barrier integrity. All piglets 14 days aged were infected with PEDV at the dose of 104 viral particles per

²³ Molecular characteristics of the Porcine Epidemic Diarrhea Virus strains isolated in different regions of Ukraine / D. Masiuk et al. *Biopolymers and Cell*. 2019. Vol. 35, No. 6. P. 486–494.

²⁴ Porcine Epidemic Diarrhea Virus: an updated overview of virus epidemiology, virulence variation patterns and virus-host interactions / Y. Zhang et al. *Viruses*. 2022. Vol. 14, No. 11. Art. 2434.

²⁵ Jung K., Saif L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Veterinary Journal*. 2015. Vol. 204, No. 2. P. 134–143.

²⁶ Cellular entry of the porcine epidemic diarrhea virus / W. Li et al. *Virus Research*. 2016. Vol. 226, No. 2. P. 117–127.

²⁷ BST2 suppresses porcine epidemic diarrhea virus replication by targeting and degrading virus nucleocapsid protein with selective autophagy / N. Kong et al. *Autophagy*. 2020. Vol. 16, No. 10. P. 1737–1752.

²⁸ The influence of porcine epidemic diarrhea virus on pig small intestine mucosal epithelial cell function / X. Y. Wang et al. *Archives of Virology*. 2019. Vol. 164, No. 1. P. 83–90.

²⁹ LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of alix after Porcine Epidemic Diarrhea Virus infection / W. Qin et al. *Journal of Virology*. 2023. Vol. 97, No. 3. Art. e0188422.

³⁰ Alterations of suckling piglet jejunal microbiota due to infection with Porcine Epidemic Diarrhea Virus and protection against infection by *Lactobacillus salivarius* / W. Dong et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 771411.

³¹ Oral administration of Lactic acid bacteria inhibits PEDV infection in young piglets / S. Yang et al. *Virology*. 2023. Vol. 579. P. 1–8.

animal. The piglets of both groups were fed with a whole milk replacer, and the experimental group was additionally fed with 3% isotonic protein mixture (IPM) by free-access manner.

To assess the infection process in control and experimental group of piglets, rectal swabs and blood serum were collected from piglets on day 14 (before virus infection) and on the 21st, 28th, 35th and 42nd day of life. According to this scheme 3 piglets of each subgroup were euthanized to study dynamic changes of small intestine cells. Samples of duodenum tissue were taken from the piglets and stored frozen at -18 to -22°C for examination.

All experimental procedures with piglets were carried out in compliance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.2006 “On the Protection of Animals from Brutal Treatment”, in accordance with the basic principles of the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), and approved by the Local Commission for Bioethics and Animal Experiments of Dnipro State Agrarian and Economic University.

qPCR. The content of PEDV genetic material was determined in rectal swabs by qPCR method.

Nucleic acids were extracted from rectal swabs in several stages. Firstly, rectal swabs were placed in a test tube to which lysis buffer solution ATL Buffer (Biosellal, France) and quartz beads were added according the recommendations of producer. The test tube was placed in the rotor of the FastPrep-24 homogenizer, where the swabs were crushed. A volume of 200 µL was collected from each homogenate sample. Nucleic acids (NA) were extracted using a commercial reagent kit “BioExtract Premium Mag” on a KingFisher Duo automatic nucleic acid extraction device.

The cDNA content was determined in the NA extracts using the “oneMIX & PEDV” test kit (Exolpol, Spain). Amplification and detection of the results were performed using a CFX 96 instrument (BioRad, USA). Thermal cycling was performed according to the test kit instructions.

All samples were analysed in technical triplicate, including the corresponding negative controls (distilled water without DNase/RNase). The PCR efficiency and correlation coefficients of the standard curves were in the 89.20–111.00% range, and R² was from 0.988 to 0.999, indicating a sufficiently high level of linear dependence. The sensitivity limit for qPCR was assessed as 10–100 copies of cDNA from three independent tests.

To verify the qPCR linearity and dynamic range, standard curves were constructed by making 10 times serial dilutions of a positive PEDV cDNA control sample with a certain copy number. The qPCR results were calculated

and analysed using the CFX Manager software, and the amplification efficiency (E) was calculated using the formula $E = (10^{-1/\text{slope}}) - 1$.

ELISA. Serum immunoglobulins of class G (IgG) specific for PEDV antigens were determined by an indirect ELISA method using a commercial diagnostic system "ID Screen® PEDV Indirect" (ID, France) on an ELx800 photometer analyzer (BioTek, USA).

In accordance with the recommendations for the diagnostic kit, the serum was diluted 1/10 before testing. A sample was considered positive if the sum of products of deviations (SP) value was more than or equal to 60%. In accordance with the instructions of the test system used, the level of antibodies in the pig blood serum is proportional to the SP value.

Western blot. The amount of E-cadherin, fibronectin and interferon- α proteins was determined in duodenum tissues by Western blot (WB) method.

To prepare protein extracts of the aforementioned molecular markers small intestine tissue every individual was homogenized with phosphate buffer pH 7.6 containing 1% Triton X100, 0.2% sodium dodecyl sulphate and inhibitors cocktail. The total protein concentration in each sample was determined by the Bradford method (Bradford, 1976). Protein extract samples were separated by electrophoresis in a polyacrylamide gel (PAAG) with an acrylamide gradient of 7–18% (Laemmli, 1970). To control the efficiency of individual polypeptide separation, several tracks were additionally stained with a 0.1% Coomassie G-250 solution. The transfer of proteins from PAAG to nitrocellulose was carried out using an electric field for 120 min at a current of 250 mA. The blocking of unspecific absorbance onto nitrocellulose was performed in a 3% solution of skimmed milk powder at 60 min. After blocking, the nitrocellulose membranes were treated with a 1:25 00 dilution of specific rabbit antiserum (E-cadherin, fibronectin and interferon- α Santa-Cruz), washed in phosphate buffer and incubated for 60 min in a solution of peroxidase-labelled secondary antibodies (anti-mouse Santa-Cruz). To visualize the polypeptide zones, the nitrocellulose membrane was treated with a 50 mM tris-HCl pH 7.4 solution containing 0.05% diaminobenzidine and 0.01% hydrogen peroxide. The results of total protein content and immunoblotting were expressed as a percentage of the control group.

The results were statistically processed using the specialized software Statistica 6 (StatSoft Inc, USA). The differences significance was assessed by the Student's t-test. The sample parameters presented in the paper had the following designations: M – sample mean; m – standard error of the mean. Changes in indicators were considered significant at $P < 0.05$ (including $P < 0.01$ and $P < 0.001$).

2. Results on PEDV excretion dynamics

The results of the PEDV PCR study indicate that before infection both control and experimental group had no virus RNA in rectal swabs (Fig. 1).

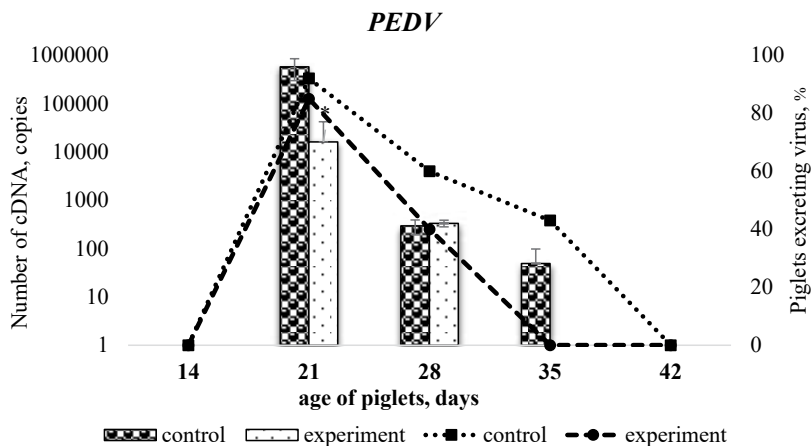


Fig. 1. Duration of PEDV excretion into infected piglets' feces of control group and group consumed IPM ($M \pm SEM$, $n = 16$)

The highest amount of virus was detected in rectal swabs of piglets on the 21st day of life. Over time, the virus excretion level decreases, as indicated by a small amount of cDNA in rectal swabs of control piglets on the 28th and 35th day of life. Complete virus excretion stops only on day 42 after infection. In the experimental group, the virus was detected only at the 21st and 28th day of life. In 21-day-old piglets of the experimental group, the amount of virus in rectal swabs was significantly lower by 10 times, and the number of virus-positive animals was 7% less than in the control group.

There was no difference found between the experimental and control group piglets in the amount of excreted virus on day 28 of life. Besides, there was a significant difference between the percentage of animals with the virus replicating in their bodies. Thus, in the experimental group of piglets, 20% fewer animals containing PEDV cDNA in rectal swabs were detected compared to the values of the control group.

The lowest level of animals excreting PEDV was found in the control group on day 35 of the life. At the same time, the amount of PEDV cDNA in rectal swabs from these animals was the lowest for the entire study period. Besides, there was no PEDV cDNA detected in the rectal swabs of the experimental

group which exposed to IPM. Moreover, all examined rectal swabs from piglets on the 42nd day of life did not contain PEDV cDNA.

The results of the intestinal barrier molecular markers study in the duodenum tissues characterize the changes in the content of E-cadherin, fibronectin and INF- α in piglets of control and experimental groups affected with PED infection (Table 1).

Table 1

Relative content of the molecular markers in the duodenum tissues of the piglets infected with PEDV ($M \pm m$, $n=3$)

Age of piglets, days	E-cadherin		Fibronectin		Interferon- α	
	control	experiment	control	experiment	control	experiment
21	101,2 $\pm$ 3,2	114,3 $\pm$ 5,8	99,7 $\pm$ 3,1	103,1 $\pm$ 5,2	107 $\pm$ 4,7	105 $\pm$ 5,5
28	99,5 $\pm$ 4,7	116,4 $\pm$ 6,2*	98,9 $\pm$ 4,5	106,4 $\pm$ 6,1	195 $\pm$ 7,6	291 $\pm$ 8,2**
35	99,7 $\pm$ 4,5	122,8 $\pm$ 6,4**	101,2 $\pm$ 4,2	112,7 $\pm$ 3,9*	316 $\pm$ 9,2	217 $\pm$ 7,1***
42	102,3 $\pm$ 3,9	111,4 $\pm$ 4,5*	99,4 $\pm$ 3,8	109,2 $\pm$ 3,7*	221 $\pm$ 6,4	151 $\pm$ 5,3**

*Note: changes are considered to be statistically significant at * – $P < 0,05$, ** – $P < 0,05$, *** – $P < 0,001$ relative to the parameters of the animals of the control group.*

There was found that the content of E-cadherin in the piglets' intestinal tissues of the experimental group on day 28, 35 and 42 of life was significantly higher by 17%, 23% and 9% compared to the values of the control group.

Similar results were obtained for fibronectin content alteration into intestine tissue. The level of fibronectin in the duodenum tissues was significantly higher in piglets of the experimental group at 35 and 42 days of age about 11.5% and 9.8% ($P < 0.05$) accordingly compared to the values of the control group.

The results of the IFN- α study showed that its increase in piglets of the experimental group was almost 2 times ($P < 0.05$) on day 28 of life compared to the values of the control group. At 35 and 42 days of life, the IFN- α level in the duodenum tissues decreased sharply and was significantly lower by 2.0 and 1.7 times compared to the values of the control group of animals.

The study results of the PEDV antigen-specific IgG levels in the blood showed a nonlinear rising dynamics of seroconversion processes in piglets from day 21 to day 42 of the modelled infection (Fig. 2).

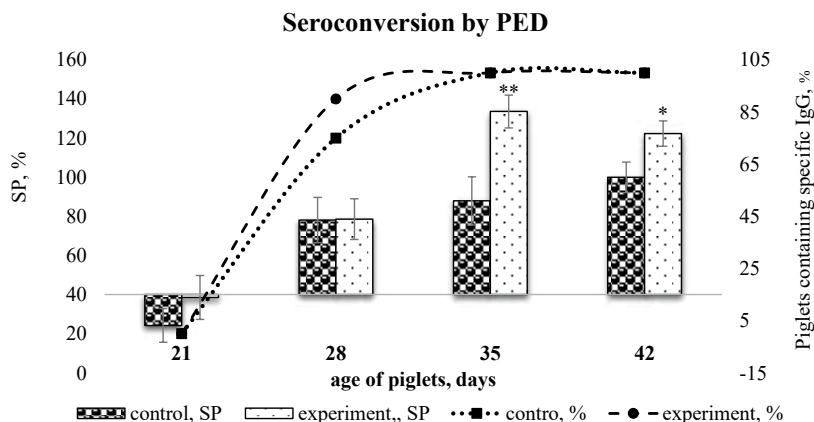


Fig. 2. Dynamics of IgG seroconversion specific against PEDV antigens in the piglet blood of control group and group consumed IPM (M±SEM, n = 16)

The feeding of the experimental piglet group with an IPM increases the number of immune animals by 15% at day 28 compared to the control group. The SP score in piglets of this age did not differ significantly between the experimental and control groups. All piglets of the experimental and control groups developed specific IgG to PEDV antigens in the blood at the 35th and 42nd day of life. The SP value in animals of the experimental group at the 35th and 42nd day of life was significantly higher by 52% and 22% ($P < 0.05$; $P < 0.05$), accordingly, compared to the piglets of the control group.

3. The protective effect of Isotonic Protein Formulation on PEDV proliferation

The obtained results characterize the changes in molecular markers content that the intestinal barrier in piglets at different stages of the infectious process remodeled with experimental PEDV infection. The course of the infectious process was assessed by the timing of virus elimination into environment from the piglets as well as with modulation of immune response in both the blood serum and duodenum tissue.

The PCR results showed that PEDV was excreted in feces up to the 35th day of life. Similar results have been demonstrated in several studies³². It was

³² Immune responses to porcine epidemic diarrhea virus (PEDV) in swine and protection against subsequent infection / V. D. Krishna et al. *PLoS ONE*. 2020. Vol. 15, No. 4. Art. e0231723.

found that the excretion of PEDV in feces lasted for 28 days after inoculation, and the virus persisted in intestinal tissues for a longer time, which, according to the authors, poses additional risks of virus spread into a pig farm.

We have found that the use of IPM in piglets reduces the PEDV excretion time by 7 days and inhibits the excreted virus content by 10 times. Similar results were demonstrated in the study on the treatment piglets with glycyrrhiza extract presented by Gao et al. (2020)³³. The authors note that glycyrrhizin suppresses PEDV infection and reduces the period of virus excretion by attenuating the secretion of proinflammatory cytokines.

Given the presented results and literature data, it can be assumed that the increased rate of PEDV elimination from the infected piglets may be due to intensified immunobiological response and faster restoration of the intestinal barrier integrity³⁴.

This hypothesis is confirmed by the results of present study. Obtained in our study data have shown that the shortening of virus excretion was accompanied by the immune response activation in piglets affected with PEDV infection. Furthermore, immune response could be associated with amelioration in epithelial cell adhesion via modulation tight junction molecular markers in the duodenum.

The results of our study demonstrated dynamic time-dependent changes in expression all of studied molecular markers. The revealed trend towards a more progressive increase in the content of E-cadherin and fibronectin in the experimental piglets' duodenum tissues is due to more significant proliferative processes in the intestine during PEDV infection against the background of feeding piglets with an IPM³⁵. Recently, there was reported that PEDV replication in enterocytes leads to the loss of tight junction proteins, including ZO-1, ZO-2, occludin and some claudins, indicating a disruption of intestinal integrity³⁶. Given that E-cadherin and fibronectin proteins are responsible for intercellular and cell-matrix contacts and ensure the integumentary barrier density³⁷, our results indicate a protective effect of the IPM and enhancement of repair processes in intestinal tissues³⁸.

³³ Glycyrrhizin inhibits PEDV infection and proinflammatory cytokine secretion via the HMGB1/TLR4-MAPK p38 pathway / R. Gao et al. *International Journal of Molecular Sciences*. 2020. Vol. 21, No. 8. Art. 2961.

³⁴ Wiarda J. E., Loving C. L. Intraepithelial lymphocytes in the pig intestine: T cell and innate lymphoid cell contributions to intestinal barrier immunity. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1048708.

³⁵ LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of alix after Porcine Epidemic Diarrhea Virus infection / W. Qin et al. *Journal of Virology*. 2023. Vol. 97, No. 3. Art. e0188422.

³⁶ Jung K., Saif L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Veterinary Journal*. 2015. Vol. 204, No. 2. P. 134–143.

³⁷ Schoutz I., Keita A. V. The intestinal barrier and current techniques for the assessment of gut permeability. *Cells*. 2020. Vol. 9, No. 8. Art. 1909.

³⁸ Developmental changes in intercellular junctions and Kv channels in the intestine of piglets during the suckling and post-weaning periods / J. Wang et al. *Journal of Animal Science and Biotechnology*. 2016. Vol. 7, No. 1. Art. 4.

Taking into the account that a wide range of entero-pathogens destroy the integumentary barrier, protective effect of IPM targeted to the support of enterocytes viability and their ability to form strong adhesive contacts as well as to enhance the proliferation into the intestine affected areas³⁹. All of aforementioned cellular activity alone or together require metabolic energy expenditure. The isotonic protein formulation biologically active components simultaneously stimulate feed intake, cellular energy metabolism, and local immune responses which is confirmed by observed results in present study⁴⁰.

We have found that in an experimental PEDV infection feeding piglets with IPM during all studied period of the suckling from day 14 of life contributes to a rapid increase in IFN- α levels by day 28 compared to the values of control group. Since interferons type I regulate a wide range of nonspecific protective responses in the intestine, it can be assumed that stimulation of IFN- α production could be a useful marker of increased innate immune activity⁴¹.

Feeding piglets with an IPM to enhance immune response against a progress of PEDV infection can restrict the period of seroconversion and reduces the period of immunological tolerance. Obtained results have shown that enhanced IFN- α synthesis in the experimental group piglets' intestinal tissues and increase in the level of seroconversion can reflect the protective effect of IPM exposure. This hypothesis is confirmed with observed data in respect to higher SP value in experimental group of piglets on days 35 and 42 of life. Similar results were obtained in a recent study of experimental infection of pigs with European strains of PEDV⁴². The authors observed the appearance of blood serum IgG specific to PEDV antigens on the 18th and 10th day after virus inoculation. Such changes are usually associated with antigen-stimulated seroconversion⁴³.

Therefore, the intestinal barrier is a critical component in ensuring the pig's health as it has a significant impact on permeability selectivity and the intestinal immune system⁴⁴. Effective recovery of the intestinal barrier integrity leads to a reduction in the period of recovery and viral shedding, while

³⁹ Assessing intestinal health. In vitro and ex vivo gut barrier models of farm animals: benefits and limitations / F. Ghiselli et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 723387.

⁴⁰ Glycyrrhizin inhibits PEDV infection and proinflammatory cytokine secretion via the HMGB1/TLR4-MAPK p38 pathway / R. Gao et al. *International Journal of Molecular Sciences*. 2020. Vol. 21, No. 8. Art. 2961.

⁴¹ Single-blinded study highlighting the differences between the small intestines of neonatal and weaned piglets / C. Yuan et al. *Animals*. 2021. Vol. 11, No. 2. Art. 271.

⁴² Experimental infection of pigs with recent European porcine epidemic diarrhea viruses / C. M. Lazov et al. *Viruses*. 2022. Vol. 14, No. 12. Art. 2751.

⁴³ Tran T. H. T., Everaert N., Bindelle J. Review on the effects of potential prebiotics on controlling intestinal enteropathogens *Salmonella* and *Escherichia coli* in pig production. *Journal of Animal Physiology and Animal Nutrition*. 2018. Vol. 102, No. 1. P. 17–32.

⁴⁴ Wiarda J. E., Loving C. L. Intraepithelial lymphocytes in the pig intestine: T cell and innate lymphoid cell contributions to intestinal barrier immunity. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1048708.

enhancing the body's immune responses and shortening the seroconversion period⁴⁵. Taking into the account aforementioned, the estimation of protein markers including E-cadherin and fibronectin constructs usable way to monitor the enterotropic drugs effect on the intestinal condition⁴⁶. Moreover, this strategy is prospective tool to determine the level of damage and the effectiveness of the intestinal barrier recovery in pigs under the influence of various alternative factors, including PEDV.

CONCLUSIONS

The peculiarities of PEDV excretion by piglets infected with a dose of 104 viral particles per animal showed that starting from 14 days of life, excretion progressively increased for the 21st day and then decreased until the 35th day of age. Starting from the 42nd day of life, the PEDV excretion in feces was finished completely.

Feeding piglets with an IPM stimulate the content increase in the adhesive proteins E-cadherin and Fibronectin in the duodenum tissues by an average of 17% and 11%, accordingly, and enhanced the synthesis of IFN- α and IgG specific to the PEDV antigen by 2 and 1.5 times, accordingly. Thus, the decrease in PEDV excretion may be due to the improvement of barrier function and innate immunity in the animals' intestines induced by the consumption of IPM.

SUMMARY

The intestinal barrier is an extremely important structure to maintain piglet health. However, various enteropathogens, including coronaviruses, are especially aggressive factors for the intestinal barrier destruction. That is why the aim of present study was to clarify the role of adhesive proteins in the piglet intestinal barrier maintaining affected with coronavirus enteric infection. For this purpose, we formed two groups of 14-day-old piglets to construct experimental model enteric disorder. Each animal was infected with porcine epidemic diarrhea virus (PEDV) at a dose of 104 virions. The piglets of the experimental group were additionally fed of isotonic protein mixture (IPM). The intensity of the infectious process was assessed by the duration of virus elimination from the piglets' body, the amount of excreted virus, the immune response efficacy. The level immune response was detected by specific IgG in the blood serum and α -interferon (IFN- α) in the intestinal tissues. The intestinal barrier integrity was assessed by the level of proteins

⁴⁵ Regulation of the intestinal barrier function by host defense peptides / K. Robinson et al. *Frontiers in Veterinary Science*. 2015. Vol. 2. Art. 57.

⁴⁶ Effect of riboflavin on intestinal development and intestinal epithelial cell function of weaned piglets / Y. Qin et al. *Journal of Animal Physiology and Animal Nutrition*. 2023. Vol. 107, No. 2. P. 518–528.

in the tissues that form tight intercellular contacts especially E-cadherin and fibronectin in the duodenum. The results have shown a variability in the duration of the PEDV elimination period in piglets' feces. The exposure to IPM provides a protective effect that enhances the recovery processes in the intestinal tissues. That was also reflected in an increase in the amount of E-cadherin and fibronectin in the duodenum. Furthermore, the isotonic protein mixture's protective effect was reflected in an increase in the body's immune responses into the intestinal level. This effect was evidenced by an increase in the amount of IFN- α in the duodenum and an increase in the synthesis of specific IgG in the blood serum. Obtained results showed a protective effect of IPM treatment while the intestinal barrier and immune responses were enhanced. IPM treatment induced about 10 times decrease in the amount of PEDV excreted in the feces in experimental animals and contributed to a 7-day reduction in the virus excretion period.

Bibliography

1. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health / B. Catry et al. *International Journal of Antimicrobial Agents*. 2015. Vol. 46, No. 3. P. 297–306. DOI: <https://doi.org/10.1016/j.ijantimicag.2015.06.005>.
2. Alterations of suckling piglet jejunal microbiota due to infection with Porcine Epidemic Diarrhea Virus and protection against infection by *Lactobacillus salivarius* / W. Dong et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 771411. DOI: <https://doi.org/10.3389/fvets.2021.771411>.
3. Glycyrrhizin inhibits PEDV infection and proinflammatory cytokine secretion via the HMGB1/TLR4-MAPK p38 pathway / R. Gao et al. *International Journal of Molecular Sciences*. 2020. Vol. 21, No. 8. Art. 2961. DOI: <https://doi.org/10.3390/ijms21082961>.
4. Assessing intestinal health. In vitro and ex vivo gut barrier models of farm animals: benefits and limitations / F. Ghiselli et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 723387. DOI: <https://doi.org/10.3389/fvets.2021.723387>.
5. Apple Polyphenols Improve Intestinal Antioxidant Capacity and Barrier Function by Activating the Nrf2/Keap1 Signaling Pathway in a Pig Model / T. Huang et al. *Journal of Agricultural and Food Chemistry*. 2022. Vol. 70, No. 24. P. 7576–7585. DOI: <https://doi.org/10.1021/acs.jafc.2c02495>.
6. Protective effect of berberine against LPS-induced injury in the intestine: a review / F. Izadparast et al. *Cell Cycle*. 2022. Vol. 21, No. 22. P. 2365–2378. DOI: <https://doi.org/10.1080/15384101.2022.2100682>.

7. Jung K., Saif L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Veterinary Journal*. 2015. Vol. 204, No. 2. P. 134–143. DOI: <https://doi.org/10.1016/j.tvjl.2015.02.017>.
8. Combined effects of cadmium and ochratoxin A on intestinal barrier dysfunction in human Caco-2 cells and pig small intestinal epithelial cells / S. H. Kim et al. *Mycotoxin Research*. 2022. Vol. 38, No. 4. P. 253–264. DOI: <https://doi.org/10.1007/s12550-022-00464-0>.
9. BST2 suppresses porcine epidemic diarrhea virus replication by targeting and degrading virus nucleocapsid protein with selective autophagy / N. Kong et al. *Autophagy*. 2020. Vol. 16, No. 10. P. 1737–1752. DOI: <https://doi.org/10.1080/15548627.2019.1707487>.
10. Immune responses to porcine epidemic diarrhea virus (PEDV) in swine and protection against subsequent infection / V. D. Krishna et al. *PLoS ONE*. 2020. Vol. 15, No. 4. Art. e0231723. DOI: <https://doi.org/10.1371/journal.pone.0231723>.
11. Experimental infection of pigs with recent European porcine epidemic diarrhea viruses / C. M. Lazov et al. *Viruses*. 2022. Vol. 14, No. 12. Art. 2751. DOI: <https://doi.org/10.3390/v14122751>.
12. Lee M. A. A global comparison of the nutritive values of forage plants grown in contrasting environments. *Journal of Plant Research*. 2018. Vol. 131, No. 4. P. 641–654. DOI: <https://doi.org/10.1007/s10265-018-1024-y>.
13. Cellular entry of the porcine epidemic diarrhea virus / W. Li et al. *Virus Research*. 2016. Vol. 226, No. 2. P. 117–127. DOI: <https://doi.org/10.1016/j.virusres.2016.05.031>.
14. Protecting intestinal epithelial cells against deoxynivalenol and E. coli damage by recombinant porcine IL-22 / Y. Li et al. *Veterinary Microbiology*. 2019. Vol. 231. P. 154–159. DOI: <https://doi.org/10.1016/j.vetmic.2019.02.027>.
15. Molecular characteristics of the Porcine Epidemic Diarrhea Virus strains isolated in different regions of Ukraine / D. Masiuk et al. *Biopolymers and Cell*. 2019. Vol. 35, No. 6. P. 486–494. DOI: <https://doi.org/10.7124/bc.000A1B>.
16. McEwen S. A., Collignon P. J. Antimicrobial resistance: one health perspective. *Microbiology Spectrum*. 2018. Vol. 6, No. 2. DOI: <https://doi.org/10.1128/microbiolspec.ARBA-0009-2017>.
17. Effects of supplemental myo-inositol on growth performance and apparent total tract digestibility of weanling piglets fed reduced protein high-phytate diets and intestinal epithelial cell proliferation and function / T. Z. Ogunribido et al. *Journal of Animal Science*. 2022. Vol. 100, No. 7. Art. skac187. DOI: <https://doi.org/10.1093/jas/skac187>.
18. Petry A. L., Patience J. F. Xylanase supplementation in corn-based swine diets: a review with emphasis on potential mechanisms of action.

Journal of Animal Science. 2020. Vol. 98, No. 11. Art. skaa318. DOI: <https://doi.org/10.1093/jas/skaa318>.

19. LncRNA446 regulates tight junctions by inhibiting the ubiquitinated degradation of alix after Porcine Epidemic Diarrhea Virus infection / W. Qin et al. *Journal of Virology*. 2023. Vol. 97, No. 3. Art. e0188422. DOI: <https://doi.org/10.1128/jvi.01884-22>.

20. Chlorogenic acid improves intestinal morphology by enhancing intestinal stem-cell activity / Y. Qin et al. *Journal of the Science of Food and Agriculture*. 2023. Vol. 103, No. 7. P. 3287–3294. DOI: <https://doi.org/10.1002/jsfa.12469>.

21. Effect of riboflavin on intestinal development and intestinal epithelial cell function of weaned piglets / Y. Qin et al. *Journal of Animal Physiology and Animal Nutrition*. 2023. Vol. 107, No. 2. P. 518–528. DOI: <https://doi.org/10.1111/jpn.13725>.

22. Regulation of the intestinal barrier function by host defense peptides / K. Robinson et al. *Frontiers in Veterinary Science*. 2015. Vol. 2. Art. 57. DOI: <https://doi.org/10.3389/fvets.2015.00057>.

23. Schoultz I., Keita Å. V. The intestinal barrier and current techniques for the assessment of gut permeability. *Cells*. 2020. Vol. 9, No. 8. Art. 1909. DOI: <https://doi.org/10.3390/cells9081909>.

24. Weaning stress and intestinal health of piglets: A review / X. Tang et al. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1042778. DOI: <https://doi.org/10.3389/fimmu.2022.1042778>.

25. Tasho R. P., Cho J. Y. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment*. 2016. Vol. 563–564. P. 366–376. DOI: <https://doi.org/10.1016/j.scitotenv.2016.04.140>.

26. Tran T. H. T., Everaert N., Bindelle J. Review on the effects of potential prebiotics on controlling intestinal enteropathogens Salmonella and Escherichia coli in pig production. *Journal of Animal Physiology and Animal Nutrition*. 2018. Vol. 102, No. 1. P. 17–32. DOI: <https://doi.org/10.1111/jpn.12666>.

27. Multidrug-resistant bacteria and alternative methods to control them: an overview / R. Vivas et al. *Microbial Drug Resistance*. 2019. Vol. 25, No. 6. P. 890–908. DOI: <https://doi.org/10.1089/mdr.2018.0319>.

28. Developmental changes in intercellular junctions and Kv channels in the intestine of piglets during the suckling and post-weaning periods / J. Wang et al. *Journal of Animal Science and Biotechnology*. 2016. Vol. 7, No. 1. Art. 4. DOI: <https://doi.org/10.1186/s40104-016-0063-2>.

29. The influence of porcine epidemic diarrhea virus on pig small intestine mucosal epithelial cell function / X. Y. Wang et al. *Archives of Virology*. 2019. Vol. 164, No. 1. P. 83–90. DOI: <https://doi.org/10.1007/s00705-018-4061-x>.

30. Intestinal single-cell atlas reveals novel lymphocytes in pigs with similarities to human cells / J. E. Wiarda et al. *Life Science Alliance*. 2022. Vol. 5, No. 10. Art. e202201442. DOI: <https://doi.org/10.26508/lsa.202201442>.
31. Wiarda J. E., Loving C. L. Intraepithelial lymphocytes in the pig intestine: T cell and innate lymphoid cell contributions to intestinal barrier immunity. *Frontiers in Immunology*. 2022. Vol. 13. Art. 1048708. DOI: <https://doi.org/10.3389/fimmu.2022.1048708>.
32. Combined Soluble Fiber-Mediated Intestinal Microbiota Improve Insulin Sensitivity of Obese Mice / C. Xu et al. *Nutrients*. 2020. Vol. 12, No. 2. Art. 351. DOI: <https://doi.org/10.3390/nu12020351>.
33. Oral administration of Lactic acid bacteria inhibits PEDV infection in young piglets / S. Yang et al. *Virology*. 2023. Vol. 579. P. 1–8. DOI: <https://doi.org/10.1016/j.virol.2022.12.005>.
34. Yefimov V., Kostiushkevych K., Rakytiatskyi V. Effect of feeding treated peat as a supplement on the parameters of cellular immunity, antioxidant status and performance of piglets in early post-weaning period. *Human & Veterinary Medicine*. 2016. Vol. 8, No. 3. P. 133–136. URL: <http://dspace.dsau.dp.ua/jspui/handle/123456789/591>.
35. Changes in progenitors and differentiated epithelial cells of neonatal piglets / L. Yin et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 265–276. DOI: <https://doi.org/10.1016/j.aninu.2021.10.008>.
36. Single-blinded study highlighting the differences between the small intestines of neonatal and weaned piglets / C. Yuan et al. *Animals*. 2021. Vol. 11, No. 2. Art. 271. DOI: <https://doi.org/10.3390/ani11020271>.
37. Potential of aromatic plant-derived essential oils for the control of foodborne bacteria and antibiotic resistance in animal production: a review / L. Zhang et al. *Antibiotics (Basel)*. 2022. Vol. 11, No. 11. Art. 1673. DOI: <https://doi.org/10.3390/antibiotics11111673>.
38. Porcine Epidemic Diarrhea Virus: an updated overview of virus epidemiology, virulence variation patterns and virus-host interactions / Y. Zhang et al. *Viruses*. 2022. Vol. 14, No. 11. Art. 2434. DOI: <https://doi.org/10.3390/v14112434>.
39. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks / J. Zhao et al. *Poultry Science*. 2010. Vol. 89, No. 10. P. 2147–2156. DOI: <https://doi.org/10.3382/ps.2009-00608>.
40. Effects of dietary addition of heat-killed *Mycobacterium phlei* on growth performance, immune status and anti-oxidative capacity in early weaned piglets / J. F. Zhong et al. *Archives of Animal Nutrition*. 2016. Vol. 70, No. 4. P. 249–262. DOI: <https://doi.org/10.1080/1745039X.2016.1183365>.

41. MicroRNA-29a mediates the impairment of intestinal epithelial integrity induced by intrauterine growth restriction in pig / Y. Zhu et al. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. 2017. Vol. 312, No. 5. P. 434–442. DOI: <https://doi.org/10.1152/ajpgi.00020.2017>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,
Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,

Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Kokariiev Andrii,

Candidate of Veterinary Sciences, Associate Professor,
Head of the Department of Immunochemical and Molecular Genetic
Analysis of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

**CURRENT APPROACHES IN MAINTENANCE GUT
HEALTH IN FARMING ANIMALS.
PART 2. GLYCERIDES BLEND MODULATES
INTESTINAL BARRIER FUNCTIONING IN PIGLETS**

Masiuk Dmytro, Nedzvetsky Victor, Kokariev Andrii
DOI <https://doi.org/10.30525/978-9934-26-714-7-9>

INTRODUCTION

The issue of the keeping sustainable piglet intestinal health is becoming increasingly important to both livestock specialists and the scientific community. Sustainable intestinal development and maintaining piglet health are key factors for successful pig breeding. One of the innovative approaches to improve piglet health is to maintain the barrier function of the intestinal system¹. An effective intestinal barrier consists of a columnar epithelial cell layer that performs two interdependent functions, namely creating a protective barrier against harmful substances and acting as a selective filter for nutrients^{2, 3}. Both of these functions are aimed at maintaining homeostatic conditions in the intestine and forming communication pathways between cells and the environment. In addition, the protective property of the intestinal system is the requirement to prevent potentially harmful substances invasion, including pathogens, antigens, and pro-inflammatory factors, from entering the external environment to the intestinal tissue. The intestinal barrier's selective capabilities contribute to the development of the intestinal immune system and the formation of immune tolerance^{4, 5}.

¹ Integrity of the intestinal barrier: The involvement of epithelial cells and microbiota – a mutual relationship / M. Gierynska et al. *Animals*. 2022. Vol. 12, No. 2. Art. 145.

² Interaction between the gut microbiome and mucosal immune system / N. Shi et al. *Military Medical Research*. 2017. Vol. 4, No. 1. Art. 14.

³ Paone P., Cani P. D. Mucus barrier, mucins and gut microbiota: the expected slimy partners / P. Paone, P. D. Cani. *Gut*. 2020. Vol. 69, No. 12. P. 2232–2243.

⁴ Turner J. R. Intestinal mucosal barrier function in health and disease / J. R. Turner. *Nature Reviews Immunology*. 2009. Vol. 9, No. 11. P. 799–809.

⁵ Sardinha-Silva A., Alves-Ferreira E. V. C., Grigg M. E. Intestinal immune responses to commensal and pathogenic protozoa / A. Sardinha-Silva, E. V. C. Alves-Ferreira, M. E. Grigg. *Frontiers in Immunology*. 2022. Vol. 13. Art. 963723.

A disorder of the intestinal protective mechanisms, namely mucus loss and destruction of epithelial cells contributes to the transport of both beneficial and potentially harmful substances from the intestinal lumen to the internal environment, thus causing an over-activated immune response⁶. Intestinal barrier function depends on the interaction between several barrier components, including the intestinal mucus layer, cytokines and chemokines, antibacterial peptides, and intercellular adhesions⁷.

The basic junctions form a continuous and dense branched network between the basolateral cell membranes, which leads to an increase in the compactness of the apical intercellular space. The epithelial ability to maintain barrier function depends on four types of intercellular junctions: desmosomes (DSM), adherens junctions (AJ), tight junctions (TJ), and gap junctions (GJ)⁸. Adhesive contacts and desmosomes are anchor junctions that link actin filaments of the cytoskeleton to the plasma membrane at the sites of intercellular adhesion. Tight junctions create a seal in the plasma membrane to regulate paracellular transport and polarize cells by keeping proteins in their correct compartments⁹. Gap junctions containing connexin electrically connect cells and allow small molecules to diffuse from one cell to another cell.

One of the main representatives of intercellular adhesive contacts is the cadherin subfamily, with E-cadherin being the epithelial-specific protein. This transmembrane protein is involved in cellular processes such as signaling of intercellular contact inhibition, cell proliferation, actin cytoskeleton remodeling, and cell polarity¹⁰. The functioning of the intestinal epithelial layer requires stable intercellular adhesion to ensure barrier function¹¹. A decrease in the content of molecular markers of barrier function can provoke a violation of the epithelial layer integrity and initiate the intestinal barrier destruction¹².

⁶ Shen L., Turner J. R. Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: tight junction dynamics exposed / L. Shen, J. R. Turner. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. 2006. Vol. 290, No. 4. P. 577–582.

⁷ Suzuki T. Regulation of the intestinal barrier by nutrients: The role of tight junctions / T. Suzuki. *Animal Science Journal*. 2020. Vol. 91, No. 1. Art. e13357.

⁸ Green K. J., Jaiganesh A., Broussard J. A. Desmosomes: Essential contributors to an integrated intercellular junction network / K. J. Green, A. Jaiganesh, J. A. Broussard. *F1000Research*. 2019. Vol. 8. Art. F1000 Faculty Rev-2150.

⁹ Johnson J. L., Najor N. A., Green K. J. Desmosomes: regulators of cellular signaling and adhesion in epidermal health and disease / J. L. Johnson, N. A. Najor, K. J. Green. *Cold Spring Harbor Perspectives in Medicine*. 2014. Vol. 4, No. 11. Art. a015297.

¹⁰ Maître J. L., Heisenberg C. P. Three functions of cadherins in cell adhesion / J. L. Maître, C. P. Heisenberg. *Current Biology*. 2013. Vol. 23, No. 14. P. 626–633.

¹¹ Garcia M. A., Nelson W. J., Chavez N. Cell-cell junctions organize structural and signaling networks / M. A. Garcia, W. J. Nelson, N. Chavez. *Cold Spring Harbor Perspectives in Biology*. 2018. Vol. 10, No. 4. Art. a029181.

¹² Yulis M., Kusters D. H. M., Nusrat A. Cadherins: cellular adhesive molecules serving as signalling mediators / M. Yulis, D. H. M. Kusters, A. Nusrat. *The Journal of Physiology*. 2018. Vol. 596, No. 17. P. 3883–3898.

In spite of the progress in research on the role of adhesive proteins in intestinal barrier function, the important component of barrier function is the extracellular matrix (ECM), which is considered to be a key mediator in many intercellular interactions. There are various components that regulate and activate many cellular processes and ultimately affect the cellular homeostasis. An essential component of the ECM is fibronectin, a glycoprotein involved in tissue integrity through cell-ECM adhesion, proliferation and migration and produced by several types of intestinal epithelial cells¹³.

To maintain the protective properties of intestinal system, the biological effects of dietary supplements with short-chain fatty acids and monoglycerides (SCA-M) as the cytoprotector for intestinal barrier have been actively studied recently. Similar supplements contain formic, propionic, citric, and acetic acids, which exhibit antimicrobial properties, confirming the prospects of their usage as supportive agents. Organic acids are used by human as a feed preservative due to their strong antibacterial and antifungal effects. An important point for fruitful application of these compounds is that they have multifactorial effects on the microbiome state, immune response and key intestinal components interactions. Unfortunately, the molecular and cellular mechanisms of SCA-M effect on piglet intestinal health are not well defined. Besides, the growth recent data support the evidence that maintaining the functionality of the intestinal system is a critical requirement for pig breeding to achieve high productive rates and ensure the sustainable development of this field.

The aim of our study was to determine the SCA-M effect on fibronectin content in the small intestine of piglets, total protein content in a blood and average weight gain.

1. Methodological approaches to study the bioeffects of fatty acids blend

The research was carried out in the laboratory of the Research Centre for Biosafety and Environmental Control of Agricultural Resources (Biosafety Centre) of the Dnipro State Agrarian and Economic University (DSAE). The work was carried out on piglets from the 42nd to the 77th day of life in an industrial pig farm. 24 animals were included into the experiment, 2 groups were formed – control and experimental, each consisting of 12 piglets. The experimental group piglets were raised according to the standard protocol of the farm. At the same time, the piglets of the experimental group received SCA-M (C3–C12) from the 42nd to the 77th day of life at a dose of 0.5 kg of the drug per ton of feed. On day 42 of life, control weighing of piglets of

¹³ Dhanani K. C. H., Samson W. J., Edkins A. L. Fibronectin is a stress responsive gene regulated by HSF1 in response to geldanamycin. *Scientific Reports*. 2017. Vol. 7. Art. 17617.

both groups was carried out. For weighing, 4 animals from each group were randomly selected. For laboratory studies, 4 pigs from each group were selected on 42nd day of life.

All procedures with piglets were carried out in accordance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.06 “On the Protection of Animals from Brutal Treatment”, in accordance with the basic principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986) and approved by the Commission on Bioethics of Animal Experiments of Dnipro State Agrarian and Economic University. After euthanasia, the abdominal cavity was opened and the fragments of the duodenum were isolated.

Protein extract samples were obtained by intestinal tissue homogenizing in 25 mM Tris buffer (pH 7.4) containing 0.2% sodium dodecyl sulfate and a mixture of protease inhibitors. Homogenates were incubated for 60 min at +4°C to extract structural proteins and were centrifuged¹⁴. The concentration of total protein in each sample was determined by the Bradford method¹⁵. Samples were mixed in 1:1 ratio with Lemmli buffer containing 0.1 M dithiothreitol and boiled for 5 min. Protein samples were frozen and stored at -20°C for up to two weeks before analysis.

Fibronectin content was determined by immunoblotting as described previously¹⁶. Anti-fibronectin (Abcam, ab-194586) and anti-GAPDH (Santa-Cruz, sc-69879) antibodies diluted 1/1000 were used in this study. Densitometric analysis of the results was performed using Total Lab TL120 software. The molecular weight of stained polypeptide bands was identified by extrapolating the relative mobility of each polypeptide on the pre-stained proteins with known molecular weights (PageRuler Prestained Protein Ladder, Fermentas, Germany).

Total protein content was measured with Bradford method (Bradford 1976). The results of total protein content in blood serum and tissue extracts were expressed as a percentage relative to the control group. The obtained results are presented as an average $\pm$ standard deviation (SD) on histograms. The results were analyzed with using one-way analysis of variance (ANOVA) followed by

¹⁴ Low doses of imidacloprid induce disruption of intercellular adhesion and initiate proinflammatory changes in Caco-2 cells / V. S. Nedzvetsky et al. *Regulatory Mechanisms in Biosystems*. 2021. Vol. 12, No. 3. P. 430–437.

¹⁵ Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding / M. M. Bradford. *Analytical Biochemistry*. 1976. Vol. 72, No. 1–2. P. 248–254.

¹⁶ Water-soluble C60 fullerene ameliorates astroglial reactivity and TNF- α production in retina of diabetic rats / V. S. Nedzvetsky et al. *Regulatory Mechanisms in Biosystems*. 2019. Vol. 10, No. 4. P. 513–519.

Bonferroni's post-hoc test. Changes in indicators were considered significant at $P < 0.05$.

2. The results on bioeffects of fatty acids blend

To evaluate the effect of the drug SCA-M on productive parameters, the average daily weight gain of animals in the control and experimental groups was analyzed. The results showed a non-statistically significant increase in the piglet weight of the experimental group compared to that of the control group (Fig. 1).

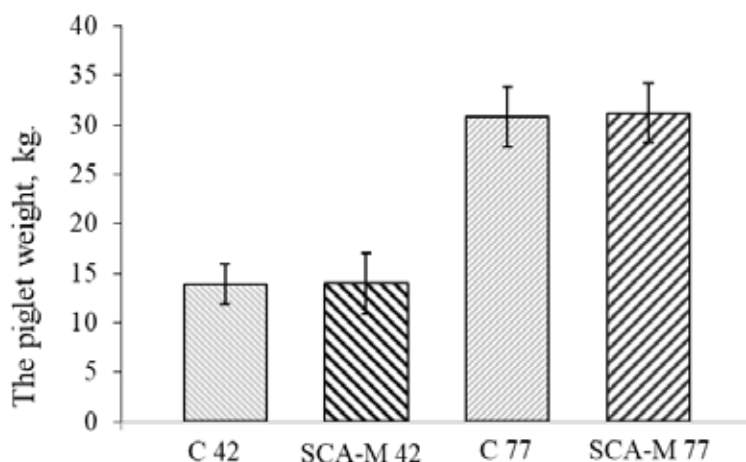


Fig. 1. Average weight gain of animals from 42nd to 77th days in the control group (C) and the group of piglets treated with SCA-M (SCA-M)

The total protein content in the blood serum and small intestine tissue extracts of piglets was determined to control the possible effect of SCA-M on protein metabolism and to normalize the immunoblot results corresponding to individual data. The results of total protein determination did not reveal a significant difference between the control and experimental groups in both blood serum and piglet intestinal tissue extracts (Fig. 2).

The fibronectin content in the small intestine tissue of piglets was determined to identify the SCA-M effect on the state of the extracellular matrix. The obtained results showed a significant increase in fibronectin content in the group of piglets consuming SCA-M compared to the control group (Fig. 3).

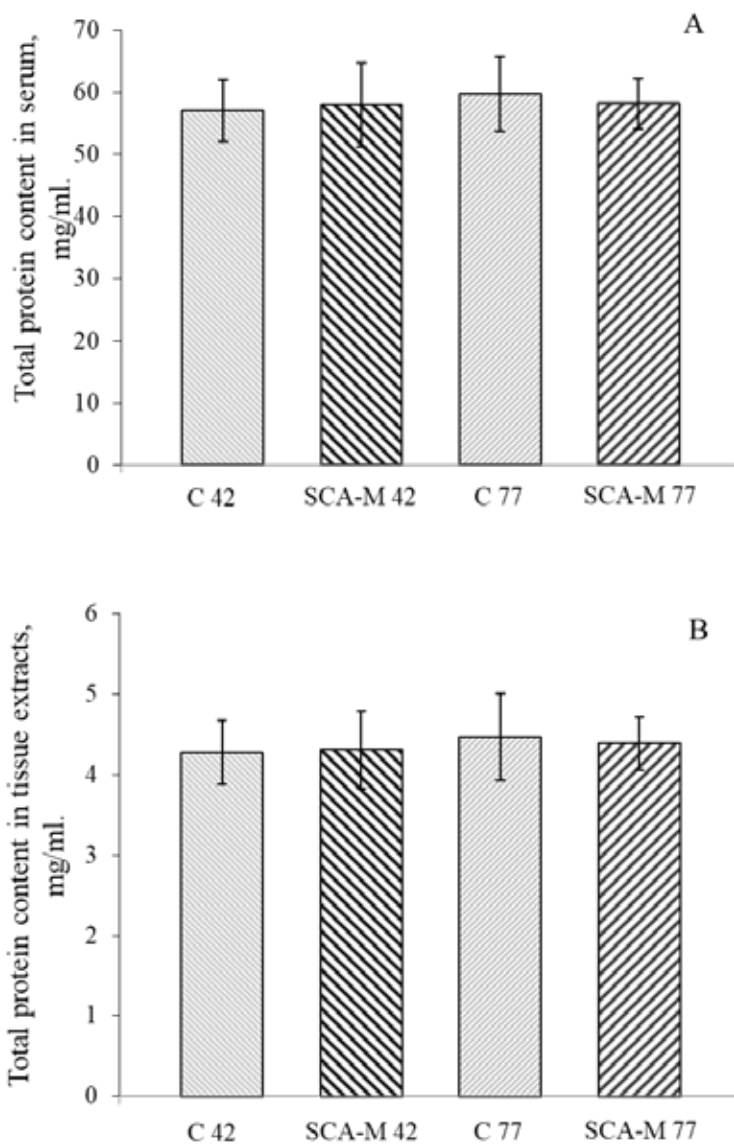


Fig. 2. Total protein content in blood serum (A) and small intestine tissue extracts (B) from control group piglets (C) and piglets treated with SCA-M

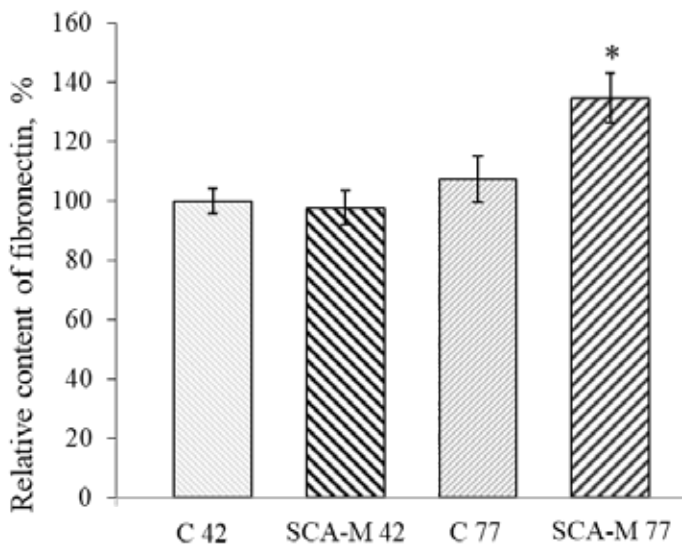


Fig. 3. Fibronectin content in the small intestine of the control group (C) and the group of piglets fed SCA-M. Significance of the differences in compare to 42th day SCA-M group: * – $P < 0,05$

In general, obtained results indicate that the consumption of SCA-M initiates an increase in the biosynthesis of ECM proteins, in particular, fibronectin.

3. The protective effects of fatty acids blend supplementation

The main tasks in the context of limited antibiotics usage are to ensure human and animal health, food safety, and increase the efficiency and quality of animal production. All these tasks cannot be achieved without understanding the molecular and cellular mechanisms of antibiotic alternatives actions. An equally important issue is the need to integrate research in nutrition, health and veterinary medicine. Both model experiments and field trials of possible antibiotic alternatives in different livestock production systems are considered necessary to address these challenges¹⁷. Such research should be effective if it is based on the study of fundamental indicators, namely, molecular markers of central pathways for cellular function control and whole organism viability support¹⁸.

¹⁷ Alternatives to antibiotics: a symposium on the challenges and solutions for animal health and production / T. R. Callaway et al. *Antibiotics (Basel)*. 2021. Vol. 10, No. 5. Art. 471.

¹⁸ Morphology of inner cell mass: a better predictive biomarker of blastocyst viability / S. Sivanantham et al. *Peer J*. 2022. Vol. 10. Art. e13935.

In recent years, large number research has been conducted to identify functional feed additives with similar beneficial effects as antibiotic growth promoters. The focus has been on prebiotics, probiotics and synbiotics, which are proposed as means of supporting intestinal health in various animals, including pigs¹⁹. A significant number of studies have shown some beneficial effects of such agents when using these functional feed additives as an alternative to antibiotics. The use of these agents can inhibit the pathogenic microflora growth and promote the commensal microbiota taxa progress. It has been shown that the suppression of *Lawsonia intracellularis* through the feed additive of the probiotic *Bacillus pumilus* can be one of the ways to maintain piglet health after weaning²⁰.

Recent results have shown that the use of probiotics can be beneficial in controlling intestinal infections in post-weaning piglets. It has also been suggested that a mixture of probiotics can be used to increase microbiota diversity, which mitigates the impact of *L. intracellularis* pathogenic infection²¹. The use of preparations containing Lactobacilli in piglets infected with pathogenic *Escherichia coli* K88 showed a beneficial effect associated with the villi morphology maintenance and expression of tight junction genes that regulate intestinal barrier function and permeability²².

A special place among the probiotics suggested as a replacement for antibiotics is occupied by Lactobacillus, which are well known and used by humans to make yoghurts and cheeses²³. Lactobacillus delbrueckii has been shown to inhibit the proliferation of various pathogenic taxa including Clostridiales²⁴. In addition, Lactobacillus delbrueckii improves the morphology of the mucosal layer, the length of villi and increases the content of butyrates, isobutyl and isovaleric acids, which provide antimicrobial effects in the animal intestinal system.

¹⁹ Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs / D. Shin et al. *PLoS ONE*. 2019. Vol. 14, No. 8. Art. e0220843.

²⁰ *Bacillus pumilus* probiotic feed supplementation mitigates *Lawsonia intracellularis* shedding and lesions / T. Opriessnig et al. *Veterinary Research*. 2019. Vol. 50, No. 1. Art. 85. DOI: <https://doi.org/10.1186/s13567-019-0696-1>.

²¹ Muwonge A., Karuppannan A. K., Opriessnig T. Probiotics mediated gut microbiota diversity shifts are associated with reduction in histopathology and shedding of *Lawsonia intracellularis* / A. Muwonge, A. K. Karuppannan, T. Opriessnig. *Animal Microbiome*. 2021. Vol. 3, No. 1. Art. 22.

²² Effect of *Lactobacillus plantarum* on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic *Escherichia coli* K88 / K. M. Yang et al. *Journal of Animal Science*. 2014. Vol. 92, No. 4. P. 1496–1503.

²³ Effects of varying dietary folic acid during weaning stress of piglets / L. Wang et al. *Animal Nutrition*. 2021a. Vol. 7, No. 1. P. 101–110.

²⁴ Effects of dietary supplementation of *Lactobacillus delbrueckii* on gut microbiome and intestinal morphology in weaned piglets / X. L. Wang et al. *Frontiers in Veterinary Science*. 2021b. Vol. 8. Art. 692389.

Another strategy based on the use of enzymatically processed plant products, in particular, soy has shown beneficial effects on feed transformation, immune response and intestinal barrier function in post-weaning piglets fed an antibiotic-free diet²⁵. Consumption of such product improved the small intestinal villi morphometric parameters and the expression of enterocyte adhesion proteins, including Z-occludin 1²⁶.

Unfortunately, there is currently no experimental evidence that individual antibiotic substitution strategies can be effective in terms of growth promotion and antimicrobial protection. On the other hand, given the individual properties of certain antibiotic candidates, combining individual strategies is a promising direction in developing an effective alternative to antibiotics. For example, a beneficial effect on intestinal health as an alternative to antibiotics has been shown as a result of the probiotic's combined usage with yeast hydrolysate²⁷.

The multifactorial beneficial effect has also been investigated in the study of the fatty acid mixture effects on the intestinal health of productive animals. The study of the action mechanisms of short- and medium-chain fatty acids on the functions of the animal intestinal system is one of the priority areas of livestock production, as well as poultry production. In recent years, significant progress in improving organic acid regimens for productivity has been shown in many research studies on broiler chickens^{28,29,30}. However, the rejection of the antibiotic usage as a growth stimulant in livestock production necessitates the search for alternative agents that simultaneously exhibit antimicrobial properties and provide sufficient animal growth intensity. A wide range of compounds have been suggested and investigated as potential alternatives to antibiotics. In particular, prebiotics, probiotics, organic acids, emulsifiers, enzymes, essential

²⁵ Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets / S. Long et al. *Animals*. 2021. Vol. 11, No. 9. Art. 2600.

²⁶ The effects of enzymatically treated soybean meal on growth performance and intestinal structure, barrier integrity, inflammation, oxidative status, and volatile fatty acid production of nursery pigs / L. A. Ruckman et al. *Translational Animal Science*. 2020. Vol. 4, No. 3. Art. txaa170.

²⁷ Effects of dietary *Bacillus coagulans* and yeast hydrolysate supplementation on growth performance, immune response and intestinal barrier function in weaned piglets / R. Fu et al. *Journal of Animal Physiology and Animal Nutrition*. 2021. Vol. 105, No. 5. P. 898–907.

²⁸ Potential feed additives as antibiotic alternatives in broiler production / H. Ayalew et al. *Frontiers in Veterinary Science*. 2022. Vol. 9. Art. 916473.

²⁹ Organic acid blends improve intestinal integrity, modulate short-chain fatty acids profiles and alter microbiota of broilers under necrotic enteritis challenge / A. Kumar et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 82–90.

³⁰ Effect of supplemental glycerol monolaurate and oregano essential oil blend on the growth performance, intestinal morphology, and amino acid digestibility of broiler chickens / S. A. Amer et al. *BMC Veterinary Research*. 2021. Vol. 17, No. 1. Art. 312.

oils, medium-chain fatty acids, and monoglycerides have received particular attention in the last decade^{31, 32, 33}.

Data on the combined effect of natural compounds as an alternative to the antibiotic use in weaned piglets are extremely limited. In particular, the combined use of benzoic acid, *Bacillus coagulans*, and oregano oil had beneficial effects on the immune status, microbiome, and intestinal barrier function in piglets³⁴.

The post-weaning piglet's intestinal system is extremely vulnerable to enteropathogens due to antibody deficiencies, lack of immune response and intestinal barrier function. Therefore, immune response maintenance, intestinal morphology and barrier function are considered to be the main issues for maintaining piglet health after weaning^{35, 36}. Unfortunately, the number of studies on the effects of short-chain fatty acids and monoglycerides as an alternative strategy to antibiotics in pig production is extremely limited. In particular, it has been shown that folic acid feed supplementation can improve growth performance and intestinal morphology of weaned piglets by maintaining the epithelial cell renewal balance³⁷.

Intestinal epithelial cells provide the most critical functions of the intestinal system, namely nutrient transport and barrier properties to toxins and pathogens³⁸. Given this fact, maintaining and rehabilitating damaged epithelial cells is a promising area for ensuring intestinal health. Recent results have demonstrated the beneficial effect of a monoglyceride blend on the transmission and spread of porcine epidemic diarrhea virus (PEDV) in piglets during early weaning³⁹. It should be noted that the monoglycerides mixture in this study

³¹ Role of yeast and yeast-derived products as feed additives in broiler nutrition / R. M. Bilal et al. *Animal Biotechnology*. 2023. Vol. 34, No. 2. P. 392–401.

³² Organic acid blends improve intestinal integrity, modulate short-chain fatty acids profiles and alter microbiota of broilers under necrotic enteritis challenge / A. Kumar et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 82–90.

³³ Progress on gut health maintenance and antibiotic alternatives in broiler chicken production / Q. Zhu et al. *Frontiers in Nutrition*. 2021. Vol. 8. Art. 692839.

³⁴ Effects of benzoic acid, *Bacillus coagulans* and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets / J. Pu et al. *Animal Nutrition*. 2020. Vol. 6, No. 2. P. 152–159.

³⁵ Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets / S. Long et al. *Animals*. 2021. Vol. 11, No. 9. Art. 2600.

³⁶ Bacteriophage as an alternative to antibiotics promotes growth performance by regulating intestinal inflammation, Intestinal barrier function and gut microbiota in weaned piglets / Y. Zeng et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 623899.

³⁷ Effects of dietary supplementation of *Lactobacillus delbrueckii* on gut microbiome and intestinal morphology in weaned piglets / X. L. Wang et al. *Frontiers in Veterinary Science*. 2021b. Vol. 8. Art. 692389.

³⁸ Beneficial effects of phytochemical feed additives on epithelial barrier integrity in an in vitro co-culture model of the piglet gut / D. Wendner et al. *Molecules*. 2023. Vol. 28, No. 3. Art. 1026.

³⁹ Monoglyceride reduces viability of porcine epidemic diarrhoea virus in feed and prevents disease transmission to post-weaned piglets / F. C. Phillips et al. *Transboundary and Emerging Diseases*. 2022. Vol. 69, No. 1. P. 121–127.

was constructed with using an integrated analysis of the individual properties of the individual components, which ensured a sufficiently high efficiency in preventing viral disease transmission to piglets from contaminated feed. Thus, the combination of different monoglycerides may be a promising direction for finding alternative strategies to antibiotics.

Another useful property of monoglycerides is their soft antimicrobial effect. Mixtures of free fatty acids and monoglycerides can destabilize bacterial cell membranes, causing a wide range of direct and indirect inhibitory effects⁴⁰. Recent studies have shown that the mechanisms of fatty acids and monoglycerides impact are aimed at remodeling bacterial phospholipid membranes and can cause irreversible damage that inhibits the growth of various microorganisms⁴¹. Recent studies have shown that the activity of fatty acids and their derivatives, including monoglycerides, is directed against various bacteria types⁴².

At the same time, the antibacterial activity is largely dependent on the composition of fatty acids and remains not fully understood. The variation of monoglyceride compositions in feed additives is an important factor to find out optimal bioeffect. Observed in our study results demonstrated beneficial effect on fibronectin content in intestinal tissue that could be initiated by SCA-M. In this line SCA-M activates cell metabolism via indirect mechanism related to multiple regulatory pathways as well as cell reactivity. In spite of recent reports, the chronic effect of various fatty acids and monoglycerides on the piglet intestinal system cells, in particular, on epithelial cells as the main target of enteropathogens and toxins, remains unexplored. Also, the possible toxic effects of these compounds on the intestinal epithelial barrier function remain unclear. Given the fact that epithelial cells form intercellular connections with the help of specialized tight junction proteins, adhesion proteins make a major contribution to the intestinal barrier function. The interaction of epithelial cells with ECM also plays a significant role in the intestinal barrier formation⁴³. In our study, the fibronectin content was determined to assess the state of ECM as an indicator of the cell-ECM adhesion strength. The results of the present study indicate a stimulating effect of the SCA-M mixture on the fibronectin content

⁴⁰ Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications / B. K. Yoon et al. *International Journal of Molecular Sciences*. 2018. Vol. 19, No. 4. Art. 1114.

⁴¹ Competing interactions of fatty acids and monoglycerides trigger synergistic phospholipid membrane remodeling / B. K. Yoon et al. *The Journal of Physical Chemistry Letters*. 2020. Vol. 11, No. 13. P. 4951–4957.

⁴² Churchward C. P., Alany R. G., Snyder L. A. S. Alternative antimicrobials: the properties of fatty acids and monoglycerides / C. P. Churchward, R. G. Alany, L. A. S. Snyder. *Critical Reviews in Microbiology*. 2018. Vol. 44, No. 5. P. 561–570.

⁴³ Beneficial effects of phytogetic feed additives on epithelial barrier integrity in an in vitro co-culture model of the piglet gut / D. Wendner et al. *Molecules*. 2023. Vol. 28, No. 3. Art. 1026.

and, accordingly, on the cell-ECM adhesion. The strength of such adhesion is a component of piglet health after weaning because this period is characterized as a trigger for the mature organism functions formation. Stimulation of ECM protein synthesis can promote cell fixation and activation of their specialized functions⁴⁴. Thus, an increase in fibronectin content can stimulate the cells of the intestinal system directly through interaction with the ECM, as well as modulate intercellular interaction of epithelial cells. The increase in fibronectin content determined in our study may reflect an increase in the epithelial cell-ECM adhesion strength, which enhances the integrative properties of the intestinal barrier. At the same time, the consumption of SCA-M had a slight effect on the productive parameters, which may be the result of a short-term exposure to the drug.

CONCLUSIONS

Consumption of SCA-M by piglets during 5 weeks contributes to an increase in fibronectin content in the small intestine tissue. Upregulation in fibronectin content could be a part of ECM strengthening and intestinal barrier maintaining. The further study with respect to molecular biomarkers of intestinal barrier modulation with organic acids is required.

SUMMARY

Searching for modern strategies to ensure productive parameters in the context of modern requirements for the relatively limited use of antibiotics as growth stimulants is relevant for the pig breeding industry. First and foremost, maintaining intestinal health is the main goal of feed additive application that have beneficial effects on the productive animals' health. Monitoring the intestinal system state and timely responding to the development of enteropathogens is essential in the context of limited antibiotics usage. Molecular markers of the intestinal system are promising tool for monitoring the basic functions of the intestine, including the intestinal barrier. Given the fact that protection against enteropathogens is provided by specialized cell-cell and cell-extracellular matrix adhesions, changes in the content of intercellular adhesion proteins may reflect the intestinal barrier density. Fatty acid mixtures are considered to be one of the promising alternatives to the antibiotics use in animal farming. In order to determine the intestinal barrier function status, the modulation of fibronectin content as the extracellular matrix marker in piglets was determined after consumption of a short-chain

⁴⁴ Marlar S., Abdellatef S. A., Nakanishi J. Reduced adhesive ligand density in engineered extracellular matrices induces an epithelial-mesenchymal-like transition / S. Marlar, S. A. Abdellatef, J. Nakanishi. *Acta Biomaterialia*. 2016. Vol. 39. P. 106–113.

fatty acid-monoglyceride mixture (SCA-M). Control group of animals consumed a standard diet. The experimental group exposed to a mixture of SCA-M at a dose of 5 g/kg for 4 weeks. The fibronectin content was determined by immunoblotting in the small intestine tissue of the control and experimental groups piglets. It was shown that the fibronectin content in piglets of the experimental group was significantly higher ($P < 0.05$) compared to the control group. The determination of total protein in the blood serum and intestinal tissue extracts of piglets did not showed a significant difference between the control and experimental groups. Comparative analysis of the average daily weight gain found out a tendency to a more progressive increase in piglet weight. In general, the obtained results indicate a beneficial SCA-M effect on the fibronectin content modulation and intestinal barrier density maintenance in piglets. Comprehensive molecular markers studies of the piglet intestinal system will be useful for the advance of antibiotic-free strategies in accordance with modern pig breeding requirements.

Bibliography

1. Effect of supplemental glycerol monolaurate and oregano essential oil blend on the growth performance, intestinal morphology, and amino acid digestibility of broiler chickens / S. A. Amer et al. *BMC Veterinary Research*. 2021. Vol. 17, No. 1. Art. 312. DOI: <https://doi.org/10.1186/s12917-021-03022-5>.
2. Potential feed additives as antibiotic alternatives in broiler production / H. Ayalew et al. *Frontiers in Veterinary Science*. 2022. Vol. 9. Art. 916473. DOI: <https://doi.org/10.3389/fvets.2022.916473>.
3. Role of yeast and yeast-derived products as feed additives in broiler nutrition / R. M. Bilal et al. *Animal Biotechnology*. 2023. Vol. 34, No. 2. P. 392–401. DOI: <https://doi.org/10.1080/10495398.2021.1942028>.
4. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding / M. M. Bradford. *Analytical Biochemistry*. 1976. Vol. 72, No. 1–2. P. 248–254. DOI: <https://doi.org/10.1006/abio.1976.9999>.
5. Alternatives to antibiotics: a symposium on the challenges and solutions for animal health and production / T. R. Callaway et al. *Antibiotics (Basel)*. 2021. Vol. 10, No. 5. Art. 471. DOI: <https://doi.org/10.3390/antibiotics10050471>.
6. Churchward C. P., Alany R. G., Snyder L. A. S. Alternative antimicrobials: the properties of fatty acids and monoglycerides / C. P. Churchward, R. G. Alany, L. A. S. Snyder. *Critical Reviews in Microbiology*. 2018. Vol. 44, No. 5. P. 561–570. DOI: <https://doi.org/10.1080/1040841X.2018.1467875>.
7. Dhanani K. C. H., Samson W. J., Edkins A. L. Fibronectin is a stress responsive gene regulated by HSF1 in response to geldanamycin.

Scientific Reports. 2017. Vol. 7. Art. 17617. DOI: <https://doi.org/10.1038/s41598-017-18061-y>.

8. Effects of dietary *Bacillus coagulans* and yeast hydrolysate supplementation on growth performance, immune response and intestinal barrier function in weaned piglets / R. Fu et al. *Journal of Animal Physiology and Animal Nutrition*. 2021. Vol. 105, No. 5. P. 898–907. DOI: <https://doi.org/10.1111/jpn.13529>.

9. Garcia M. A., Nelson W. J., Chavez N. Cell-cell junctions organize structural and signaling networks / M. A. Garcia, W. J. Nelson, N. Chavez. *Cold Spring Harbor Perspectives in Biology*. 2018. Vol. 10, No. 4. Art. a029181. DOI: <https://doi.org/10.1101/cshperspect.a029181>.

10. Integrity of the intestinal barrier: The involvement of epithelial cells and microbiota – a mutual relationship / M. Gierynska et al. *Animals*. 2022. Vol. 12, No. 2. Art. 145. DOI: <https://doi.org/10.3390/ani12020145>.

11. Green K. J., Jaiganesh A., Broussard J. A. Desmosomes: Essential contributors to an integrated intercellular junction network / K. J. Green, A. Jaiganesh, J. A. Broussard. *F1000Research*. 2019. Vol. 8. Art. F1000 Faculty Rev-2150. DOI: <https://doi.org/10.12688/f1000research.20942.1>.

12. Johnson J. L., Najor N. A., Green K. J. Desmosomes: regulators of cellular signaling and adhesion in epidermal health and disease / J. L. Johnson, N. A. Najor, K. J. Green. *Cold Spring Harbor Perspectives in Medicine*. 2014. Vol. 4, No. 11. Art. a015297. DOI: <https://doi.org/10.1101/cshperspect.a015297>.

13. Organic acid blends improve intestinal integrity, modulate short-chain fatty acids profiles and alter microbiota of broilers under necrotic enteritis challenge / A. Kumar et al. *Animal Nutrition*. 2022. Vol. 8, No. 1. P. 82–90. DOI: <https://doi.org/10.1016/j.aninu.2021.04.003>.

14. Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets / S. Long et al. *Animals*. 2021. Vol. 11, No. 9. Art. 2600. DOI: <https://doi.org/10.3390/ani11092600>.

15. Maitre J. L., Heisenberg C. P. Three functions of cadherins in cell adhesion / J. L. Maitre, C. P. Heisenberg. *Current Biology*. 2013. Vol. 23, No. 14. P. 626–633. DOI: <https://doi.org/10.1016/j.cub.2013.06.019>.

16. Marlar S., Abdellatef S. A., Nakanishi J. Reduced adhesive ligand density in engineered extracellular matrices induces an epithelial-mesenchymal-like transition / S. Marlar, S. A. Abdellatef, J. Nakanishi. *Acta Biomaterialia*. 2016. Vol. 39. P. 106–113. DOI: <https://doi.org/10.1016/j.actbio.2016.05.006>.

17. Muwonge A., Karuppannan A. K., Opriessnig T. Probiotics mediated gut microbiota diversity shifts are associated with reduction in histopathology and shedding of *Lawsonia intracellularis* / A. Muwonge, A. K. Karuppannan,

T. Opriessnig. *Animal Microbiome*. 2021. Vol. 3, No. 1. Art. 22. DOI: <https://doi.org/10.1186/s42523-021-00084-6>.

18. Low doses of imidacloprid induce disruption of intercellular adhesion and initiate proinflammatory changes in Caco-2 cells / V. S. Nedzvetsky et al. *Regulatory Mechanisms in Biosystems*. 2021. Vol. 12, No. 3. P. 430–437. DOI: <https://doi.org/10.15421/022159>.

19. Water-soluble C60 fullerene ameliorates astroglial reactivity and TNF- α production in retina of diabetic rats / V. S. Nedzvetsky et al. *Regulatory Mechanisms in Biosystems*. 2019. Vol. 10, No. 4. P. 513–519. DOI: <https://doi.org/10.15421/021975>.

20. Paone P., Cani P. D. Mucus barrier, mucins and gut microbiota: the expected slimy partners / P. Paone, P. D. Cani. *Gut*. 2020. Vol. 69, No. 12. P. 2232–2243. DOI: <https://doi.org/10.1136/gutjnl-2020-322260>.

21. Monoglyceride reduces viability of porcine epidemic diarrhoea virus in feed and prevents disease transmission to post-weaned piglets / F. C. Phillips et al. *Transboundary and Emerging Diseases*. 2022. Vol. 69, No. 1. P. 121–127. DOI: <https://doi.org/10.1111/tbed.14353>.

22. Effects of benzoic acid, Bacillus coagulans and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets / J. Pu et al. *Animal Nutrition*. 2020. Vol. 6, No. 2. P. 152–159. DOI: <https://doi.org/10.1016/j.aninu.2020.02.004>.

23. The effects of enzymatically treated soybean meal on growth performance and intestinal structure, barrier integrity, inflammation, oxidative status, and volatile fatty acid production of nursery pigs / L. A. Ruckman et al. *Translational Animal Science*. 2020. Vol. 4, No. 3. Art. txaa170. DOI: <https://doi.org/10.1093/tas/txaa170>.

24. Sardinha-Silva A., Alves-Ferreira E. V. C., Grigg M. E. Intestinal immune responses to commensal and pathogenic protozoa / A. Sardinha-Silva, E. V. C. Alves-Ferreira, M. E. Grigg. *Frontiers in Immunology*. 2022. Vol. 13. Art. 963723. DOI: <https://doi.org/10.3389/fimmu.2022.963723>.

25. Shen L., Turner J. R. Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: tight junction dynamics exposed / L. Shen, J. R. Turner. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. 2006. Vol. 290, No. 4. P. 577–582. DOI: <https://doi.org/10.1152/ajpgi.00439.2005>.

26. Interaction between the gut microbiome and mucosal immune system / N. Shi et al. *Military Medical Research*. 2017. Vol. 4, No. 1. Art. 14. DOI: <https://doi.org/10.1186/s40779-017-0122-9>.

27. Bacillus pumilus probiotic feed supplementation mitigates Lawsonia intracellularis shedding and lesions / T. Opriessnig et al. *Veterinary Research*. 2019. Vol. 50, No. 1. Art. 85. DOI: <https://doi.org/10.1186/s13567-019-0696-1>.

28. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs / D. Shin et al. *PLoS ONE*. 2019. Vol. 14, No. 8. Art. e0220843. DOI: <https://doi.org/10.1371/journal.pone.0220843>.
29. Morphology of inner cell mass: a better predictive biomarker of blastocyst viability / S. Sivanantham et al. *Peer J*. 2022. Vol. 10. Art. e13935. DOI: <https://doi.org/10.7717/peerj.13935>.
30. Suzuki T. Regulation of the intestinal barrier by nutrients: The role of tight junctions / T. Suzuki. *Animal Science Journal*. 2020. Vol. 91, No. 1. Art. e13357. DOI: <https://doi.org/10.1111/asj.13357>.
31. Turner J. R. Intestinal mucosal barrier function in health and disease / J. R. Turner. *Nature Reviews Immunology*. 2009. Vol. 9, No. 11. P. 799–809. DOI: <https://doi.org/10.1038/nri2653>.
32. Effects of varying dietary folic acid during weaning stress of piglets / L. Wang et al. *Animal Nutrition*. 2021a. Vol. 7, No. 1. P. 101–110. DOI: <https://doi.org/10.1016/j.aninu.2020.12.002>.
33. Effects of dietary supplementation of *Lactobacillus delbrueckii* on gut microbiome and intestinal morphology in weaned piglets / X. L. Wang et al. *Frontiers in Veterinary Science*. 2021b. Vol. 8. Art. 692389. DOI: <https://doi.org/10.3389/fvets.2021.692389>.
34. Beneficial effects of phytogetic feed additives on epithelial barrier integrity in an in vitro co-culture model of the piglet gut / D. Wendner et al. *Molecules*. 2023. Vol. 28, No. 3. Art. 1026. DOI: <https://doi.org/10.3390/molecules28031026>.
35. Bacteriophage as an alternative to antibiotics promotes growth performance by regulating intestinal inflammation, Intestinal barrier function and gut microbiota in weaned piglets / Y. Zeng et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 623899. DOI: <https://doi.org/10.3389/fvets.2021.623899>.
36. Progress on gut health maintenance and antibiotic alternatives in broiler chicken production / Q. Zhu et al. *Frontiers in Nutrition*. 2021. Vol. 8. Art. 692839. DOI: <https://doi.org/10.3389/fnut.2021.692839>.
37. Effect of *Lactobacillus plantarum* on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic *Escherichia coli* K88 / K. M. Yang et al. *Journal of Animal Science*. 2014. Vol. 92, No. 4. P. 1496–1503. DOI: <https://doi.org/10.2527/jas.2013-6619>.
38. Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications / B. K. Yoon et al. *International Journal of Molecular Sciences*. 2018. Vol. 19, No. 4. Art. 1114. DOI: <https://doi.org/10.3390/ijms19041114>.
39. Competing interactions of fatty acids and monoglycerides trigger synergistic phospholipid membrane remodeling / B. K. Yoon et al. *The Journal*

of Physical Chemistry Letters. 2020. Vol. 11, No. 13. P. 4951–4957. DOI: <https://doi.org/10.1021/acs.jpcllett.0c01138>.

40. Yulis M., Kusters D. H. M., Nusrat A. Cadherins: cellular adhesive molecules serving as signalling mediators / M. Yulis, D. H. M. Kusters, A. Nusrat. *The Journal of Physiology*. 2018. Vol. 596, No. 17. P. 3883–3898. DOI: <https://doi.org/10.1113/JP275328>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,
Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,

Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Kokariiev Andrii,

Candidate of Veterinary Sciences, Associate Professor,
Head of the Department of Immunochemical and Molecular Genetic
Analysis of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

**CURRENT APPROACHES IN MAINTENANCE GUT
HEALTH IN FARMING ANIMALS.
PART 3. THE PROSPECTS OF ORGANIC ACIDS
SUPPLEMENTATION IN POULTRY FEED ADDITIVES**

Masiuk Dmytro, Nedzvetsky Victor, Kokariev Andrii
DOI <https://doi.org/10.30525/978-9934-26-714-7-10>

INTRODUCTION

Poultry farming is the most widespread form of livestock production in the world, due to its relatively low cost and short production cycle. At the current stage of poultry development, the best possible conditions have been created to fulfil the genetic potential of animals. According to N. Haulisah et al. (2021)¹, one of the key methods of increasing broiler productivity since the 1950s has been the use of antibiotics as growth promoters (GPs). However, the use of these substances has brought the issue of antibiotic resistance to a new level that threatens humanity. The most widely used antibiotics, as GPs, have been used to improve feed conversion and health status of poultry². The abandonment of antibiotics has initiated an increase in the number of studies on effective alternative methods of monitoring and correcting animal health, welfare and productivity, including in poultry farming.

A wide range of products have been proposed to replace antibiotics in feed, among which organic acids (OAs) show promising results³. The pool of carboxylic acids that are most common in living organisms is represented by free fatty acids and those that are part of the lipids of living organisms. According to D. Venegas et al. (2019)⁴, according to the chain length, fatty acids

¹ High levels of antibiotic resistance in isolates from diseased livestock / N. A. Haulisah et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 652351.

² Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass yields, muscle amino acid and fatty acid profiles, and antioxidant indices of broilers supplemented with synbiotic and/or organic acids / A. S. Salah, O. A. Ahmed-Farid, M. S. El-Tarabany. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52.

³ In vitro investigation of the antibacterial activity of nine commercial water disinfectants, acidifiers, and glyceride blends against the most important poultry zoonotic bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381. DOI: <https://doi.org/10.3390/pathogens12030381>.

⁴ Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277.

are divided into short-chain fatty acids (SCFAs) – containing up to 6 carbon atoms, medium-chain fatty acids (MCFAs) – containing 6–12 carbon atoms, and long-chain fatty acids (LCFAs) – containing 13–21 carbon atoms. There is also (very long) elongated chain fatty acids (VLCFAs), which have over 22 carbon atoms. All SCFAs have varying degrees of water solubility, which distinguishes them from LCFAs, which are insoluble. In the poultry industry, SCFAs are used more frequently and somewhat less frequently than MCFAs as an alternative to antibacterial preparations and growth stimulants. G. Galli et al. (2021)⁵ note that the effectiveness of SCFAs and MCFAs is influenced by a considerable number of factors, among which the key ones are the chemical structure, form of additive, its amount and method of supplementation, buffer capacity of feed, and its nutritional value.

Organic acids are widely used as additives in drinking water or as feed additives. They are often used in the salt forms (sodium, potassium, or calcium and/or partially esterified). According to M. Aljumaah et al. (2020)⁶, the advantage of using salts is their odourlessness, solid form (the purest organic salts are volatile), lower corrosivity, and better solubility. The efficiency of an organic acid depends on its molecular weight, pKa value and form (undissociated or dissociated), which together determine the difference in antimicrobial activity and bioavailability. Several OAs have a specific bioavailability. The bioavailability of OAs can be improved by choosing the correct form of additive that promotes OA adsorption by cells. F. Mannelli et al. (2019)⁷, N. Qi et al. (2023)⁸ pointed out that the use of fatty acid salts is the simplest solution, as these salts are solid, which facilitates the technology of feed production and improves their organoleptic properties. Microencapsulation is considered a promising way to deliver organic acids to the digestive tract. Therewith, the composition of the coating that envelops the OA ensures a more efficient release of these substances in the right place in the digestive tract.

The purpose of this study was to summarise the current comprehension and prospects for the use of short- and medium-chain organic acids in poultry farming as safe substitutes for antimicrobial agents and/or growth promoters based on the analysis of available literature.

⁵ Growth performance and meat quality of broilers fed with microencapsulated organic acids / G. M. Galli et al. *Animal Feed Science and Technology*. 2021. Vol. 271. Art. 114706.

⁶ Organic acid blend supplementation increases butyrate and acetate production in *Salmonella enterica* serovar Typhimurium challenged broilers / M. R. Aljumaah et al. *PLoS ONE*. 2020. Vol. 15, No. 6. Art. e0232831.

⁷ Effect of chestnut tannins and short chain fatty acids as anti-microbials and as feeding supplements in broilers rearing and meat quality / F. Mannelli et al. *Animals*. 2019. Vol. 9, No. 9. Art. 659.

⁸ Study of microencapsulated fatty acid antimicrobial activity in vitro and its prevention ability of *Clostridium perfringens* induced necrotic enteritis in broiler chicken / N. Qi et al. *Gut Pathogens*. 2023. Vol. 15. Art. 1.

1. Application of SCFAs in poultry farming

OAs are organic compounds with acidic properties, the most common of which are carboxylic acids, whose acidity is related to their carboxylic group – COOH. These acids are weak, partially dissociated and have a pKa (pH at which the acid is half dissociated) of 3 to 5. SCFAs used in poultry production include oil, vinegar, propionic, dairy, formic, apple, wine, fumaric, and sorbic⁹.

SCFAs (acetate, propionate, and butyrate) are produced by bacterial fermentation in the intestine and have certain effects on metabolism and the immune system. Apart from being important cross-nutrition products, microbial metabolites also have a positive effect on the mucous membrane of the digestive tract. SCFAs play an important role in the metabolic and immune homeostasis of the digestive tract, as well as in the integrity of the intestinal barrier, which opens great opportunities for therapeutic development¹⁰.

The intestinal microbiota releases SCFAs (mainly acetate, propionate, and butyrate) in the colon during fermentation, from where they are absorbed by the colon epithelium through passive and active transport. The pathways of formation of various SCFAs and the corresponding microbial producers were identified. The products of microbial fermentation of SCFAs are the main source of energy for colonocytes¹¹. At the same time, all commensal gut taxa ferment pyruvate to produce butyrate, while pathogenic bacteria, such as *Fusobacterium*, use different pathways, such as glutamate (4-aminobutyrate) and lysine, which are associated with the release of harmful by-products, such as ammonia.

The key factors that limit the production of SCFAs in the digestive tract include pH, growth factors, and gas levels. Carbohydrates, which lead to high production of SCFAs, lower the pH in the colon, which affects the microbial composition and production of SCFAs. The availability of growth factors such as iron is crucial for the production of SCFAs. Significantly lower concentrations of butyrate and propionate were found in the faeces of iron-deficient rats. Iron deficiency induces an increase in the number of *Lactobacilli* and *Enterobacteriaceae* species and, conversely, a decrease in *Roseburia* and *Eubacterium rectale* species, which are the main producers of butyrate. Oxygen and Hydrogen levels affect the fermentation process and the production of SCFAs¹². Overall, only 4 SCFAs are currently widely used in poultry production (Table 1).

⁹ Ricke S. C., Dittoe D. K., Richardson K. E. Formic acid as an antimicrobial for poultry production: A review. Richardson. *Frontiers in Veterinary Science*. 2020. Vol. 7. Art. 533419.

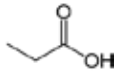
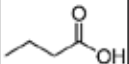
¹⁰ Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277.

¹¹ Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? / S. Deleu et al. *EBioMedicine*. 2021. Vol. 66. Art. 103293.

¹² Louis P., Flint H. J. Formation of propionate and butyrate by the human colonic microbiota / P. Louis, H. J. Flint. *Environmental Microbiology*. 2017. Vol. 19, No. 1. P. 29–41.

Table 1

Short-chain fatty acids used in poultry production

Name		Formula		Mass (g/mol)	Diagram
Common	Systematic	Molecular	Structural		
Formic acid	Methanoic acid	CH_2O_2	COOH	46.0	
Acetic acid	Ethanoic acid	$\text{C}_2\text{H}_4\text{O}_2$	CH_3COOH	60.1	
Propionic acid	Propanoic acid	$\text{C}_3\text{H}_6\text{O}_2$	$\text{CH}_3\text{CH}_2\text{COOH}$	74.1	
Butyric acid	Butanoic acid	$\text{C}_4\text{H}_8\text{O}_2$	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$	88.1	

Source: compiled by the authors of this study based on the literature data

Acetic, butyric, and propionic acids are the most abundant SCFAs in the colon, where they have a positive effect on energy status by providing a carbon source for the intestinal microbiota through the activation of glyoxylate pathway enzymes. In commercial poultry production, SCFAs are mainly associated with antimicrobial activity and increased productivity¹³. The antimicrobial effect of propionic and butyric acids is conditioned by the acid dissociation constant ($\text{pK}_a=3-5$). Once in the intestine, they lower the pH, which inhibits potentially pathogenic bacteria, and increase the level of calcium, phosphorus, and magnesium in the blood serum due to improved absorption¹⁴.

Lactic acid is used in poultry production both in pure form and in the form of butyrates, coated/encapsulated butyrate salts¹⁵ and butyrate glycerols (butyrene). Each product formulation has its own advantages and limitations in terms of bioavailability, cost, biosafety, stability, processing temperature/pressure, and digestive release. The use of an encapsulated form of lactic acid in laying hens showed better results than butyrate.

¹³ Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: A Review / F. Scicutella et al. *Antibiotics*. 2021. Vol. 10, No. 8. Art. 1010.

¹⁴ Effect of supplementation of salts of organic acids on serum and haematological parameters of broilers / V. Us et al. *International Journal of Current Microbiology and Applied Sciences*. 2017. Vol. 6, No. 11. P. 4211–4218.

¹⁵ Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers / D. H. Nguyen et al. *Poultry Science*. 2018. Vol. 97, No. 12. P. 4351–4358.

Butyric acid is one of the main acids that are successfully used in industrial poultry farming. This acid modulates the state of symbiotic microbiota and improves immunological homeostasis in the intestine. Butyrates (calcium or sodium salts) are easily converted to butyric acid in the digestive tract and are considered safe. Compared to other MCFAs, butyrate has a slight antibacterial effect, but is not expensive. The use of butyrate reduces the concentration of total circulating triglycerides and cholesterol in broilers¹⁶. Sodium butyrate is the most common form of butyric acid. There (are a series) is a number of studies showing that the addition of butyric acid, in various doses, from 0.2 g/kg to 0.6 g/kg, to broiler chickens' diets improves performance, digestibility, and nutrient absorption, and reduces the incidence of disease. The mechanism of butyrate effect is that when sodium butyrate enters the stomach, it releases the Na ion¹⁷. Due to the low pH, butyrate is rapidly converted to the undissociated form of butyric acid¹⁸.

Butyric acid is formed by microbial fermentation in the colon and is the main source of energy for colonocytes, affects their proliferation, differentiation, gene expression, and protein synthesis, and improves its absorption. Butyric acid reduces bacterial colonisation of the intestinal wall by lowering the pH, which reduces the tendency to diarrhoea. The addition of butyric acid or butyric acid glycerides to turkey diets was shown to have a beneficial effect on feed conversion, intestinal morphology, and bird health by reducing pathogen concentrations in faeces¹⁹. The use of feed additives based on butyric acid inhibits the spread of Salmonella infection and contamination of bedding with pathogens.

The addition of 0.5% acetic, lactic, or formic acid to drinking water limits the growth of *S. Typhimurium* in chickens²⁰. However, sublethal concentrations of undissociated acetic acid may not always stimulate the acid resistance of *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage²¹. A relatively new

¹⁶ Effects of dietary short-and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens / A. Khatibjoo et al. *Journal of Applied Animal Research*. 2018. Vol. 46, No. 1. P. 492–498.

¹⁷ Dietary sodium butyrate and/or vitamin D3 supplementation alters growth performance, meat quality, chemical composition, and oxidative stability in broilers / H. Gao et al. *Food Chemistry*. 2022. Vol. 390. Art. 133138.

¹⁸ Effect of sodium butyrate on intestinal health of poultry – a review / S. S. Elnesr et al. *Annals of Animal Science*. 2020. Vol. 20, No. 1. P. 29–41.

¹⁹ Makowski Z., Lipiński K., Mazur-Kuśnerek M. The effects of different forms of butyric acid on the performance of turkeys, carcass quality, incidence of footpad dermatitis and economic efficiency / Z. Makowski, K. Lipiński, M. Mazur-Kuśnerek. *Animals*. 2022. Vol. 12, No. 11. Art. 1458.

²⁰ The control of poultry salmonellosis using organic agents: An updated overview / M. T. El-Saadony et al. *Poultry Science*. 2022. Vol. 101, No. 4. Art. 101716.

²¹ Gavriil A., Thanasoulia A., Skandamis P. N. Sublethal concentrations of undissociated acetic acid may not always stimulate acid resistance in *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage Type 4: Implications of challenge substrate associated factors / A. Gavriil, A. Thanasoulia, P. N. Skandamis. *PLoS ONE*. 2020. Vol. 15, No. 7. Art. e0234999.

area is the use of dietary fermented fibres to produce bioactive fatty acids in the intestines of animals²². The addition of 1% wheat bran with a particle size of 280 µm to the feed leads to its rapid fermentation in the digestive tract with the formation of butyric acid. These feed additives improve the performance of broiler chickens. Glycerol monolaurate is successfully used in poultry feeding, which not only improves meat quality, but also has antibacterial properties at a dose of 300 mg/kg against *Escherichia coli* and *Eimeria* spp.²³. The expressed antimicrobial effect of LCFAs formed as a result of fermentation of cranberry processing waste was shown. The beneficial effect of high doses of α -linolenic acid (21.0%) and linoleic acid (39.7%) obtained from fermentation products was aimed at preventing encephalomalacia, improving the immune response against infectious bursal disease virus (IBDV) and Newcastle disease²⁴.

2. Effect of SCFA on metabolism and immune defence

SCFAs are transported through the basolateral mucosa of the colon to the portal bloodstream, probably with the participation of SCFA-specific receptors. SCFA receptors, which are activated by acetate and propionate, were found on intestinal endocrine L cells. The GPR 109A receptor, which is expressed by colon epithelial cells, is activated by butyrate and provides IL10 production and an anti-inflammatory response. The OLFR 78 receptor is expressed in renal blood vessels and is involved in blood pressure regulation²⁵. Despite a considerable amount of information on the effect of SCFAs on metabolism and energy homeostasis, the mechanisms of transport and biological activity of SCFAs are still understudied.

Along with the studied protective effects, there is an evidence of the damaging effect of butyrate on the intestinal barrier in the inflammation presence²⁶. Comparable results were obtained regarding the protection of colon stem cells from microbial metabolites. Some studies have shown conflicting data on the positive effect of butyrate on the intestinal barrier. For instance,

²² Microbial short-chain fatty acids: A bridge between dietary fibers and poultry gut health – A review / Q. Ali et al. *Animal Bioscience*. 2022. Vol. 35, No. 10. P. 1461–1478.

²³ Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: Impact on health, performance and meat quality / B. F. Fortuoso et al. *Microbial Pathogenesis*. 2019. Vol. 129. P. 161–167.

²⁴ Dietary organic cranberry pomace influences multiple blood biochemical parameters and cecal microbiota in pasture-raised broiler chickens / M. R. Islam et al. *Journal of Functional Foods*. 2020. Vol. 72. Art. 104053.

²⁵ The olfactory G protein-coupled receptor (Olfir-78/OR51E2) modulates the intestinal response to colitis / K. Kotlo et al. *American Journal of Physiology-Cell Physiology*. 2020. Vol. 318, No. 3. P. 502–513.

²⁶ Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis / M. Vancamelbeke et al. *Journal of Crohn's & Colitis*. 2019. Vol. 13, No. 10. P. 1351–1361.

butyrate promotes the growth of SCFA-producing bacteria in patients with digestive disorders²⁷. Oral administration of butyrate to mice aggravated colitis, while its intraperitoneal administration improved the condition of the animal health. On the other hand, inhibition of butyrate-producing microorganisms caused an exacerbation of colitis in mice. Acetate supplementation in mice plays a crucial role in the intestinal response to tissue damage and repair in colitis²⁸. Undoubtedly, further study (more research) is needed on both microbiome-derived and dietary-supplemented SCFAs pool to apply SCFAs in digestive tract diseases. Despite the aforementioned data, the use of SCFAs for therapeutic purposes, their optimal therapeutic doses and indications for the use of SCFA-producing bacteria or SCFAs are still unresolved.

In vitro studies have provided evidence of the beneficial effects of butyrate on the intestinal barrier. Butyrate contributes to the Butyrate contributes to the intestinal barrier integrity by activating genes whose products maintain transepithelial electrical resistance (TEER). Butyrate has been shown to stimulate the energy metabolism of intestinal epithelial cells, modulate the production of hypoxia inducible factor 1 (HIF-1) and transcription factors, which together support the intestinal barrier function. Particularly important are the results of SCFAs' impact on the intestinal barrier and the immune system. SCFAs can modulate the adaptive immune system by stimulating macrophages and dendritic cells. SCFAs can initiate the transformation of naïve T cells into regulatory T cells²⁹. Apart from polarising regulatory T cells, SCFAs also affect the polarisation and activation of Th1, Th2, and Th17 cells by inhibiting histone deacetylase (HDAC). Acetate, propionate, and butyrate also promote Th1 and Th17 differentiation in vitro. HDAC inhibition by butyrate and propionate promotes IFN- γ expression, which enhances antiviral immunity³⁰.

The effect of SCFAs on certain types of immune cells (neutrophils, monocytes, and macrophages) is also achieved through a decrease in histone deacetylase (HDAC) levels³¹. The effects of SCFA on cytokine transcription are conditioned by the activation of the nuclear factor kappa B cell (NF- κ B). Thus, SCFAs are involved in the regulation of cellular response genes expression

²⁷ Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease / S. Facchin et al. *Neurogastroenterology & Motility*. 2020. Vol. 32, No. 10. Art. e13914.

²⁸ A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice / M. Laffin et al. *Scientific Reports*. 2019. Vol. 9. Art. 12294.

²⁹ Oliveira L. M., Teixeira F. M. E., Sato M. N. Impact of retinoic acid on immune cells and inflammatory diseases / L. M. Oliveira, F. M. E. Teixeira, M. N. Sato. *Mediators of Inflammation*. 2018. Vol. 2018. Art. 3067126.

³⁰ Regulation of the effector function of CD8⁺ T cells by gut microbiota-derived metabolite butyrate / M. Luu et al. *Scientific Reports*. 2018. Vol. 8. Art. 14430.

³¹ Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs) / W. Ratajczak et al. *Acta Biochimica Polonica*. 2019. Vol. 66, No. 1. P. 1–12.

to damage, including infectious agents. Stimulation of cytokine production is a universal mechanism that ensures an adequate cellular response. In this response, it is critical to ensure a balance between pro- and anti-inflammatory factors. The ability of SCFAs to regulate anti-inflammatory cytokines and pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, and IFN- γ was found in broilers and piglets³². Furthermore, SCFAs affect neutrophil chemotaxis induced by inflammatory mediators. SCFAs can affect the differentiation and function of dendritic cells, specifically, *in vitro* studies showed butyrate inhibiting their maturation during incubation with various inflammatory inducers.

In addition to their regulatory effects, SCFAs are a complete source of energy for enterocytes. The content of SCFAs in the chicken caecum varies according to the content of the dominant microbiota³³. Microbiota modification with antibiotics in mice showed a strong association between SCFAs content and the number of *Bacteroides* in the caecum. Elevated concentrations of SCFAs are considered beneficial for gut health by lowering pH and inhibiting pathogens. However, too high a SCFAs content can inhibit beneficial taxa together with pathogens. This undesirable effect of organic acids supplementation to the diet reduces feed intake and inhibits weight gain in broiler chickens.

SCFAs inhibit cholesterol synthesis by inhibiting the activity of the enzymes 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Thus, SCFAs are involved in the indirect regulation of energy metabolism by modulating cholesterol-dependent hormone synthesis and lipid metabolism. Another way in which SCFAs are involved in metabolic regulation is by reducing plasma glucose levels through the activation of the fatty acid receptors Ffar2 and Ffar3. The lack of butyrate-producing bacteria in the microbiome induced a decrease in FFAR2/3 receptor signalling, suppressed mucin formation, and increased intestinal permeability³⁴. Considering all the above-mentioned data, the use of SCFAs in poultry production requires a detailed (understanding) comprehension of the protective mechanisms in the intestinal system cells, as well as the possible risks of inhibiting intestinal function.

3. Use of medium-chain fatty acids

Medium-chain fatty acids (MCFAs) are considered a promising alternative to antibiotics due to their beneficial effects on digestion. MCFAs exhibit antibacterial activity, activate absorption, inhibit lipase production by bacteria

³² Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers / W. Wu et al. *PLoS ONE*. 2018. Vol. 13, No. 5. Art. e0197762.

³³ 16S rRNA sequencing analysis of the gut microbiota in broiler chickens prophylactically administered with antimicrobial agents / M. Cuccato et al. *Antibiotics*. 2021. Vol. 10, No. 2. Art. 146.

³⁴ Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function / S. P. Mishra et al. *JCI Insight*. 2024. Vol. 9, No. 3. Art. e168443.

and have a lower ability to dissociate³⁵. The mechanisms of action of MCFA are still unclear. MCFAs can act as non-ionic surfactants, incorporating into the bacterial double layer of lipids to form pores, leading to a critical increase in permeability and cell destruction. MCFAs are rapidly absorbed in the small intestine, transported to the liver as free fatty acids, and enter the mitochondria independently of fatty acyl-CoA carnitine transferases³⁶. The administration of MCFAs reduces lipogenesis, lipid uptake, fatty acid biosynthesis, and increases their oxidation.

It is assumed that the antibacterial efficacy of MCFAs is comparable to that of SCFAs, but their mechanism of impact is different in respect to that of SCFAs. MCFAs have a pKa value of about 4.9 and their efficiency decreases with molecular weight magnification. An important feature of MCFAs is their ability to easily penetrate dense peptidoglycan (Gram+ bacteria) and/or phospholipid (Gram- bacteria) membranes in undissociated form³⁷. The MCFAs adsorbed by the cells dissociate in the protoplasm, lowering the pH, which initiates critical damage to microbial cells. This property of MCFAs makes them promising substitutes for antibiotics, especially against gram-positive cocci and *Escherichia coli*. *Campylobacter* spp. infections cause an estimated 250,000 cases of gastroenteritis in the EU and a cost of over EUR 2.4 billion annually (The European Union One Health 2018; Zoonoses Report, 2019)³⁸. Despite the widespread occurrence of *Campylobacter* in warm-blooded animals, the main source of infection is birds, specifically broiler chickens³⁹. Addition of MCFAs to the feed for 3 days reduces *Campylobacter* colonisation in 27-day-old broilers experimentally infected with *C. jejuni* at 15 days of age. Feed additives with MCFA significantly reduce *Campylobacter* carriage in broiler chickens.

MCFAs, namely caproic, caprylic, and capric acids, are digested and absorbed faster than long-chain fatty acids, improve digestion, absorption, and

³⁵ The combined use of triacylglycerols containing medium-chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition: I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets / N. A. Dierick et al. *Livestock Production Science*. 2002. Vol. 75, No. 2. P. 129–142.

³⁶ Roopashree P. G., Shetty S. S., Suchetha Kumari N. Effect of medium chain fatty acid in human health and disease / P. G. Roopashree, S. S. Shetty, N. Suchetha Kumari. *Journal of Functional Foods*. 2021. Vol. 87. Art. 104724.

³⁷ Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids / D. Hermans et al. *Poultry Science*. 2010. Vol. 89, No. 6. P. 1144–1155.

³⁸ The European Union One Health 2018 Zoonoses Report. *EFSA Journal*. 2019. Vol. 17, No. 12. Art. e05926.

³⁹ Peh E., Kittler S., Reich F., Kehrenberg C. Antimicrobial activity of organic acids against *Campylobacter* spp. and development of combinations – A synergistic effect? / E. Peh et al. *PLoS ONE*. 2020. Vol. 15, No. 9. Art. e0239312.

lipid transport. Furthermore, MCFAs prevent the adsorption of bacteria to the intestinal wall and reduce invasion by inhibiting the production of bacterial lipases. The beneficial effect of MCFAs (C6-C12) alone and their mixture with SCFAs (C2-C6) was shown in (relation) respect to performance, carcass characteristics, haematological and biochemical parameters of broiler chickens' serum. Monoglycerides synthesised using MCFAs are also considered as a promising alternative. Recently, the results of their beneficial effects on performance, intestinal histomorphology, amino acid digestibility, and blood chemistry of broiler chickens have been presented. A metagenomic analysis of organic acid use has shown a stimulating effect on the diversity of beneficial microorganisms, nutrient digestion, and muscle growth⁴⁰.

4. Application of organic acid mixtures in poultry farming

Numerous studies on feed additives applying have shown that mixtures of organic acids (two or more) have significantly better efficacy than any one acid alone⁴¹. Specifically, SCFAs enhances the antimicrobial effect of MCFAs, which supports the gut microbiota of piglets and feed conversion in laying hens. The polymodal effects of a mixture of fumaric, citric, malic acids with capric and caprylic acids on productivity, *Lactobacillus* content, IgG concentration and inhibition of *E. coli* growth were shown. The mixture of phosphoric acid (0.2 g/kg) and lactic acid (0.3 g/kg) increases the pH of the pectoralis major and thigh muscle within 24 hours. The use of a mixture of microencapsulated organic acids with MCFAs has a positive effect on egg production, egg strength, calcium concentration, and the content of *Lactobacillus* and *E. coli* in the faeces of laying hens.

There is a fairly considerable number of results showing the beneficial effect of organic acid mixtures on the intestinal function and health of productive poultry. Specifically, the use of mixtures of butyric, fumaric, and lactic acids in different proportions had a positive effect on poultry body weight gain, feed conversion rate, and increase in the height of villi in the small intestine. A positive effect of sodium butyrate supplementation with MCFAs salts on the intestinal health of broiler chickens has been shown⁴². The addition of SCFAs and MCFAs to the broiler diet reduces serum cholesterol levels, abdominal fat, and thigh fat percentage and improves meat quality. Combined use of MCFA

⁴⁰ A comparison study of the caecum microbial profiles, productivity and production quality of broiler chickens fed supplements based on medium chain fatty and organic acids / A. Dauksiene et al. *Animals*. 2021. Vol. 11, No. 3. Art. 610.

⁴¹ Effects of short- and medium-chain fatty acids on production, meat quality, and microbial attributes – A Review / R. T. Szabó et al. *Molecules*. 2023. Vol. 28, No. 13. Art. 4956.

⁴² Impact of dietary supplementation with sodium butyrate protected by medium-chain fatty acid salts on gut health of broiler chickens / M. Sadurní et al. *Animals*. 2022. Vol. 12, No. 19. Art. 2496.

and organic acids increases duodenal villi height and crypt depth in broiler chickens. In addition to blends of single organic acids, blends of essential oils with organic acids have recently been proposed. Testing of such mixtures has shown their effectiveness against pathogen contamination of poultry feed⁴³. However, the scheme of feed additives use is of fundamental significance. For instance, the use of a mixture of organic acids and essential oils throughout the production cycle (35 days) with the addition of MCFAs for 5 days immediately before slaughter negatively affected broiler performance⁴⁴. The authors suggest that this result may be due to changes in the microbiota of the small intestine caused by prolonged exposure to acid. Recent results of a study in Ukraine on the effects of the original SCFAs blend have shown the vital role of adhesion proteins and extracellular matrix in the barrier function of the small intestine⁴⁵. Therewith, mixtures of SCFAs with MCFAs have been shown to be effective in treating poultry infected with *Clostridium perfringens*, *Eimeria* spp., and *Salmonella typhimurium*. At the same time, mixtures of formic acid, propionic acid, and sodium formate, varying in ratio, showed no differences in effectiveness against *Salmonella*.

CONCLUSIONS

According to the current regulatory framework, specifically EU regulations, the use of antibiotics in poultry farming is critically limited. Currently, OAs are successfully used as a prophylactic alternative to antibiotics. Research has shown that OAs are a cost-effective means of ensuring productivity, antimicrobial protection, and maintaining poultry health. Recent studies have shown that certain OAs improve digestion, immune response, and suppress pathogenic microflora. On the other hand, mixtures of OAs may have more powerful protective and stimulating effects depending on the composition and relative content of each component.

SCFAs have a wide range of effects on metabolism and immune defence in poultry, but despite the available data respectively their effects on metabolism and energy homeostasis, the mechanisms of transport and biological activity is still understudied. Butyrate has been shown to have a positive effect on the intestinal barrier and stimulates the metabolism of enterocytes. SCFAs can modulate the adaptive immune system by stimulating macrophages and dendritic

⁴³ Synergistic effects of essential oils and organic acids against *Aspergillus flavus* contamination in poultry feed / T. Satterlee et al. *Toxins*. 2023. Vol. 15, No. 11. Art. 635.

⁴⁴ An investigation of the effect of water additives on broiler growth and the caecal microbiota at harvest / G. Greene et al. *Pathogens*. 2022. Vol. 11, No. 8. Art. 932.

⁴⁵ Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8.

cells. SCFAs are involved in the regulation of gene expression in the cellular response to damage, including infectious agents. Therewith, there is an evidence of the damaging effect of butyrate on the intestinal barrier during inflammation. Considering the above data, the use of SCFAs in poultry production requires a detailed understanding of the protective mechanisms of action on the cells of the intestinal system, as well as the possible risks of inhibiting intestinal function. The total use of SCFAs requires further studies of their effect on the efficiency of the intestinal barrier function and the expression of molecular markers of epithelial cell intercellular adhesion. Along with determining the expression, a significant criterion for assessing the beneficial effect of SCFAs is the production of pro- and anti-inflammatory cytokines by cells of the intestinal system, fatty acid receptors, energy metabolism, and proliferation of intestinal epithelial cells.

Recently, MCFAs have been increasingly considered as an alternative to antibiotics due to their high antibacterial activity, stimulation of absorption, inhibition of lipase production by bacteria and lower dissociation ability compared to other surfactants. However, the mechanisms of MCFAs effect are still unclear. A significant feature of MCFAs is their ability to easily penetrate microbial membranes in undissociated form, which is effective against Gram-positive cocci. The study of the antimicrobial effects of MCFAs mixtures is a crucial component of creating a modern antibiotic-free strategy.

Mixtures of OAs are more effective than their individual use. Mixtures of SCFAs with MCFAs have the most significant beneficial effect since SCFAs act better as growth stimulants and MCFAs have higher antibacterial properties. At the same time, the optimal content of individual OAs in the mixture, doses, and exposure times stays a compromise issue. The use of specific molecular markers to evaluate the effectiveness of OA mixtures will allow the development of criteria for the formation of antimicrobial feed additives with unique properties and a focus that meets the challenges in poultry production. Further research into the mechanisms of action of the OAs will allow the development of their mixtures with optimised properties in terms of antimicrobial protection, intestinal functions, and metabolic stimulation. In addition, OA mixtures can be useful for the prevention of chronic diseases, such as bacterial chondronecrosis and osteomyelitis in broiler chickens.

SUMMARY

The use of antibiotics in poultry farming is critically limited, which leads to the search for and research of alternative compounds to replace antibiotics. Organic acids are considered one such alternative, but the antimicrobial and metabolic effects of fatty acid blends are still controversial and understudied.

All this necessitates a systematic analysis of the current data on effective antibiotic replacement strategies. The purpose of this study was to analyze and summarize current ideas on the use of organic acid mixtures as an alternative strategy for sustainable poultry production. An analysis of current literature showed that one of the most promising alternatives to the use of antibiotics in poultry farming is mixtures of organic acids and their derivatives, which have antibacterial effects, lower pH, are involved in energy metabolism, and all this together contributes to intestinal function. Organic acids have a positive effect on physiological functions, namely, digestion and the immune system, are the main source of energy for colonocytes, and reduce the pathogenic bacterial load on the digestive tract. Mixtures of organic acids were shown to be more effective than their individual use, specifically, mixtures of short- and medium-chain fatty acids were shown to be highly effective in supporting the intestinal barrier, microbiome, and immunity, with the former acting better as growth promoters and the latter having higher antibacterial properties. The efficiency of organic acids alone or in mixtures depends on many factors, depending on the type of molecule, form, and dose. The expediency of further studies of the effects of organic acids was substantiated, which will be useful for the development of antibiotic-free strategies using the synergistic effects of their mixtures and multidirectional cytoprotective effect. The findings of this study will be useful for scientists and veterinarians to learn about the prospects of using organic acid compositions as antibiotic alternatives, specifically for the development of technological approaches to minimize their applying.

Bibliography

1. Microbial short-chain fatty acids: A bridge between dietary fibers and poultry gut health – A review / Q. Ali et al. *Animal Bioscience*. 2022. Vol. 35, No. 10. P. 1461–1478. DOI: <https://doi.org/10.5713/ab.21.0562>.
2. Organic acid blend supplementation increases butyrate and acetate production in *Salmonella enterica* serovar Typhimurium challenged broilers / M. R. Aljumaah et al. *PLoS ONE*. 2020. Vol. 15, No. 6. Art. e0232831. DOI: <https://doi.org/10.1371/journal.pone.0232831>.
3. 16S rRNA sequencing analysis of the gut microbiota in broiler chickens prophylactically administered with antimicrobial agents / M. Cuccato et al. *Antibiotics*. 2021. Vol. 10, No. 2. Art. 146. DOI: <https://doi.org/10.3390/antibiotics10020146>.
4. A comparison study of the caecum microbial profiles, productivity and production quality of broiler chickens fed supplements based on medium chain fatty and organic acids / A. Dauksiene et al. *Animals*. 2021. Vol. 11, No. 3. Art. 610. DOI: <https://doi.org/10.3390/ani11030610>.

5. Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? / S. Deleu et al. *EBioMedicine*. 2021. Vol. 66. Art. 103293. DOI: <https://doi.org/10.1016/j.ebiom.2021.103293>.
6. The combined use of triacylglycerols containing medium-chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition: I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets / N. A. Dierick et al. *Livestock Production Science*. 2002. Vol. 75, No. 2. P. 129–142. DOI: [https://doi.org/10.1016/S0301-6226\(01\)00303-7](https://doi.org/10.1016/S0301-6226(01)00303-7).
7. Effect of sodium butyrate on intestinal health of poultry – a review / S. S. Elnesr et al. *Annals of Animal Science*. 2020. Vol. 20, No. 1. P. 29–41. DOI: <https://doi.org/10.2478/aoas-2019-0077>.
8. The control of poultry salmonellosis using organic agents: An updated overview / M. T. El-Saadony et al. *Poultry Science*. 2022. Vol. 101, No. 4. Art. 101716. DOI: <https://doi.org/10.1016/j.psj.2022.101716>.
9. Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease / S. Facchin et al. *Neurogastroenterology & Motility*. 2020. Vol. 32, No. 10. Art. e13914. DOI: <https://doi.org/10.1111/nmo.13914>.
10. Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: Impact on health, performance and meat quality / B. F. Fortuoso et al. *Microbial Pathogenesis*. 2019. Vol. 129. P. 161–167. DOI: <https://doi.org/10.1016/j.micpath.2019.02.005>.
11. Growth performance and meat quality of broilers fed with microencapsulated organic acids / G. M. Galli et al. *Animal Feed Science and Technology*. 2021. Vol. 271. Art. 114706. DOI: <https://doi.org/10.1016/j.anifeedsci.2020.114706>.
12. Dietary sodium butyrate and/or vitamin D3 supplementation alters growth performance, meat quality, chemical composition, and oxidative stability in broilers / H. Gao et al. *Food Chemistry*. 2022. Vol. 390. Art. 133138. DOI: <https://doi.org/10.1016/j.foodchem.2022.133138>.
13. Gavriil A., Thanasoulia A., Skandamis P. N. Sublethal concentrations of undissociated acetic acid may not always stimulate acid resistance in *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage Type 4: Implications of challenge substrate associated factors / A. Gavriil, A. Thanasoulia, P. N. Skandamis. *PLoS ONE*. 2020. Vol. 15, No. 7. Art. e0234999. DOI: <https://doi.org/10.1371/journal.pone.0234999>.
14. An investigation of the effect of water additives on broiler growth and the caecal microbiota at harvest / G. Greene et al. *Pathogens*. 2022. Vol. 11, No. 8. Art. 932. DOI: <https://doi.org/10.3390/pathogens11080932>.

15. High levels of antibiotic resistance in isolates from diseased livestock / N. A. Haulisah et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 652351. DOI: <https://doi.org/10.3389/fvets.2021.652351>.
16. Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids / D. Hermans et al. *Poultry Science*. 2010. Vol. 89, No. 6. P. 1144–1155. DOI: <https://doi.org/10.3382/ps.2010-00717>.
17. Dietary organic cranberry pomace influences multiple blood biochemical parameters and cecal microbiota in pasture-raised broiler chickens / M. R. Islam et al. *Journal of Functional Foods*. 2020. Vol. 72. Art. 104053. DOI: <https://doi.org/10.1016/j.jff.2020.104053>.
18. Effects of dietary short-and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens / A. Khatibjoo et al. *Journal of Applied Animal Research*. 2018. Vol. 46, No. 1. P. 492–498. DOI: <https://doi.org/10.1080/09712119.2017.1345741>.
19. The olfactory G protein-coupled receptor (Olf-78/OR51E2) modulates the intestinal response to colitis / K. Kotlo et al. *American Journal of Physiology-Cell Physiology*. 2020. Vol. 318, No. 3. P. 502–513. DOI: <https://doi.org/10.1152/ajpcell.00454.2019>.
20. A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice / M. Laffin et al. *Scientific Reports*. 2019. Vol. 9. Art. 12294. DOI: <https://doi.org/10.1038/s41598-019-48749-2>.
21. Louis P, Flint H. J. Formation of propionate and butyrate by the human colonic microbiota / P. Louis, H. J. Flint. *Environmental Microbiology*. 2017. Vol. 19, No. 1. P. 29–41. DOI: <https://doi.org/10.1111/1462-2920.13589>.
22. Regulation of the effector function of CD8+ T cells by gut microbiota-derived metabolite butyrate / M. Luu et al. *Scientific Reports*. 2018. Vol. 8. Art. 14430. DOI: <https://doi.org/10.1038/s41598-018-32860-x>.
23. Makowski Z., Lipiński K., Mazur-Kuśnerek M. The effects of different forms of butyric acid on the performance of turkeys, carcass quality, incidence of footpad dermatitis and economic efficiency / Z. Makowski, K. Lipiński, M. Mazur-Kuśnerek. *Animals*. 2022. Vol. 12, No. 11. Art. 1458. DOI: <https://doi.org/10.3390/ani12111458>.
24. Effect of chestnut tannins and short chain fatty acids as anti-microbials and as feeding supplements in broilers rearing and meat quality / F. Mannelli et al. *Animals*. 2019. Vol. 9, No. 9. Art. 659. DOI: <https://doi.org/10.3390/ani9090659>.
25. In vitro investigation of the antibacterial activity of nine commercial water disinfectants, acidifiers, and glyceride blends against the most important poultry zoonotic bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381. DOI: <https://doi.org/10.3390/pathogens12030381>.

26. Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8. DOI: <https://doi.org/10.32819/2023.11006>.

27. Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function / S. P. Mishra et al. *JCI Insight*. 2024. Vol. 9, No. 3. Art. e168443. DOI: <https://doi.org/10.1172/jci.insight.168443>.

28. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers / D. H. Nguyen et al. *Poultry Science*. 2018. Vol. 97, No. 12. P. 4351–4358. DOI: <https://doi.org/10.3382/ps/pey339>.

29. Oliveira L. M., Teixeira F. M. E., Sato M. N. Impact of retinoic acid on immune cells and inflammatory diseases. *Mediators of Inflammation*. 2018. Vol. 2018. Art. 3067126. DOI: <https://doi.org/10.1155/2018/3067126>.

30. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277. DOI: <https://doi.org/10.3389/fimmu.2019.00277>.

31. Peh E., Kittler S., Reich F., Kehrenberg C. Antimicrobial activity of organic acids against *Campylobacter* spp. and development of combinations – A synergistic effect? / E. Peh et al. *PLoS ONE*. 2020. Vol. 15, No. 9. Art. e0239312. DOI: <https://doi.org/10.1371/journal.pone.0239312>.

32. Study of microencapsulated fatty acid antimicrobial activity in vitro and its prevention ability of *Clostridium perfringens* induced necrotic enteritis in broiler chicken / N. Qi et al. *Gut Pathogens*. 2023. Vol. 15. Art. 1. DOI: <https://doi.org/10.1186/s13099-022-00526-9>.

33. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs) / W. Ratajczak et al. *Acta Biochimica Polonica*. 2019. Vol. 66, No. 1. P. 1–12. DOI: https://doi.org/10.18388/abp.2018_2648.

34. Ricke S. C., Dittoe D. K., Richardson K. E. Formic acid as an antimicrobial for poultry production: A review. *Frontiers in Veterinary Science*. 2020. Vol. 7. Art. 533419. DOI: <https://doi.org/10.3389/fvets.2020.00563>.

35. Roopashree P. G., Shetty S. S., Suchetha Kumari N. Effect of medium chain fatty acid in human health and disease / P. G. Roopashree, S. S. Shetty, N. Suchetha Kumari. *Journal of Functional Foods*. 2021. Vol. 87. Art. 104724. DOI: <https://doi.org/10.1016/j.jff.2021.104724>.

36. Impact of dietary supplementation with sodium butyrate protected by medium-chain fatty acid salts on gut health of broiler chickens / M. Sadurní

et al. *Animals*. 2022. Vol. 12, No. 19. Art. 2496. DOI: <https://doi.org/10.3390/ani12192496>.

37. Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass yields, muscle amino acid and fatty acid profiles, and antioxidant indices of broilers supplemented with synbiotic and/or organic acids / A. S. Salah, O. A. Ahmed-Farid, M. S. El-Tarabany. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52. DOI: <https://doi.org/10.1111/jpn.12994>.

38. Synergistic effects of essential oils and organic acids against *Aspergillus flavus* contamination in poultry feed / T. Satterlee et al. *Toxins*. 2023. Vol. 15, No. 11. Art. 635. DOI: <https://doi.org/10.3390/toxins15110635>.

39. Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: A Review / F. Scicutella et al. *Antibiotics*. 2021. Vol. 10, No. 8. Art. 1010. DOI: <https://doi.org/10.3390/antibiotics10081010>.

40. Effects of short- and medium-chain fatty acids on production, meat quality, and microbial attributes – A Review / R. T. Szabó et al. *Molecules*. 2023. Vol. 28, No. 13. Art. 4956. DOI: <https://doi.org/10.3390/molecules28134956>.

41. The European Union One Health 2018 Zoonoses Report. *EFSA Journal*. 2019. Vol. 17, No. 12. Art. e05926. DOI: <https://doi.org/10.2903/j.efsa.2019.5926>.

42. Effect of supplementation of salts of organic acids on serum and haematological parameters of broilers / V. Us et al. *International Journal of Current Microbiology and Applied Sciences*. 2017. Vol. 6, No. 11. P. 4211–4218. DOI: <https://doi.org/10.20546/ijcmas.2017.611.493>.

43. Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis / M. Vancamelbeke et al. *Journal of Crohn's & Colitis*. 2019. Vol. 13, No. 10. P. 1351–1361. DOI: <https://doi.org/10.1093/ecco-jcc/jjz064>.

44. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers / W. Wu et al. *PLoS ONE*. 2018. Vol. 13, No. 5. Art. e0197762. DOI: <https://doi.org/10.1371/journal.pone.0197762>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,

Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics

Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,
Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Kokariiev Andrii,
Candidate of Veterinary Sciences, Associate Professor,
Head of the Department of Immunochemical and Molecular Genetic
Analysis of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

**CURRENT APPROACHES IN MAINTENANCE GUT
HEALTH IN FARMING ANIMALS.
PART 4. THE CURRENT PROGRESS
IN MONOGLYCERIDES SUPPLEMENTATION TO PROVIDE
ANTIBIOTICS RESTRICTION IN SWINE HUSBANDRY**

Masiuk Dmytro, Nedzvetsky Victor, Kokariev Andrii
DOI <https://doi.org/10.30525/978-9934-26-714-7-11>

INTRODUCTION

Global swine production is currently in a recovery phase following a crisis caused by the spread of African swine fever, which reduced pig populations and pork production volumes in many countries, as well as the COVID-19 pandemic, which temporarily impacted market activity. Global pork production is showing growth in both volume and prices, operating within a framework of intense competition, technological transformation, and innovative progress¹. The intensification of pork production has facilitated the spread of diseases, complicating the maintenance of productivity without the use of antimicrobial agents. Since the 1950s, growth promoters, including antimicrobial agents, have played a crucial role in enhancing animal productivity². However, their use has led to a significant increase in antibiotic resistance. The European Union has banned growth promoters in 2006 (European Commission, 2006) and introduced additional restrictions on the prophylactic use of antibiotics in 2018, which took effect in 2022. These restrictions initiated an active search for antibiotic alternatives³.

In current livestock practices, there is a growing interest in using bioactive compounds as alternatives to antibiotics in animal feed⁴. Among these

¹ Mykhalko O. H. Current state and ways of pig production in the world and Ukraine. *Bulletin of Sumy National Agrarian University. The Series: Livestock*. 2021. Iss. 3 (46). P. 61–77.

² High levels of antibiotic resistance in isolates from diseased livestock / N. A. Haulisah et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 652351.

³ Effects of short- and medium-chain fatty acids on production, meat quality, and microbial attributes – A Review / R. T. Szabó et al. *Molecules*. 2023. Vol. 28, No. 13. Art. 4956.

⁴ Effects of medium-chain fatty acids as alternatives to ZnO or antibiotics in nursery pig diets / P. L. Dahmer et al. *Translational Animal Science*. 2020. Vol. 4, No. 3. P. 1–8.

compounds, monoglycerides (MGs) are of particular interest. MGs offer several advantages in combating animal infectious diseases compared to antibiotics. Firstly, they possess a broad spectrum of antimicrobial activity, inhibiting both viruses and bacteria, whereas antibiotics are only effective against bacteria. In the second place, pathogens encounter significant barriers in developing resistance to MGs, making them effective not only for treatment but also for disease prevention⁵. Third, MGs are more effective than fatty acids and are safer compared to other agents, such as formaldehyde⁶.

In present review, we provide the comprehensive views of current data on the molecular mechanisms, the potential, and effectiveness of monoglyceride application in swine production and to determine the impact of monoglycerides on the physiological state, productivity, and overall health of pigs. In addition, we also discuss about the monoglyceride effects on immune response, microbiome state and intestinal morphology.

1. General characteristics of monoglycerides

Monoglycerides are a class of glycerides consisting of a glycerol molecule linked to a fatty acid via an ester bond. Due to the presence of both primary and secondary hydroxyl groups in glycerol, there are two types of monoglycerides: 1-monoacylglycerols, where the fatty acid is attached to the first alcohol group, and 2-monoacylglycerols, where it is attached to the second alcohol group. Owing to their structure, monoglycerides possess both hydrophilic (water-attracting) and hydrophobic properties. Mono- and di-glycerides are generally produced through the transesterification (glycerolysis) of triglycerides with glycerol⁷. In industry, monoglycerides are primarily used as surfactants, typically in the form of emulsifiers. However, their most promising application appears to be as biologically active additives, providing a safe alternative to antibiotics and hormonal products.

Monoglycerides of short-chain and medium-chain fatty acids (SCFA & MCFA) have well-documented direct antimicrobial effect *in vitro* and are known to enhance immune competence in animals, improve gut health, and reduce disease transmission risk in the post-antibiotic era. SCFA α -monoglycerides, such as α -monopropionin and α -monobutyryn (derivatives of propionic and butyric acids), are primarily active against gram-negative bacteria. In contrast,

⁵ Churchward C. P., Alany R. G., Snyder L. A. S. Alternative antimicrobials: the properties of fatty acids and monoglycerides. *Critical Reviews in Microbiology*. 2018. Vol. 44, No. 5. P. 561–570.

⁶ Jackman J. A., Lavergne T. A., Elrod C. C. Antimicrobial monoglycerides for swine and poultry applications. *Frontiers in Animal Science*. 2022. Vol. 3. Art. 1019320.

⁷ Moonen H., Bas H. Mono- and Diglycerides. *Emulsifiers in Food Technology*. 2nd ed. / ed. by R. Whitehurst, C. Law. Chichester : Wiley-Blackwell, 2014. P. 73–92.

MCFA α -monoglycerides, such as α -monocaprylin, α -monocaprin, and α -monolaurin, are more effective against gram-positive bacteria. Numerous studies have confirmed the antibacterial effects of α -monoglycerides in vitro and in vivo. Additionally, recent findings indicate that MCFA α -monoglycerides, particularly α -monolaurin, exhibit antiviral properties. The biological activity of butyric acid is associated with the modulation of certain cellular responses, including histone deacetylase inhibition and G-protein-coupled receptor activation in various cell types⁸.

Medium-chain fatty acids (MCFAs) represent a class of saturated fatty acids containing from 6 to 12 carbon atoms. This class includes caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12), which found in natural products such as coconut oil, palm kernel oil, and milk⁹. Mono-, di-, and triglycerides of MCFAs are formed via esterification with glycerol as a rule. Apart from serving as an energy source, MCFAs demonstrated potential for improving gut morphology, stimulating growth, preventing infections, regulating immune response, and functioning as an alternative to antibiotics^{10, 11}. Several results demonstrated that both mono- and tri-acylglycerol of DL-3-hydroxybutyrate is non-toxic, stimulate metabolic energy production upon intravenous administration as well as they can be used as nutrients for farming animal feeding¹².

The primary structure that provides the biological activity of MGs are micelles – self-organized particles that form stable spheres at the content above the critical micelle concentration (CMC). MGs with longer hydrocarbon chains have lower CMC values due to stronger hydrophobic interactions, that promote micelle self-assembly¹³. The non-ionic nature of MGs allows them to form micelles at lower concentrations in compare with MCFAs, which are anionic and prone to electrostatic repulsion. This accounts for their greater efficacy, as MGs can form micelles at lower concentrations.

⁸ Salvi P. S., Cowles R. A. Butyrate and the Intestinal Epithelium: Modulation of Proliferation and Inflammation in Homeostasis and Disease. *Cells*. 2021. Vol. 10, No. 7. Art. 1775.

⁹ Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms / C. B. Huang et al. *Archives of Oral Biology*. 2011. Vol. 56, No. 7. P. 650–654.

¹⁰ Effects of medium-chain fatty acids as alternatives to ZnO or antibiotics in nursery pig diets / P. L. Dahmer et al. *Translational Animal Science*. 2020. Vol. 4, No. 3. P. 1–8.

¹¹ Hanczakowska E., Świątkiewicz M., Natonek-Wisniewska M., Okoń K. Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets. *Livestock Science*. 2016. Vol. 192. P. 1–7.

¹² Birkhahn R. H., McCombs C., Clemens R., Hubbs J. Potential of the monoglyceride and triglyceride of dl-3-hydroxybutyrate for parenteral nutrition: Synthesis and preliminary biological testing in the rat. *Nutrition*. 1997. Vol. 13, No. 3. P. vi–219.

¹³ Competing Interactions of Fatty Acids and Monoglycerides Trigger Synergistic Phospholipid Membrane Remodeling / B. K. Yoon et al. *The Journal of Physical Chemistry Letters*. 2020. Vol. 11, No. 13. P. 4951–4957.

MGs can inhibit a wide range of membrane-containing viruses and bacteria via damaging their membranes. Fatty acids and monoglycerides exhibit significantly higher activity in micellar form, whereas their activity in monomeric form is considerably lower or absent¹⁴. The antibacterial effect of MGs accompanied by increased bacterial membrane permeability, local damage, and disruption of membrane functions, including electron transport, oxidative phosphorylation, and enzyme activity disturbance¹⁵. MGs are also effective against membrane-bound viruses, where membrane disruption is a key factor of their antiviral activity.

2. Effect of monoglycerides on performance and feed conversion in pigs

The supplementation of monoglycerides in pig feeding attracts special attention due to their potential to improve productivity and feed conversion efficiency. This section examines the effects of monoglycerides on growth, feed conversion, and the overall health of pigs based on recent scientific research. There is a significant amount of conflicting data regarding the lack of effects of SCFA- or MCFA-containing monoglycerides on the performance of weaned piglets^{16, 17, 18}. However, recent studies indicate that adding a monoglyceride blend to piglet diets reduces the growth-to-feed ratio in Enterotoxigenic *Escherichia coli* (ETEC) infected pigs. The addition of capric acid to piglets' diets increased their body weight gain and exhibited moderate antibacterial activity against *E. coli*¹⁹. At the same time, studies have shown that a blend of monoglycerides (butyric, caprylic, and capric acids) did not affect the performance of broilers infected with necrotic enteritis²⁰. Conversely, feed additives containing α -glycerol monolaurate (α -GML) improved the growth performance of broilers,

¹⁴ Spectrum of Membrane Morphological Responses to Antibacterial Fatty Acids and Related Surfactants / B. K. Yoon et al. *Langmuir*. 2015. Vol. 31, No. 37. P. 10223–10232.

¹⁵ Casillas-Vargas G., Ocasio-Malavé C., Medina S., Morales-Guzmán C., Del Valle R. G., Carballeira N. M., Sanabria-Ríos D. J. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*. 2021. Vol. 82. Art. 101093.

¹⁶ Low-Protein Diet Supplemented with Medium-Chain Fatty Acid Glycerides Improves the Growth Performance and Intestinal Function in Post-Weaning Piglets / Z. Cui et al. *Animals*. 2020. Vol. 10, No. 10. Art. 1852.

¹⁷ Dietary Supplementation with Mono-Lactate Glyceride Enhances Intestinal Function of Weaned Piglets / H. Li et al. *Animals*. 2023. Vol. 13, No. 8. Art. 1303.

¹⁸ Evaluation of different blends of medium-chain fatty acids, lactic acid, and monolaurin on nursery pig growth performance / L. L. Thomas et al. *Translational Animal Science*. 2020. Vol. 4, No. 2. P. 548–557.

¹⁹ Hanczakowska E., Świątkiewicz M., Natonek-Wisniewska M., Okoń K. Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets. *Livestock Science*. 2016. Vol. 192. P. 1–7.

²⁰ Monoglyceride Blend Reduces Mortality, Improves Nutrient Digestibility, and Intestinal Health in Broilers Subjected to Clinical Necrotic Enteritis Challenge / A. Kumar et al. *Animals*. 2021. Vol. 11, No. 5. Art. 1432.

that attributed to increased feed intake and enhanced gut health²¹. α -GML application demonstrated potential for enhancing antimicrobial activity in sows and promoting the growth of suckling piglets. The addition of α -GML to sow diets increases ($P < 0.05$) piglet body weight at weaning and overall digestibility of crude fat in sows²².

Recent data have shown that dose of 0.1% α -GML improves the performance and growth of weaned piglets and reduces post-weaning diarrhea, which the authors attribute to improved nutrient digestibility, reduced systemic inflammation and intestinal permeability, and stable gut microbiota in piglets²³.

The addition of glycerol monolaurate to sow diets improves gut health in suckling piglets. Specifically, the results of GML supplementation demonstrated a significant increase in fat, lactose, and protein content in colostrum of sow, while piglets exhibited a reduction in IL-12 concentration in the duodenum, TNF- α , IL-1 β , and IL-12 in the jejunum, and IL-1 β in the ileum. These changes accompanied by increase in villus height, crypt depth, and the villus-to-crypt ratio (V/C) in the jejunum and V/C in the ileum in the experimental animals in compare with control group²⁴. Together, these factors contribute to the growth and development of piglets.

The other results suggest that the addition of MCFA to pig diets enhances growth performance, provides residual mitigating activity against PEDV, and does not significantly alter fecal microbial composition²⁵.

The application of monoglycerides with lysolecithin in finishing pig diets reduces metabolic energy costs, improves pig weight uniformity within groups, and shortens the time to reach the target slaughter weight, although it worsens feed conversion²⁶. Similar results in implementing feeding strategies to minimize feed costs and improve pig performance have been obtained by

²¹ Effects of alpha-glycerol monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens / J. Lan et al. *Poultry Science*. 2021. Vol. 100, No. 3. Art. 100918.

²² Supplementation with alpha-glycerol monolaurate during late gestation and lactation enhances sow performance, ameliorates milk composition, and improves growth of suckling piglets / L. Li et al. *Journal of Animal Science and Biotechnology*. 2023. Vol. 14, No. 1. Art. 38.

²³ Effects of combined application of benzoic acid and 1-monolaurin on growth performance, nutrient digestibility, gut microflora and systemic inflammation levels of weaned piglets / K. Wei et al. *Porc Health Manag*. 2023. 9. P. 46.

²⁴ Maternal supplementation with glycerol monolaurate improves the intestinal health of suckling piglets by inhibiting the NF-kppB/MAPK pathways and improving oxidative stability / H. Zhao et al. *Food & Function*. 2023. Vol. 14, No. 7. P. 3290–3303.

²⁵ Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage / J. T. Gebhardt et al. *Journal of Animal Science*. 2020. Vol. 98, No. 1. Art. skz358.

²⁶ Gonzalez Sanchez D., Toth S., Desbruslais A. P30. Supplementing lysolecithin with emulsifier and monoglycerides to diets reformulated to lower energy on performance and lean-fat deposition in fattening pigs. *Animal – Science Proceedings*. 2022. Vol. 13, No. 2. P. 166.

other researchers²⁷. At the same time, the supplementation complex mixture containing lysolecithins, monoglycerides, and synthetic emulsifier in a dose 750 g/t to post-weaning piglet diet significantly improves their performance, enhances the digestibility of protein, fat, and fiber without increase in feed intake²⁸.

Thus, the use of monoglycerides and their derivatives in pig feeding has a beneficial effect on animal performance and health, especially in animals challenged with infectious diseases. The inclusion of monoglycerides in pig diets demonstrates antimicrobial properties and enhances resistance to infectious diseases via reducing diarrhea incidence and improving intestinal wall morphology.

3. Effectiveness of monoglycerides in the treatment and prevention of swine diseases

Nowadays, monoglycerides are widely proposed as feed additive in pig production to improve performance and feed conversion efficiency. They have the potential to positively impact animal health due to their antimicrobial properties, which help reduce disease incidence and improve metabolic processes. Experimental studies have shown that MCFAs exhibit high bacteriostatic activity against various pathogens, including gram-positive and gram-negative bacteria, viruses, fungi, algae, and protozoa.

The study of fatty acids in respect with its antimicrobial properties is well-documented in the literature. One of the most common diseases in piglets is post-weaning diarrhea (PWD), often caused by the adhesion and proliferation of ETEC F18 or F4 in the small intestine. Clinical signs include profuse diarrhea, growth retardation, dehydration, and lethargy²⁹. It has been shown that butyrate and valerate glycerides alleviate diarrhea symptoms and modulate immune response in piglets co-infected with ETEC³⁰. Specifically, pigs fed with glycerides exhibited higher body weight, reduced diarrhea severity, and lower levels of neutrophils and TNF- α in serum on day 4 post-inoculation. The use of

²⁷ Wealleans A., Vasanthakumari B., Desbruslais A. P5. Improvements in digestibility and growth of growing-finishing pigs following supplementation with a combination of lysolecithins, monoglycerides and a synthetic emulsifier. *Animal – Science Proceedings*. 2022. Vol. 13, No. 2. P. 158.

²⁸ A combination of lysolecithins, a synthetic emulsifier, and monoglycerides, supports piglet performance, nutrient digestibility, and faecal consistency / A. Desbruslais et al. *Animal – Science Proceedings*. 2023. Vol. 14, No. 6. P. 810.

²⁹ Effects of a blend of essential oils, medium-chain fatty acids, and a toxin-adsorbing mineral on diarrhea and gut microbiome of weanling pigs experimentally infected with a pathogenic *Escherichia coli* / Y. He et al. *Journal of Animal Science*. 2022. Vol. 100, No. 1. Art. skab365.

³⁰ Dietary butyrate and valerate glycerides impact diarrhea severity and immune response of weaned piglets under ETEC F4-ETEC F18 coinfection conditions / L. Kovanda et al. *Journal of Animal Science*. 2023. Vol. 101. Art. skad401.

an antimicrobial MCFAs blend (0.5%) reduced the incidence of severe diarrhea in weaned piglets infected with F18 ETEC and improved their feed efficiency at the final stage of the experiment. This blend also altered the microbiota diversity in feces and ileal mucosa³¹. Comparative analyses have shown that adding monoglyceride mixtures can enhance disease resistance in weaned piglets by reducing diarrhea severity and mitigating intestinal and systemic inflammation³². However, monoglyceride efficiency remains not comparable to high doses of zinc oxide to treat diarrhea symptoms.

The inclusion of glycerol butyrate in pig diets reduces release of pro-inflammatory factors (TNF α , IL6, and IL1B) in the jejunum and ileum prior to ETEC infection by inhibiting the NF- κ B/MAPK pathway³³. The results of the other study demonstrated that monoglycerides reduce the viability of porcine epidemic diarrhea virus (PEDV) in feed and prevent the disease's transmission to post-weaning piglets³⁴. Recent studies have shown that benzoic acid, combined with probiotics or plant essential oils, can more effectively alleviate diarrhea and stimulate growth in weaned piglets than benzoic acid alone^{35, 36, 37}.

Previous studies have confirmed the antiviral activity of free MCFAs, including capric, lauric, and myristic acids, as well as long-chain unsaturated fatty acids including like oleic, linoleic, and linolenic acids, against vesicular stomatitis virus (VSV), herpes simplex virus (HSV), and Visna virus^{38, 39}.

³¹ Effects of a blend of essential oils, medium-chain fatty acids, and a toxin-adsorbing mineral on diarrhea and gut microbiome of weanling pigs experimentally infected with a pathogenic *Escherichia coli* / Y. He et al. *Journal of Animal Science*. 2022. Vol. 100, No. 1. Art. skab365

³² Effects of monoglyceride blend on systemic and intestinal immune responses, and gut health of weaned pigs experimentally infected with a pathogenic *Escherichia coli* / S. Park et al. *Journal of Animal Science and Biotechnology*. 2024. Vol. 15, No. 1. Art. 12.

³³ Glyceryl butyrate attenuates enterotoxigenic *Escherichia coli*-induced intestinal inflammation in piglets by inhibiting the NF- κ B/MAPK pathways and modulating the gut microbiota / M. Tian et al. *Food & Function*. 2022. Vol. 13, No. 11. P. 6282–6292.

³⁴ Monoglyceride reduces viability of porcine epidemic diarrhoea virus in feed and prevents disease transmission to post-weaned piglets / F. C. Phillips et al. *Transboundary and Emerging Diseases*. 2022. Vol. 69, No. 1. P. 121–127.

³⁵ Protective Effects of Benzoic Acid, *Bacillus coagulans*, and Oregano Oil on Intestinal Injury Caused by Enterotoxigenic *Escherichia coli* in Weaned Piglets / J. Pu et al. *BioMed Research International*. 2018. Vol. 2018. Art. 1829632.

³⁶ Benzoic Acid Combined with Essential Oils Can Be an Alternative to the Use of Antibiotic Growth Promoters for Piglets Challenged with *E. coli* F4 / L. M. Rodrigues et al. *Animals*. 2020. Vol. 10, No. 11. Art. 1978.

³⁷ The use of an alternative feed additive, containing benzoic acid, thymol, eugenol, and piperine, improved growth performance, nutrient and energy digestibility, and gut health in weaned piglets / C. D. Silva Júnior et al. *Journal of Animal Science*. 2020. Vol. 98, No. 5. Art. skaa119.

³⁸ Hilmarsson H., Kristmundsdóttir T., Thormar H. Virucidal activities of medium- and long-chain fatty alcohols, fatty acids and monoglycerides against herpes simplex virus types 1 and 2: comparison at different pH levels. *APMIS*. 2005. Vol. 113, No. 1. P. 58–65.

³⁹ Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides / H. Thormar et al. *Antimicrobial Agents and Chemotherapy*. 1987. Vol. 31, No. 1. P. 27–31

Furthermore, other studies have demonstrated similar antiviral activity of MCFAs and their derivatives, including alcohols and monoglycerides, against herpes simplex viruses 1 and 2.

Currently, there are no vaccines or effective drugs for treating Seneca Valley virus (SVV) infection. In vitro studies have shown that monolaurin inhibits SVV replication by 80%, while in vivo studies have demonstrated that monolaurin reduces clinical manifestations, viral load, and organ damage in SVV-infected piglets⁴⁰. Monolaurin significantly reduced inflammatory cytokine release and promoted interferon- γ production, enhancing viral clearance activity. Researchers concluded that monolaurin is a potentially effective alternative for treating SVV infection in pigs.

The antiviral activity of caprylic, capric, and lauric acids, as well as glycerol monolaurate (GML), has been investigated concerning ASFV inhibition in liquids and feed. Compounds and its dosages that can effectively inhibit ASFV proliferation have been identified⁴¹. Low GML concentrations had a minimal effect on virulence, while sufficiently high GML concentrations caused >99% virulence reduction. The onset concentration of antiviral activity corresponded to the critical micelle concentration of GML, highlighting the essential role of formulation in stimulating protective responses against ASFV⁴².

The use of modern nanotechnology enables the creation of more effective formulations without altering the chemical composition of bioactive preparations. For example, natural GML has low water solubility and limited efficacy against gram-negative bacteria. It deserves to be mentioned that nanoencapsulation not only improves bioavailability but also reduces ecotoxicity⁴³.

Fatty acids and monoglycerides have proven effective against *Neisseria gonorrhoeae*. Microemulsions with α -linolenic acid are effective in treating neonatal gonococcal ophthalmia⁴⁴. In the absence of vaccines, monocaprin may serve as a novel prophylactic agent to prevent *N. gonorrhoeae* growth and colonization, to which the bacterium cannot develop resistance⁴⁵.

⁴⁰ In vitro and in vivo antiviral activity of monolaurin against Seneca Valley virus / B. Su et al. *Frontiers in Veterinary Science*. 2023. Vol. 10. Art. 980187.

⁴¹ Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate / J. A. Jackman et al. *Journal of Animal Science and Biotechnology*. 2020. Vol. 11, No. 1. P. 1–10.

⁴² Glycerol Monolaurate Inhibits Wild-Type African Swine Fever Virus Infection in Porcine Macrophages / J. A. Jackman et al. *Pathogens*. 2023. Vol. 12, No. 10. Art. 1193.

⁴³ Ecotoxicology of Glycerol Monolaurate nanocapsules / L. Q. Lopes et al. *Ecotoxicology and Environmental Safety*. 2017. Vol. 139. P. 73–77.

⁴⁴ Belagal P. Current alternative therapies for treating drug-resistant *Neisseria gonorrhoeae* causing ophthalmia neonatorum. *Future Microbiology*. 2024. Vol. 19, No. 7. P. 631–647.

⁴⁵ Churchward C. P., Calder A., Snyder L. A. S. Mutations in *Neisseria gonorrhoeae* grown in sub-lethal concentrations of monocaprin do not confer resistance. *PloS One*. 2018. Vol. 13, No. 4. Art. e0195453.

Stress associated with piglet weaning suppresses digestion, absorptive capacity, and intestinal energy provision. MCFAs can effectively improve nutrient absorption and lipid metabolism in piglets to meet their energy needs, thereby supporting the growth and development of weaned piglets⁴⁶. MCFAs, such as hexanoic and octanoic acids, have shown significant anticandidal activity, as have long-chain fatty acids, including lauric acid⁴⁷.

Thus, in a view of intense competition and increasing food safety requirements, the supplementation of monoglyceride blends could be prospective strategy to remove antibiotics in productive animals farming. Besides, both antimicrobial and metabolic effect of monoglycerides can be important factors for reducing the risk of antibiotic resistance and enhancing swine production profitability.

Monoglycerides, known for their antimicrobial and anti-inflammatory properties, are emerging as promising additives to enhance immune and antioxidant systems in pigs. The effects of monoglycerides are directed at strengthening immunity, reducing oxidative stress, and increasing the overall resilience of animals against enteric infections. Administering α -GML to piglets at a dose of 1000 mg/kg increases cytokine concentrations, particularly IL-10, and the activity of antioxidant enzymes (GSH-Px) in the serum of weaned piglets. Under these conditions, serum MDA and TNF- α concentrations decrease. However, no differences were found in T-AOC and SOD activity or IL-6 concentration in serum⁴⁸. It is suggested that α -GML enhances antioxidant capacity by modulating intestinal barrier function.

In pigs, administering 0.6% α -GML reduces malondialdehyde and hydrogen peroxide levels in the ileum and significantly increases ($p < 0.05$) superoxide dismutase and catalase activities in the ileum and colon⁴⁹.

Mechanisms of antioxidant capacity transfer from the sow to the fetus through the gut-placenta axis have also been studied, using α -GML glycerol monolaurate feed supplements during pregnancy⁵⁰. Correlational analysis demonstrated a significant negative association between plasma lipopolysaccharide levels and placental weight, oxidative stress, and inflammation.

⁴⁶ Effects of Medium-Chain Fatty Acid Glycerides on Nutrient Metabolism and Energy Utilization in Weaned Piglets / Z. Cui et al. *Frontiers in Veterinary Science*. 2022. Vol. 9. Art. 938888.

⁴⁷ Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms / C. B. Huang et al. *Archives of Oral Biology*. 2011. Vol. 56, No. 7. P. 650–654.

⁴⁸ Effects of alpha-glycerol monolaurate on intestinal morphology, nutrient digestibility, serum profiles, and gut microbiota in weaned piglets / L. Li et al. *Journal of Animal Science*. 2022. Vol. 100, No. 3. Art. skac046.

⁴⁹ Supplementation with alpha-glycerol monolaurate during late gestation and lactation enhances sow performance, ameliorates milk composition, and improves growth of suckling piglets / L. Li et al. *Journal of Animal Science and Biotechnology*. 2023. Vol. 14, No. 1. Art. 38.

⁵⁰ Enhanced Antioxidative Capacity Transfer between Sow and Fetus via the Gut-Placenta Axis with Dietary Selenium Yeast and Glycerol Monolaurate Supplementation during Pregnancy / J. Zhang et al. *Antioxidants*. 2024. Vol. 13, No. 2. Art. 177.

4. Effects of monoglycerides on intestinal morphology and microbiome in pigs

Gut health is a key factor in ensuring optimal productivity in pigs and resilience to infectious diseases. Monoglycerides have beneficial effects on the morphology of intestinal structures and can modulate the composition of the gut microbiota, thereby supporting intestinal barrier function⁵¹. Studies have shown that supplementation α -GML to piglets at a dose of 1000 mg/kg increases the villus height-to-crypt depth ratio in the duodenum and villus height in the jejunum and ileum compared to the control group, although it did not affect crypt depth in any intestinal section⁵². Additionally, GML promotes an increase in goblet cell numbers⁵³.

MCFA-containing glycerides influence intestinal morphology, immune and goblet cells in post-weaning piglets. *E. coli* infection is associated with reduced villus height and crypt depth. However, in piglets exposed to LPS, MCFA-derived glycerides restore villus morphology and increase IgA levels in blood. A similar effect was observed in the ileum, albeit with a less pronounced impact⁵⁴.

The application of α -GML to piglets in a dose 1000 mg/kg increases the relative abundance of Firmicutes by 17.87% ($P < 0.05$) and decreases the proportions of Bacteroidota and Campylobacterota ($P < 0.05$). In the cecum, the levels of unclassified Lactobacillus and Blautia species significantly increased ($P < 0.05$) while Eubacterium rectale, unclassified Campylobacter, and uncultured_bacterium Alloprevotella decreased ($P < 0.05$)⁵⁵.

Gene expression analysis indicated that linoleic acid significantly enhances the expression of genes involved in adipogenesis and pathways dependent on PPAR γ , C/EBP α , ap2, and NRF1 signaling. Cells incubated with LNA showed reduced phosphorylation of Ser 112 in PPAR γ compared to cells treated with palmitic or oleic acid. The lack of significant difference in triacylglycerol levels upon inhibition of PPAR γ Ser 112 phosphorylation suggests varied metabolic

⁵¹ Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8.

⁵² Supplementation with alpha-glycerol monolaurate during late gestation and lactation enhances sow performance, ameliorates milk composition, and improves growth of suckling piglets / L. Li et al. *Journal of Animal Science and Biotechnology*. 2023. Vol. 14, No. 1. Art. 38.

⁵³ Low-Protein Diet Supplemented with Medium-Chain Fatty Acid Glycerides Improves the Growth Performance and Intestinal Function in Post-Weaning Piglets / Z. Cui et al. *Animals*. 2020. Vol. 10, No. 10. Art. 1852.

⁵⁴ Medium-chain glycerides affect gut morphology, immune- and goblet cells in post-weaning piglets: In vitro fatty acid screening with Escherichia coli and in vivo consolidation with LPS challenge / K. De Keyser et al. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 221–230.

⁵⁵ Supplementation with alpha-glycerol monolaurate during late gestation and lactation enhances sow performance, ameliorates milk composition, and improves growth of suckling piglets / L. Li et al. *Journal of Animal Science and Biotechnology*. 2023. Vol. 14, No. 1. Art. 38.

pathways of dietary fatty acids in porcine adipocytes. LNA significantly promotes lipid accumulation, likely by regulating PPAR γ phosphorylation⁵⁶.

The supplementation of GML increases the expression of claudin-1, occludin, and ZO-1 in the jejunum and ZO-1 in the ileum, indicating modulation of the intestinal barrier⁵⁷. Administering 0.6% glycerol monolactate in pigs significantly ($p < 0.05$) increases occludin expression in the intestine (H. Li et al., 2023). Additionally, GML applying to sows enhances the levels of zonula occludens-1, occludin, and claudin-1 proteins in the small intestine ($P < 0.05$), along with increased mRNA expression of mucins (MUC-1, MUC-3, and MUC-4) and porcine beta-defensins⁵⁸.

Capric acid reduces total intestinal length, while probiotics do not affect intestinal mass or length but increase villus width and crypt depth in the epithelial cells of the small intestine. MCFAs do not impact these parameters. Both additives exhibit strong antibacterial action: capric acid against *E. coli* and probiotics against *C. perfringens*⁵⁹. Sequencing of 16S rDNA demonstrated that supplementing sows with GML alters the colon microbiome of piglets and reduces the relative abundance of *Escherichia shigella*⁶⁰.

Thus, monoglycerides can have beneficial effects on intestinal morphology, particularly by increasing villus height and improving the villus-to-crypt ratio, which promotes more efficient nutrient absorption. Additionally, monoglycerides can modulate the composition of the gut microbiota, reducing pathogenic bacteria and supporting the balance of beneficial microbiota.

CONCLUSIONS

Monoglycerides exhibit significant potential in swine production maintenance due to their antimicrobial, antiviral, and anti-inflammatory properties. They not only contribute to improved animal productivity but also provide essential protection against infectious diseases. The use of monoglycerides in pig feeding has demonstrated high effectiveness in reducing the incidence of diarrhea

⁵⁶ PAM, OLA, and LNA are Differentially Taken Up and Trafficked Via Different Metabolic Pathways in Porcine Adipocytes / C. Yu et al. *Lipids*. 2017. Vol. 52, No. 11. P. 929–938.

⁵⁷ Low-Protein Diet Supplemented with Medium-Chain Fatty Acid Glycerides Improves the Growth Performance and Intestinal Function in Post-Weaning Piglets / Z. Cui et al. *Animals*. 2020. Vol. 10, No. 10. Art. 1852.

⁵⁸ Maternal supplementation with glycerol monolaurate improves the intestinal health of suckling piglets by inhibiting the NF-kappaB/MAPK pathways and improving oxidative stability / H. Zhao et al. *Food & Function*. 2023. Vol. 14, No. 7. P. 3290–3303.

⁵⁹ Effects of monoglyceride blend on systemic and intestinal immune responses, and gut health of weaned pigs experimentally infected with a pathogenic *Escherichia coli* / S. Park et al. *Journal of Animal Science and Biotechnology*. 2024. Vol. 15, No. 1. Art. 12.

⁶⁰ Maternal supplementation with glycerol monolaurate improves the intestinal health of suckling piglets by inhibiting the NF-kappaB/MAPK pathways and improving oxidative stability / H. Zhao et al. *Food & Function*. 2023. Vol. 14, No. 7. P. 3290–3303.

caused by bacterial infections, particularly *Escherichia coli*, by stabilizing gut microbiota and strengthening the immune system. Additionally, they can improve intestinal morphological parameters, such as villus height and the villus-to-crypt ratio, which enhance nutrient absorption and overall pig health.

Monoglycerides also play a crucial role in stimulating immune and antioxidant systems, reducing oxidative stress, and helping to maintain animal health under stressful conditions, such as weaning or intensive rearing. Their antioxidant properties protect cells from damage and reduce inflammation, which is critical for supporting healthy metabolism and increasing disease resilience in animals. Given the current requirements to reduce antibiotics supplementation in animal husbandry, monoglycerides serve as an effective alternative capable of replacing traditional antimicrobial agents.

Thus, monoglycerides represent a promising and safe feed additive for enhancing the productivity, health, and overall resilience of pigs. Their application helps reduce dependence on antibiotics, supporting stable productivity and competitiveness in swine production amid global challenges, such as combating antibiotic resistance and meeting the growing demand for environmentally sustainable products.

SUMMARY

The article examines the properties and prospects of using monoglycerides in swine production as an alternative to antibiotics, aligning with modern demands for environmental safety and sustainable livestock farming. Monoglycerides are bioactive compounds which develop antimicrobial, antiviral, and anti-inflammatory effects. Furthermore, monoglycerides application confirmed to be effective tool to support the animal health, immune status, and productivity of pig farming, especially in current intensive production requirements.

The use of monoglycerides enhances the overall resistance of the organism to infections, which is crucial for minimizing disease incidence and ensuring stable productivity in scenarios with limited antibiotic use. Recent research findings indicate that monoglycerides positively impact intestinal morphology, particularly by increasing villus height and improving the villus-to-crypt ratio, which boosts nutrient absorption efficiency. In addition, monoglyceride blends can modulate the composition of the gut microbiota via the pathogenic bacteria reduction and regulation of beneficial microbial taxa balance. This effect lead to the improvement in digestion, metabolism, and overall animal health that is especially valuable during stress periods, including post-weaning complications.

Besides, monoglycerides demonstrated an ability to stimulate the immune system and enhance antioxidant defense. They reduce oxidative stress progress,

that is an essential factor to maintain cell viability and organ functionality, and promote cytokine synthesis, which is involved in regulating immune responses. Furthermore, due to their antimicrobial properties, monoglycerides can serve as an effective alternative to antibiotics in new strategy for treatment bacterial and viral infections.

Taking into the account an absent of environmental toxicity, reduction of antibiotic dependence, and positive effects on pig health, monoglycerides could become an efficient tool to build the strategy of sustainable swine production. Their application contributes to improved productivity, quality, and product safety while supporting livestock resilience to global challenges, such as the rising demand for environmentally friendly products and control over infectious diseases. Thus, monoglycerides are a promising feed additive which capable to enhance the competitiveness and efficiency of swine farms in line with contemporary standards.

Bibliography

1. Belagal P. Current alternative therapies for treating drug-resistant *Neisseria gonorrhoeae* causing ophthalmia neonatorum. *Future Microbiology*. 2024. Vol. 19, No. 7. P. 631–647. DOI: <https://doi.org/10.2217/FMB-2023-0251>.
2. Birkhahn R. H., McCombs C., Clemens R., Hubbs J. Potential of the monoglyceride and triglyceride of dl-3-hydroxybutyrate for parenteral nutrition: Synthesis and preliminary biological testing in the rat. *Nutrition*. 1997. Vol. 13, No. 3. P. vi–219. DOI: [https://doi.org/10.1016/S0899-9007\(96\)00404-2](https://doi.org/10.1016/S0899-9007(96)00404-2).
3. Casillas-Vargas G., Ocasio-Malavé C., Medina S., Morales-Guzmán C., Del Valle R. G., Carballeira N. M., Sanabria-Ríos D. J. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*. 2021. Vol. 82. Art. 101093. DOI: <https://doi.org/10.1016/j.plipres.2021.101093>.
4. Churchward C. P., Alany R. G., Snyder L. A. S. Alternative antimicrobials: the properties of fatty acids and monoglycerides. *Critical Reviews in Microbiology*. 2018. Vol. 44, No. 5. P. 561–570. DOI: <https://doi.org/10.1080/1040841X.2018.1467875>.
5. Churchward C. P., Calder A., Snyder L. A. S. Mutations in *Neisseria gonorrhoeae* grown in sub-lethal concentrations of monocaprin do not confer resistance. *PloS One*. 2018. Vol. 13, No. 4. Art. e0195453. DOI: <https://doi.org/10.1371/journal.pone.0195453>.
6. Low-Protein Diet Supplemented with Medium-Chain Fatty Acid Glycerides Improves the Growth Performance and Intestinal Function in Post-Weaning Piglets / Z. Cui et al. *Animals*. 2020. Vol. 10, No. 10. Art. 1852. DOI: <https://doi.org/10.3390/ani10101852>.

7. Effects of Medium-Chain Fatty Acid Glycerides on Nutrient Metabolism and Energy Utilization in Weaned Piglets / Z. Cui et al. *Frontiers in Veterinary Science*. 2022. Vol. 9. Art. 938888. URL: <https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2022.938888>.
8. Effects of medium-chain fatty acids as alternatives to ZnO or antibiotics in nursery pig diets / P. L. Dahmer et al. *Translational Animal Science*. 2020. Vol. 4, No. 3. P. 1–8. DOI: <https://doi.org/10.1093/tas/txaa151>.
9. Medium-chain glycerides affect gut morphology, immune- and goblet cells in post-weaning piglets: In vitro fatty acid screening with *Escherichia coli* and in vivo consolidation with LPS challenge / K. De Keyser et al. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 221–230. DOI: <https://doi.org/10.1111/jpn.12998>.
10. A combination of lysolecithins, a synthetic emulsifier, and monoglycerides, supports piglet performance, nutrient digestibility, and faecal consistency / A. Desbruslais et al. *Animal – Science Proceedings*. 2023. Vol. 14, No. 6. P. 810. DOI: <https://doi.org/10.1016/j.anscip.2023.08.081>.
11. Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage / J. T. Gebhardt et al. *Journal of Animal Science*. 2020. Vol. 98, No. 1. Art. skz358. DOI: <https://doi.org/10.1093/jas/skz358>.
12. Gonzalez Sanchez D., Toth S., Desbruslais A. P30. Supplementing lysolecithin with emulsifier and monoglycerides to diets reformulated to lower energy on performance and lean-fat deposition in fattening pigs. *Animal – Science Proceedings*. 2022. Vol. 13, No. 2. P. 166. DOI: <https://doi.org/10.1016/j.anscip.2022.03.233>.
13. Hanczakowska E., Świątkiewicz M., Natonek-Wisniewska M., Okoń K. Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets. *Livestock Science*. 2016. Vol. 192. P. 1–7. DOI: <https://doi.org/10.1016/j.livsci.2016.08.002>.
14. Effects of a blend of essential oils, medium-chain fatty acids, and a toxin-adsorbing mineral on diarrhea and gut microbiome of weanling pigs experimentally infected with a pathogenic *Escherichia coli* / Y. He et al. *Journal of Animal Science*. 2022. Vol. 100, No. 1. Art. skab365. DOI: <https://doi.org/10.1093/jas/skab365>.
15. Hilmarsson H., Kristmundsdóttir T., Thormar H. Virucidal activities of medium- and long-chain fatty alcohols, fatty acids and monoglycerides against herpes simplex virus types 1 and 2: comparison at different pH levels. *APMIS*. 2005. Vol. 113, No. 1. P. 58–65. DOI: <https://doi.org/10.1111/j.1600-0463.2005.apm1130109.x>.

16. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms / C. B. Huang et al. *Archives of Oral Biology*. 2011. Vol. 56, No. 7. P. 650–654. DOI: <https://doi.org/10.1016/j.archoralbio.2011.01.011>.
17. Glycerol Monolaurate Inhibits Wild-Type African Swine Fever Virus Infection in Porcine Macrophages / J. A. Jackman et al. *Pathogens*. 2023. Vol. 12, No. 10. Art. 1193. DOI: <https://doi.org/10.3390/pathogens12101193>.
18. Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate / J. A. Jackman et al. *Journal of Animal Science and Biotechnology*. 2020. Vol. 11, No. 1. P. 1–10. DOI: <https://doi.org/10.1186/s40104-020-00517-3>.
19. Jackman J. A., Lavergne T. A., Elrod C. C. Antimicrobial monoglycerides for swine and poultry applications. *Frontiers in Animal Science*. 2022. Vol. 3. Art. 1019320. DOI: <https://doi.org/10.3389/fanim.2022.1019320>.
20. Dietary butyrate and valerate glycerides impact diarrhea severity and immune response of weaned piglets under ETEC F4-ETEC F18 coinfection conditions / L. Kovanda et al. *Journal of Animal Science*. 2023. Vol. 101. Art. skad401. DOI: <https://doi.org/10.1093/jas/skad401>.
21. Monoglyceride Blend Reduces Mortality, Improves Nutrient Digestibility, and Intestinal Health in Broilers Subjected to Clinical Necrotic Enteritis Challenge / A. Kumar et al. *Animals*. 2021. Vol. 11, No. 5. Art. 1432. DOI: <https://doi.org/10.3390/ani11051432>.
22. Effects of alpha-glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens / J. Lan et al. *Poultry Science*. 2021. Vol. 100, No. 3. Art. 100918. DOI: <https://doi.org/10.1016/j.psj.2020.11.052>.
23. Dietary Supplementation with Mono-Lactate Glyceride Enhances Intestinal Function of Weaned Piglets / H. Li et al. *Animals*. 2023. Vol. 13, No. 8. Art. 1303. DOI: <https://doi.org/10.3390/ani13081303>.
24. Supplementation with alpha-glycerol monolaurate during late gestation and lactation enhances sow performance, ameliorates milk composition, and improves growth of suckling piglets / L. Li et al. *Journal of Animal Science and Biotechnology*. 2023. Vol. 14, No. 1. Art. 38. DOI: <https://doi.org/10.1186/s40104-023-00848-x>.
25. Effects of alpha-glycerol monolaurate on intestinal morphology, nutrient digestibility, serum profiles, and gut microbiota in weaned piglets / L. Li et al. *Journal of Animal Science*. 2022. Vol. 100, No. 3. Art. skac046. DOI: <https://doi.org/10.1093/jas/skac046>.
26. Ecotoxicology of Glycerol Monolaurate nanocapsules / L. Q. Lopes et al. *Ecotoxicology and Environmental Safety*. 2017. Vol. 139. P. 73–77. DOI: <https://doi.org/10.1016/j.ecoenv.2017.01.019>.

27. Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8. DOI: <https://doi.org/10.32819/2023.11006>.

28. Moonen H., Bas H. Mono- and Diglycerides. *Emulsifiers in Food Technology*. 2nd ed. / ed. by R. Whitehurst, C. Law. Chichester : Wiley-Blackwell, 2014. P. 73–92. DOI: <https://doi.org/10.1002/9781118921265.ch4>.

29. Mykhalko O. H. Current state and ways of pig production in the world and Ukraine. *Bulletin of Sumy National Agrarian University. The Series: Livestock*. 2021. Iss. 3 (46). P. 61–77. DOI: <https://doi.org/10.32845/bsnau.lvst.2021.3.9>.

30. Effects of monoglyceride blend on systemic and intestinal immune responses, and gut health of weaned pigs experimentally infected with a pathogenic *Escherichia coli* / S. Park et al. *Journal of Animal Science and Biotechnology*. 2024. Vol. 15, No. 1. Art. 12. DOI: <https://doi.org/10.1186/s40104-024-01103-7>.

31. Monoglyceride reduces viability of porcine epidemic diarrhoea virus in feed and prevents disease transmission to post-weaned piglets / F. C. Phillips et al. *Transboundary and Emerging Diseases*. 2022. Vol. 69, No. 1. P. 121–127. DOI: <https://doi.org/10.1111/tbed.14353>.

32. Protective Effects of Benzoic Acid, *Bacillus coagulans*, and Oregano Oil on Intestinal Injury Caused by Enterotoxigenic *Escherichia coli* in Weaned Piglets / J. Pu et al. *BioMed Research International*. 2018. Vol. 2018. Art. 1829632. DOI: <https://doi.org/10.1155/2018/1829632>.

33. Benzoic Acid Combined with Essential Oils Can Be an Alternative to the Use of Antibiotic Growth Promoters for Piglets Challenged with *E. coli* F4 / L. M. Rodrigues et al. *Animals*. 2020. Vol. 10, No. 11. Art. 1978. DOI: <https://doi.org/10.3390/ani10111978>.

34. Salvi P. S., Cowles R. A. Butyrate and the Intestinal Epithelium: Modulation of Proliferation and Inflammation in Homeostasis and Disease. *Cells*. 2021. Vol. 10, No. 7. Art. 1775. DOI: <https://doi.org/10.3390/cells10071775>.

35. The use of an alternative feed additive, containing benzoic acid, thymol, eugenol, and piperine, improved growth performance, nutrient and energy digestibility, and gut health in weaned piglets / C. D. Silva Júnior et al. *Journal of Animal Science*. 2020. Vol. 98, No. 5. Art. skaa119. DOI: <https://doi.org/10.1093/jas/skaa119>.

36. In vitro and in vivo antiviral activity of monolaurin against Seneca Valley virus / B. Su et al. *Frontiers in Veterinary Science*. 2023. Vol. 10. Art. 980187. DOI: <https://doi.org/10.3389/fvets.2023.980187>.

37. Evaluation of different blends of medium-chain fatty acids, lactic acid, and monolaurin on nursery pig growth performance / L. L. Thomas et al. *Translational Animal Science*. 2020. Vol. 4, No. 2. P. 548–557. DOI: <https://doi.org/10.1093/tas/txaa029>.
38. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides / H. Thormar et al. *Antimicrobial Agents and Chemotherapy*. 1987. Vol. 31, No. 1. P. 27–31. DOI: <https://doi.org/10.1128/AAC.31.1.27>.
39. Glyceryl butyrate attenuates enterotoxigenic *Escherichia coli*-induced intestinal inflammation in piglets by inhibiting the NF-kappaB/MAPK pathways and modulating the gut microbiota / M. Tian et al. *Food & Function*. 2022. Vol. 13, No. 11. P. 6282–6292. DOI: <https://doi.org/10.1039/D2FO01056A>.
40. Wealleans A., Vasanthakumari B., Desbruslais A. P5. Improvements in digestibility and growth of growing-finishing pigs following supplementation with a combination of lysolecithins, monoglycerides and a synthetic emulsifier. *Animal – Science Proceedings*. 2022. Vol. 13, No. 2. P. 158. DOI: <https://doi.org/10.1016/j.anscip.2022.03.208>.
41. Effects of combined application of benzoic acid and 1-monolaurin on growth performance, nutrient digestibility, gut microflora and systemic inflammation levels of weaned piglets / K. Wei et al. *Porc Health Manag*. 2023. 9. P. 46. <https://doi.org/10.1186/s40813-023-00339-5>.
42. Spectrum of Membrane Morphological Responses to Antibacterial Fatty Acids and Related Surfactants / B. K. Yoon et al. *Langmuir*. 2015. Vol. 31, No. 37. P. 10223–10232. DOI: <https://doi.org/10.1021/acs.langmuir.5b02088>.
43. Competing Interactions of Fatty Acids and Monoglycerides Trigger Synergistic Phospholipid Membrane Remodeling / B. K. Yoon et al. *The Journal of Physical Chemistry Letters*. 2020. Vol. 11, No. 13. P. 4951–4957. DOI: <https://doi.org/10.1021/acs.jpcclett.0c01138>.
44. PAM, OLA, and LNA are Differentially Taken Up and Trafficked Via Different Metabolic Pathways in Porcine Adipocytes / C. Yu et al. *Lipids*. 2017. Vol. 52, No. 11. P. 929–938. DOI: <https://doi.org/10.1007/s11745-017-4302-x>.
45. Enhanced Antioxidative Capacity Transfer between Sow and Fetus via the Gut-Placenta Axis with Dietary Selenium Yeast and Glycerol Monolaurate Supplementation during Pregnancy / J. Zhang et al. *Antioxidants*. 2024. Vol. 13, No. 2. Art. 177. DOI: <https://doi.org/10.3390/antiox13020141>.
46. Maternal supplementation with glycerol monolaurate improves the intestinal health of suckling piglets by inhibiting the NF-kappaB/MAPK pathways and improving oxidative stability / H. Zhao et al. *Food & Function*. 2023. Vol. 14, No. 7. P. 3290–3303. DOI: <https://doi.org/10.1039/D3FO00068K>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,
Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,

Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Kokariiev Andrii,

Candidate of Veterinary Sciences, Associate Professor,
Head of the Department of Immunochemical and Molecular Genetic
Analysis of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

**CURRENT APPROACHES IN MAINTENANCE GUT
HEALTH IN FARMING ANIMALS.
PART 5. MONOGLYCERIDE BLEND SUPPLEMENTATION
MODULATES INTESTINAL MOLECULAR MARKERS
EXPRESSION AND MICROBIOME STATUS
IN THE DUODENUM OF BROILER CHICKENS**

Masiuk Dmytro, Nedzvetsky Victor

DOI <https://doi.org/10.30525/978-9934-26-714-7-12>

INTRODUCTION

The antibiotics were applied in poultry as a growth promoter (GP) and microbial agent to support sustainable animal farming for many decades. However, due to the global increase in the generation of multidrug resistant strains, the antibiotics supplementation in husbandry has been banned in several developed countries¹. Various alternatives of anti-microbial agents and growth promoters were proposed last decade including probiotics, prebiotics, enzymes and organic acids^{2, 3, 4}. However, all aforementioned tools confirmed as alternatives with restricted efficiency in compare to antibiotics features. In spite of current progress in the study of antimicrobial compounds to treat productive animal diseases, the search for alternative strategies to remove antibiotics remains an actual challenge⁵. Particularly, the engineering of antibiotic-free growth promoters for poultry farming is a most important task in a view the fact that modern broilers are fast-growing birds⁶. Poultry performance has

¹ Global Restriction of Using Antibiotic Growth Promoters and Alternative Strategies in Poultry Production / H. M. Salim et al. *Science Progress*. 2018. Vol. 101, No. 1. P. 52–75.

² Nutraceuticals Induced Changes in the Broiler Gastrointestinal Tract Microbiota / E. Tolnai et al. *mSystems*. 2021. Vol. 6, No. 1. Art. e01124-20.

³ Prospects of Organic Acids as Safe Alternative to Antibiotics in Broiler Chickens Diet / R. U. Khan et al. *Environmental Science and Pollution Research International*. 2022. Vol. 29, No. 22. P. 32594–32604.

⁴ Effect of prebiotics administered during embryo development on mitochondria in intestinal and immune tissues of adult broiler chickens / A. Dunislawska et al. *Poultry Science*. 2023. Vol. 102, No. 6. Art. 102663.

⁵ The Immune Enhancing Effect of Antimicrobial Peptide LLv on Broilers Chickens / X. Liu et al. *Poultry Science*. 2024. Vol. 103, No. 2. Art. 103235.

⁶ Dietary tributyrin improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders / J. Wang et al. *Poultry Science*. 2021. Vol. 100, No. 11. Art. 101429.

been regulated with low doses of antibiotics in feed for many years, leading to disease prevention and increased body weight gain⁷. However, even with the use of low doses of antibiotics, poultry health problems still cannot be completely eliminated⁸. Besides, feed additives considered to be prospective manner to maintain profitability in poultry farming⁹.

The application of organic acids in feed is the most promising technic for replacing antibacterial drugs in industrial poultry farming¹⁰. In particular, organic acids with a short (SCFA), medium (MCFA) and long (LCFA) chains, which are able to selectively adjust the growth or activity of particular bacteria are already successfully used today¹¹. Monoglycerides synthesized with use SCFA have several advantages, including a pH-independent antimicrobial effect and improvement of the intestinal barrier function. Monolaurin supplementation exhibited pronounced antibacterial and anti-inflammatory effects¹².

Recent data demonstrated that intercellular adhesion proteins play a key role in ensuring the barrier function of the intestinal epithelium¹³. Unique adhesion proteins form tight intercellular junctions that prevent harmful substances, microorganisms and toxins penetration into the internal environment of the body¹⁴. Recent report has shown that supplementation with different levels of organic acids can stimulate expression of a significant number of genes related to tight junctions (TJ) and immune function in broilers¹⁵. Butyrate can improve intestinal barrier function via increasing Claudin-1 transcription, by improving the association between Sp1 transcription factor and the Claudin-1 promoter¹⁶.

⁷ Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass Yields, Muscle Amino Acid and Fatty Acid Profiles, and Antioxidant Indices of Broilers Supplemented with Synbiotic and/or Organic Acids. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52.

⁸ Abdelli N., Solà-Oriol D., Pérez J. F. Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges. *Animals*. 2021. Vol. 11, No. 12. Art. 3471.

⁹ Mahfuz S., Shang Q., Piao X. Phenolic compounds as natural feed additives in poultry and swine diets: a review. *Journal of Animal Science and Biotechnology*. 2021. Vol. 12, No. 1. Art. 48.

¹⁰ In Vitro Investigation of the Antibacterial Activity of Nine Commercial Water Disinfectants, Acidifiers, and Glyceride Blends against the Most Important Poultry Zoonotic Bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381.

¹¹ Desbois A. P., Smith V. J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Applied Microbiology and Biotechnology*. 2010. Vol. 85, No. 6. P. 1629–1642.

¹² Monobutyryl Reduces Live Cholesterol and Improves Intestinal Barrier Function in Rats Fed High-Fat Diets / T. D. Nguyen et al. *Nutrients*. 2019. Vol. 11, No. 2. Art. 308.

¹³ Use of Short-Chain Fatty Acids for the Recovery of the Intestinal Epithelial Barrier Affected by Bacterial Toxins / D. Pérez-Reytor et al. *Frontiers in Physiology*. 2021. Vol. 12. Art. 650313.

¹⁴ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

¹⁵ Surface Components and Metabolites of Probiotics for Regulation of Intestinal Epithelial Barrier / Q. Liu et al. *Microbial Cell Factories*. 2020. Vol. 19. Art. 23.

¹⁶ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

Organic acids such as butyric, amino acids, and pectin can affect the expression of E-cadherin. E-cadherin is a protein that plays an important role in maintaining the integrity of cell junctions in the intestinal epithelium¹⁷. The changes in E-cadherin expression can affect cell adhesion, migration, and invasion, which are important for the processes of tissue remodelling, inflammatory balance, and wound healing¹⁸. However, to date, the issue of modulation of the molecular markers expression, including E-cadherin, fibronectin (FN), interferon- α (IFN- α) and interferon- γ (IFN- γ) in the duodenum of broiler chickens remains outside the attention of the researchers. Aforementioned markers confirmed to be convenient tools to assess both intestinal barrier integrity and barrier leaking¹⁹.

Microbiome produces many various metabolites which can affect intestinal cells and modulate they functioning thorough both beneficial and detrimental effect. Among of similar metabolites, SCFA exhibit antimicrobial, metabolic, and signalling features. Furthermore, several SCFA blends possess different targeted bioactivity²⁰. However, the effect of SCFA and monoglyceride blend (SCFA-M) on the intestine health also is undiscovered. Therefore, the important field of the animal science is to clarify the effect of SCFA-M on the intestinal barrier integrity, the microbiome community and their interaction in broiler chickens. The understanding of the interaction between microbiome and intestinal cells can advance the development of new approach to increasing the productivity and resistance of animals with the minimization of the use of antibiotics.

The aim of the current work was to study the effect of SCFA-M on the modulation of molecular markers expression, the microbiome composition and to evaluate the correlations between them in broiler chickens.

1. Methodology of the study of intestinal health assessment

The study was carried out in the Research Center of biosafety and environmental control of agriculture resources (Biosafety-center) of Dnipro State Agrarian and Economic University (DSAEU) and poultry farm located in Dnipro region. The broiler chickens of the Cobb 500 cross were used to form the control and SCFA-M-exposed groups.

¹⁷ Modulation of E-cadherin Monomer Folding by Cooperative Binding of Calcium Ions / O. Courjean et al. *Biochemistry*. 2008. Vol. 47, No. 8. P. 2339–2349.

¹⁸ Modulation of E-cadherin Function and Dysfunction by N-glycosylation / S. S. Pinho et al. *Cellular and Molecular Life Sciences*. 2011. Vol. 68, No. 6. P. 1011–1020.

¹⁹ An Isotonic Protein Solution Favorably Modulated the Porcine Intestinal Immune Response and Cellular Adhesion Markers and Reduced PEDV Shedding in Vivo / D. M. Masiuk et al. *Veterinary Immunology and Immunopathology*. 2024. Vol. 271. Art. 110753.

²⁰ Desbois A. P., Smith V. J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Applied Microbiology and Biotechnology*. 2010. Vol. 85, No. 6. P. 1629–1642.

All procedures with the animals were carried out in compliance with the requirements stated in the Law of Ukraine No. 3447-IV dated 21.02.06 “On the Protection of Animals from Cruelty”, agreed with the main principles of the “Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 22 September 2010, Official Journal of the European Union L276, 20 October 2010, pp. 33–79”, adhere to ethical standards concerning animal welfare in accord “Consensus Guidelines on Animal Ethics and Welfare for Veterinary Journals – International Association of Veterinary Editors, Geneva, Switzerland, 2010”. All experimental protocols were approved by the Commission on Bioethics of Experiments on Animals of the DSAEU, Ukraine (Protocol No. 19–072023).

The treatment with SCFA-M carried out to assess SCFA-M effect on the intestinal health of productive animals in the standard conditions of the industrial poultry farming. Two groups of 7 days old chickens were kept in the separate production houses with stocking density in both groups averaged 20.6 chickens/m². The number of broiler chickens in control and SCFA-M-exposed groups was 30.000 and 36.000 chickens approximal.

Broilers were grown according to the company’s standard protocol. Poultry breeding took place according to modern technologies implemented at the enterprise as well as all procedures were performed in accord with the basic requirements for biological safety. All biological safety requirements were implemented in production in the form of relevant work instructions regarding compliance with veterinary and sanitary rules. The birds of the exposed group consumed the SCFA-M blend with drinking water trice on 16–18, 25–29, 32–36 days of life. The final concentration of SCFA-M blend was equal to 1% in a water. The used SCFA-M formulation contained fatty acids (from C-3 to C-10) and monoglycerides as the major compounds of the formulation. Diglycerides, triglycerides, and free glycerol were presented in the blend as the minor components.

Seven chickens were separated from both control and SCFA-M-exposed group. Duodenal tissue samples with contents were collected from chickens on 16th, 22nd, 29th, 36th, and 45th days. The quantitative composition of the microflora was determined by preparing tenfold dilutions according to the standard of Ukraine (ISO 6887-1:2003) and sowing them on appropriate nutrient media. To determine the number of bacteria of the *Escherichia coli* group (BECG), seeding was carried out using Endo’s medium, staphylococci – Beid-Parker’s medium, enterococci – yolk-esculin agar, clostridia – Wilson-Blair’s iron-containing sulphate agar. All studied taxa (*Staphylococcus*, *Enterococcus*, and *Escherichia coli*) were cultivated with aerobic conditions. The crops were cultivated for 20–24 hours in a thermostat at a temperature of

37±1°C. Determination and interpretation of results performed in accordance with ISO 7218. The washed tissue samples were frozen and store at -22°C before western blot (WB) analysis.

The study of the relative content of molecular markers carried out with use the WB method. Briefly, the thin intestinal samples were homogenized in the RIPA buffer contained protease and phosphatase inhibitor cocktail. Total protein concentration in each sample was determined by the Bradford method (Bradford, 1976). After that, the proteins of the intestinal tissue homogenate were separated by electrophoresis in a polyacrylamide gel (PAAG) with an acrylamide gradient 5–20%²¹. Protein bands separated in PAAG were stained in a 0.1% Coomassie G-250 solution to control the quality of the distribution. The transfer of proteins from PAAG to nitrocellulose membrane was carried out using an electric current at 150 mA for 60 min. The blocking of nitrocellulose membrane was performed with bovine serum albumin 1% solution in PBS. After blocking, nitrocellulose membrane was incubated with primary antibodies against E-cadherin (Santa Cruz, sc-271842), fibronectin (Santa Cruz, sc-8422), interferon- α (Abcam, ab191903), and interferon- γ (Santa Cruz, sc-74104) diluted accordingly producer recommendation. After incubation with primary mouse antibodies (anti-E-cadherin, anti-fibronectin, and anti-interferon- γ) 14 hours at 4°C, the membranes were washed 3 times with PBS and probed with secondary anti-mouse (Abcam, ab6789) horseradish peroxidase-conjugated antibody diluted accordingly producer recommendations. Membranes were then washed in PBS three times. Using the same protocol, samples were incubated with anti-rabbit antibody (Abcam, ab205718) for the detection of primary anti-IFN- α . To visualize the polypeptide zones, the nitrocellulose membrane was incubated with a Tris-HCl solution containing diaminobenzidine (0.05%) and hydrogen peroxide (0.02%). The results reflecting the total protein content and immunoblotting were displayed as percentages relative to the similar results in a control group.

Statistical analysis of the obtained data was performed using the specialized software GraphPad Prism 9. The sample parameters presented in the work had the following designations: M – sample mean; SD – standard deviation. Changes in all obtained data were considered reliable at $P < 0.05$ (including $P < 0.01$ and $P < 0.001$).

2. The results on intestinal health assessment

The content of E-cadherin and FN was determined to evaluate the integrity of the intestinal barrier of the small intestine of chickens. The results of WB

²¹ Nedzvetsky V. S., Agca C. A., Baydas G. The Peptidoglycan Fraction Enriched with Muramyl Pentapeptide from *Lactobacillus bulgaricus* Inhibits Glioblastoma U373MG Cell Migration Capability and Upregulates PARP1 and NF- κ B Levels. *Biotechnology Acta*. 2020. Vol. 13, No. 2. P. 65–79.

showed that the content of E-cadherin in the duodenum of broiler chickens of the control and experimental groups on the 16th day of life was not significantly different (Fig. 1). During the period from 16th to 45th day of chickens' life, the modulation of E-cadherin expression in the duodenum of broiler chickens of the control group demonstrated a downward trend, as indicated by a downward regression line, and the regression equation had the form $-Y = -0.1005 \cdot X + 105.4$ ($P = 0.47$).

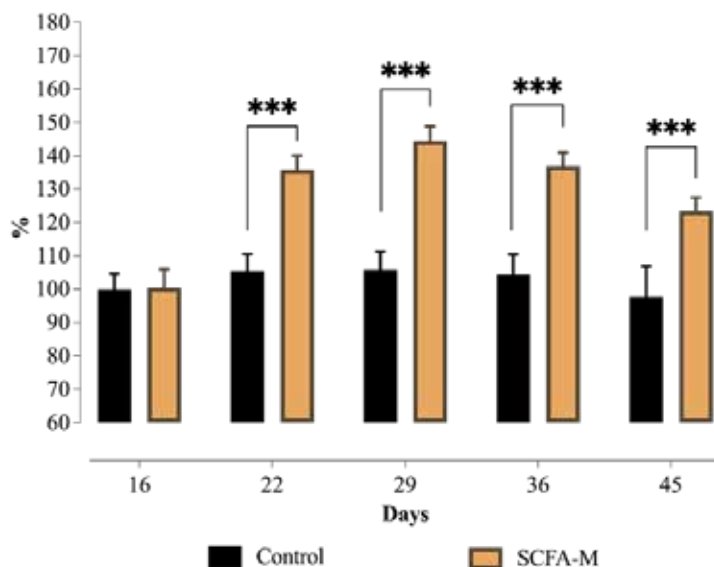


Fig. 1. Modulation of E-cadherin content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$).
Significant differences: *** $P < 0.001$

Consumption of SCFA-M by chickens of the exposed group had a significant effect on this membrane protein expression in the duodenum. In particular, from the 16th to the 22nd day of life the E-cadherin content in the duodenum of broiler chickens of the research group was increased by 35.3% ($P \leq 0.001$). After the 22nd day, the content of E-cadherin increased but somewhat slower until the 29th day of age (by 8.7%), then it gradually decreased by 21.0% ($P \leq 0.001$) until the 45th day of life. We registered higher level of E-cadherin expression in chickens of the research group throughout the entire period of research, so in the duodenum of broiler chickens 22-, 29-, 36-, and 45-day-old,

the expression of this protein was higher respectively by 30.4%, ($P \leq 0.001$), 38.8% ($P \leq 0.001$), 32.6% ($P \leq 0.001$) and 25.8% ($P \leq 0.001$) compared to the indicators of chickens of the control group. The regression equation for the content of E-cadherin in the duodenum of broiler chickens of the research group was $Y = 0.5612 \cdot X + 112.8$ ($P = 0.07$).

The content of FN in the duodenum of 16-day-old broiler chickens of the control and experimental groups did not differ (Fig. 2). During the experiment, the expression of FN in the duodenum of broiler chickens of the control group was increased by 4.8% from 16th to 45th day of life, which determined the upward regression line ($Y = 0.152 \cdot X + 99.1$; $P = 0.123$).

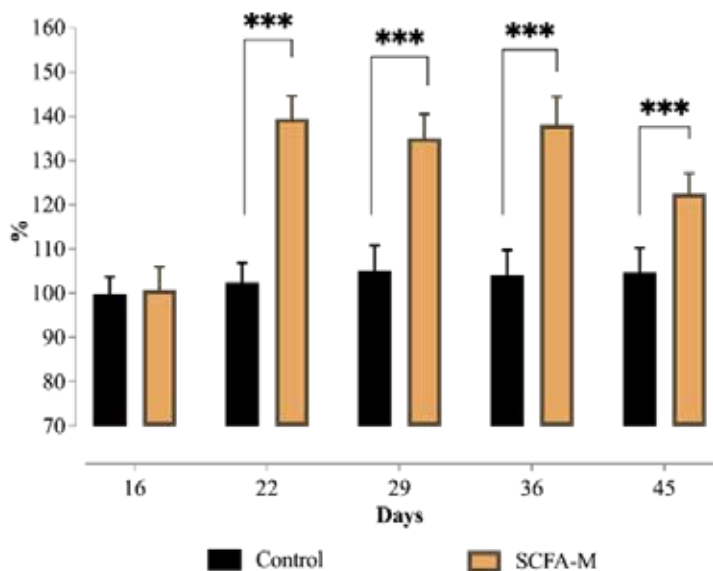


Fig. 2. Modulation of FN content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$). Significant differences: * $P < 0.001$**

The content of FN in chickens of the experimental group duodenum increased by 38.6% ($P \leq 0.001$) from the 16th to the 22nd day of life. The FN content did not change significantly from the 22nd day to the 36th day of life, however, from the 36th to the 45th day of life, the FN content decreased by 15.6% ($P \leq 0.001$). The level of FN expression in the duodenum of the 22-, 29-, 36-, and 45-day-old chickens of the experimental group was higher by 37.0% ($P \leq 0.001$), 30.0% ($P \leq 0.001$),

33.9%, respectively ($P \leq 0.001$) and 17.8% ($P \leq 0.001$) compared to the indicators of chickens of the control group. The regression equation for the content of FN in the duodenum of broiler chickens of the experimental group was $-Y = 0.500 \cdot X + 113.5$ ($P = 0.104$).

The content of IFN- α in the duodenum of 16-day-old broiler chickens of the control and experimental groups was not significantly different (Fig. 3). The expression of IFN- α in the duodenum did not change significantly from the 16th to the 45th day of life of chickens of the control group. Accordingly, the regression equation had the form $-Y = 0.072 \cdot X + 101.9$; $P = 0.628$. Along with this, the content of IFN- α in chickens of the research group duodenum increases by 78% ($P \leq 0.001$) by the 22nd day of life. After that it gradually decreases, in particular, by 29 days of age by 26% ($P \leq 0.001$), by the 36th day by 16.4% ($P \leq 0.05$) and by the 45th day by another 10.4%.

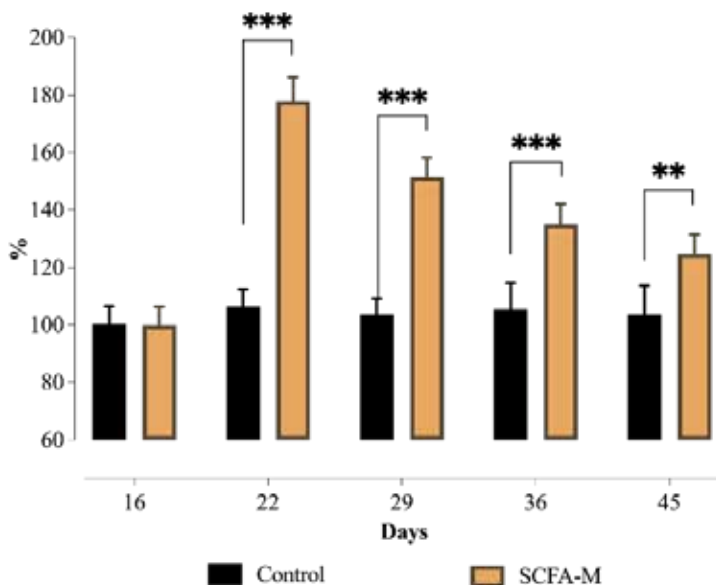


Fig. 3. IFN- α content in the duodenum of broiler chickens exposed to SCFA-M ($M \pm SD$; $n=5$). Significant differences: ** $P < 0.01$, * $P < 0.001$**

Obtained data evidence that the level of IFN- α expression in the duodenum of chickens of the experimental group of 22-, 29-, 36- and 45-days of age was significantly higher, respectively by 71.6% ($P \leq 0.001$), 47.8% ($P \leq 0.001$), 29.7% ($P \leq 0.001$) and 21.0% ($P \leq 0.01$) compared to the indicators of broiler

chickens of the control group. The regression equation for the content of this marker in the duodenum of broiler chickens of the experimental group was $Y = -0.032 \cdot X + 138.7$ ($P = 0.954$). Observed results demonstrated that the content of IFN- γ in the duodenum of broiler chickens of the control and experimental groups on the 16th day of life did not differ significantly and showed a downward trend in both groups (Fig. 4). Thus, the modulation of IFN- γ expression in the duodenum of broiler chickens of the control group was lower by 13.9% than in a control group by the 22nd day of life and, accordingly, the regression equation had the form $-Y = -0.476 \cdot X + 100.9$ ($P = 0.002$).

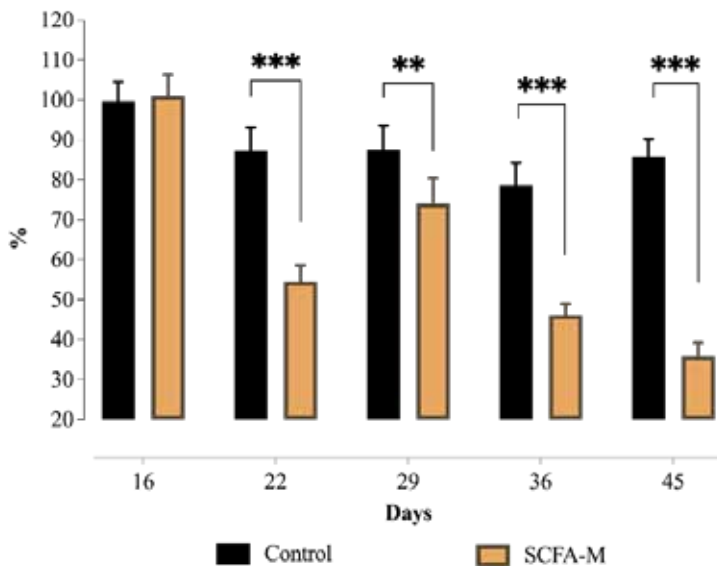


Fig. 4. IFN- γ content in the duodenum of broiler chickens exposed to SCFA-M (M $\pm$ SD; n=5). Significant differences: ** $P < 0.01$, * $P < 0.001$**

The content of IFN- γ in the duodenum of SCFA-M-exposed group decreased by 46.4% ($P \leq 0.001$) from the 16th to the 22nd day of life, after which it increases slightly (by 19.5%; $P \leq 0.01$) until the age of 29 days. The followed decrease was observed until the age of 36 days (by 28.0%; $P \leq 0.001$). Later, by the 45th day of life, the content of IFN- γ in chickens of the SCFA-M-exposed group drops by 10.2%. The level of IFN- γ expression in the duodenum of the chickens of the SCFA-M-exposed group throughout the experiment was significantly lower than that of the control group chickens, in particular at 22nd,

29th, 36th, and 45th days by 32.7% ($P \leq 0.001$), 13.5% ($P \leq 0.01$), 32.4% ($P \leq 0.001$) and 49.9% ($P \leq 0.001$) respectively. The regression equation for IFN- γ content in the duodenum of broiler chickens of the research group was $-Y = -1.89 \cdot X + 114.5$ ($P = 0.001$).

Correlational analysis of present data on the modulation of molecular markers expression in the duodenum of control group chickens didn't detected significant and/or statistically interrelations between these parameters. However, the direct correlation links between the expression of E-cadherin, FN, and IFN- α were observed in chicken duodenum equal $-r = 0.21-0.58$. On the other side, inverse correlations were found between the expression of IFN- γ and E-cadherin $-r = -0.36$, of IFN- γ and FN $-r = -0.79$, of IFN- γ and IFN- α $-r = -0.82$ (Fig. 5).

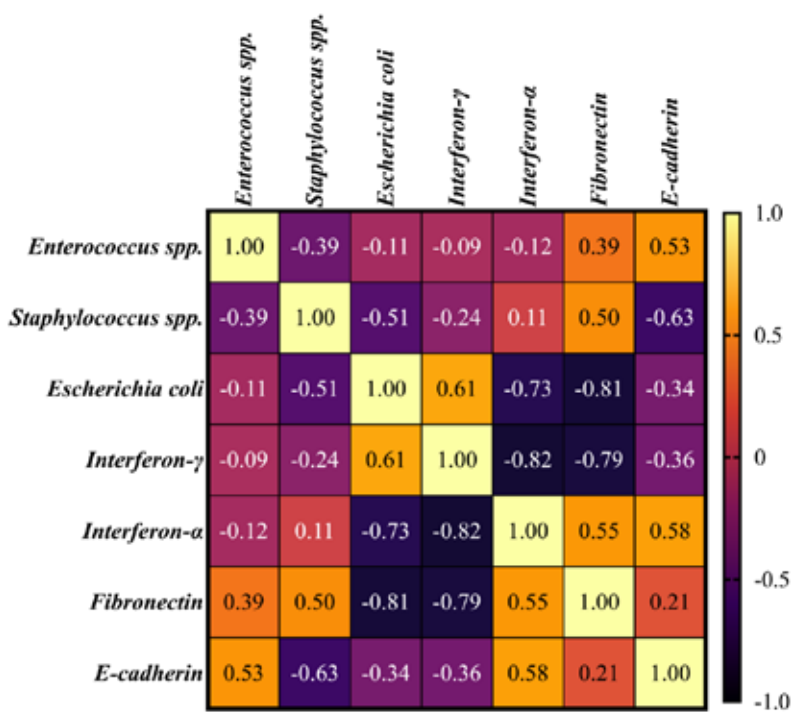


Fig. 5. Correlation matrix of interrelation between molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) and duodenal microbiome broiler chickens of the control group

Furthermore, the supplementation of SCFA-M induced increase in aforementioned correlation coefficients. Besides, statistically significant correlations in SCFA-M-exposed group were found between E-cadherin and FN – $r = 0.96$, between E-cadherin and IFN- α – $r = 0.80$, as well as between E-cadherin and IFN- γ – $r = -0.54$. Similar to the correlation between E-cadherin and other markers, the interrelations between FN and IFN- α exhibited direct dependence (Fig. 6). On the other side, the interrelation between IFN- γ and modulation of FN was decreased – $r = -0.54$ and as well as modulation IFN- α – $r = -0.41$ (Fig. 6).

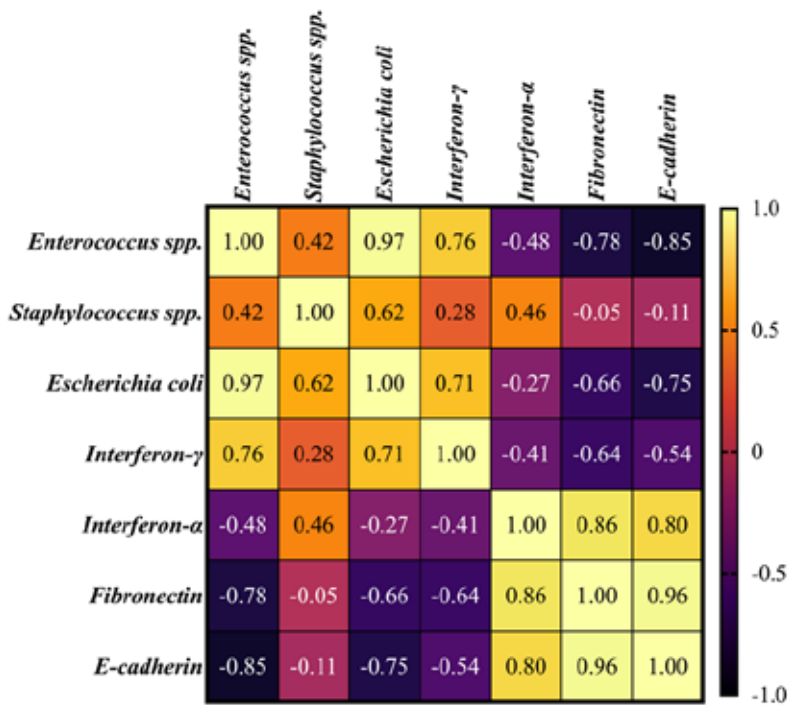


Fig. 6. Correlation matrix of interrelation between molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) and duodenal microbiome broiler chickens of the SCFA-exposed groups (r ; $n=5$)

Observed data on the changes in relative content of the main taxa including *Escherichia coli*, *Enterococcus spp.*, and *Staphylococcus spp.* evidence that all of them permanently involved in concurrent interrelations and participate in the competition to colonize the environment of gut. This fact is supported

by the results of the correlative analysis in respect with the calculated inverse correlations between *Escherichia coli* and *Enterococcus* spp. ($r = -0.11$), *Escherichia coli* and *Staphylococcus* spp. ($r = -0.51$), *Staphylococcus* spp. and *Enterococcus* spp. ($r = -0.39$). However, the SCFA-M supplementation affected multiple interactions into microbiome community and shifted the relative content several taxa. Obtained results have shown that SCFA-M significantly changed correlation between *Escherichia coli* and *Enterococcus* spp. – $r = 0.96$. Furthermore, the direct correlation was observed between *Staphylococcus* spp. and *Escherichia coli* ($r = 0.42$) relative contents as well as between *Staphylococcus* spp. and *Enterococcus* spp. ($r = 0.62$).

The results on main taxa relative content demonstrated inverse correlation in control group of chickens between *Escherichia coli* and the modulation of molecular markers including E-cadherin, FN, and IFN- α ($r = -0.81$ – 0.34). Besides, the direct correlation was observed between *Escherichia coli* and IFN- γ modulation ($r = 0.81$). Similar interrelations were detected in SCFA-M-exposed chicken group.

Direct correlations were detected between *Staphylococcus* spp. content in duodenum of chicken control group and the changes in IFN- α ($r = 0.11$) and FN ($r = 0.50$) contents. However, inverse correlation was observed between *Staphylococcus* spp. content and E-cadherin ($r = -0.63$) and IFN- γ ($r = -0.24$). The treatment with SCFA-M induced the shift of similar to control group parameters, particularly correlation between *Staphylococcus* spp. content in thin intestine SCFA-M-exposed chicken group and E-cadherin modulation was equal $r = -0.11$, while this parameter of FN was equal $r = -0.05$. On the other hand, direct correlations were observed between *Staphylococcus* spp. content and IFN- α changes ($r = 0.28$) and IFN- γ changes ($r = 0.46$).

The relative content of *Enterococcus* spp. in chicken duodenum of control group exhibited the direct correlation in respect with FN and E-cadherin modulation ($r = 0.11$ and $r = 0.50$ respectively). Invers correlation between *Enterococcus* spp. content and IFN- α and IFN- γ changes were observed comparatively low ($r = -0.12$ and $r = -0.09$ accordingly). Moreover, these interrelations were statistically insignificant. Similar to previous described correlations, the SCFA-M exposure induced an increase in inverse correlation coefficients between *Enterococcus* spp. content in thin intestine and modulation of E-cadherin, FN, and IFN- α ($r = -0.85$, $r = -0.78$, and -0.48 respectively). The direct correlation was detected between *Enterococcus* spp. content and changes in IFN- γ ($r = 0.76$) similarly to other studied taxa. Therefore, contrary to aforementioned interrelations in control group, SCFA-M application initiated the decrease in the correlation coefficients almost in all groups from 16th to 45th day of life.

3. The intestinal health maintenance is key task to provide poultry profitability

The correlation analysis demonstrated a complex nonlinear interaction between molecular markers and the duodenal microbiome community of broiler chickens. In the control group, the relationships between molecular markers are weak and statistically insignificant, which indicates the preservation of the balance of the microbiome community within the physiological norm. At the same time, the addition of SCFA-M induced in the study a significant increase in the correlation between markers and the microbiome. This effect may be due to the signaling and regulatory properties of SCFA-M in relation to individual microbiome taxa. In addition, the inclusion of SCFA-M in the energy metabolism pathways of intestinal cells stimulates the expression of individual specific proteins, including intercellular adhesion proteins. All this together can stimulate the complex interaction of the microbiome with intestinal epithelial cells and contribute to the improvement of barrier function.

The observed modulation of the *Escherichia coli* content demonstrated significant inverse relationships with E-cadherin, FN and IFN- α , which reflects the possible negative impact of this bacterium on structural and immune markers. The direct relationship of *Escherichia coli* with IFN- γ indicates the activation of a specific immune response. Similar trends persist in the SCFA-M-exposed group. The changes in *Staphylococcus* spp. content in control group had a direct relationship with the modulation of FN and IFN- α . On the other hand, this taxon had an inverse relationship with the modulation of the content of E-cadherin and IFN- γ . Thus, the results have shown multifaceted regulation of innate immune and structural markers. The effects of SCFA-M significantly modulate the correlation between the microbiome state and the content of the above-mentioned molecular markers. In general, the observed effects of SCFA-M can be associated with differential regulation of individual microbiome taxa by modifying the environment and changing the competition conditions between the main taxa. Changes in the content of *Enterococcus* spp. in the control group showed weak and unreliable relationships with the modulation of molecular markers. However, these relationships in the SCFA-M-exposed group become significant and strengthened. For example, the relative content of *Enterococcus* spp. was inversely correlated with the content of E-cadherin, FN and IFN- α . On the other hand, a direct correlation was found between the content of *Enterococcus* spp. and changes in IFN- γ . Obtained data demonstrated beneficial effect of SCFA-M supplementation, including the commensal microflora modulation via involving *Enterococcus* spp. in the regulation of both the immune system and the intestinal barrier integrity. Therefore, SCFA-M is potent to regulate

interrelation of various microbiome taxa and to switch the communication from synergic type to antagonistic suppression.

Epithelial cells of the intestine, bound together by various intercellular proteins, are the first line of defense against bacteria and their toxins. Claudins, occludins and other TJ proteins are located on the apical surface of the basolateral membrane, form intercellular tight contacts, regulate permeability through the epithelial monolayer and are responsible for cell polarity²². Structural integrity largely depends on the expression levels of intercellular junction proteins²³. We established the fundamental relationships of the modulation of the content of molecular markers (E-cadherin, FN, IFN- α , and IFN- γ) in the duodenum of broiler chickens ($r = 0.98-1.00$; $P \leq 0.001$).

The results of present study have established the dynamics of modulation of the molecular markers expression in the duodenum of broiler chickens for the first time. In particular, the modulation of E-cadherin expression in the cecum of broiler chickens of the control group increases by 5.7% from the 16th to the 22nd day, after which it gradually decreases (by 7.2%) until the 45th day of the chickens' life. At the same time, the expression of E-cadherin in the duodenum of broiler chickens was less dynamics and shown a tendency to decrease from 16th to the 45th day. Recently, it has been shown that the content of E-cadherin in the small intestine can be used as an indicator of the deterioration of the barrier function of the gut²⁴.

FN plays an important role in maintaining the small intestine health of broilers by providing cell adhesion to the extracellular matrix, protection against pathogens, and promoting tissue healing and remodeling processes^{25, 26}. Optimum housing conditions and balanced nutrition are key factors contributing to the maintenance of an adequate level of FN and ensure the health and high productivity of birds²⁷.

IFNs are a family of cytokines that exert biological activity by binding to specific cell membrane receptors and triggering a signaling pathway that

²² Use of Short-Chain Fatty Acids for the Recovery of the Intestinal Epithelial Barrier Affected by Bacterial Toxins / D. Pérez-Reytor et al. *Frontiers in Physiology*. 2021. Vol. 12. Art. 650313.

²³ Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135.

²⁴ An Isotonic Protein Solution Favorably Modulated the Porcine Intestinal Immune Response and Cellular Adhesion Markers and Reduced PEDV Shedding in Vivo / D. M. Masiuk et al. *Veterinary Immunology and Immunopathology*. 2024. Vol. 271. Art. 110753.

²⁵ The Role of the Fibronectin Synergy Site for Skin Wound Healing / I. Gimeno-Lluch et al. *Cells*. 2022. Vol. 11, No. 13. Art. 2100.

²⁶ Kanta J., Zavadakova A. Role of Fibronectin in Chronic Venous Diseases: A Review. *Vascular Medicine*. 2020. Vol. 25, No. 6. P. 588–597.

²⁷ Novel Models for Chronic Intestinal Inflammation in Chickens: Intestinal Inflammation Pattern and Biomarkers / G. C. Dal Pont et al. *Frontiers in Immunology*. 2021. Vol. 12. Art. 676628.

culminates in the induction of interferon-stimulated gene (ISG) transcription and immune enhancing effect^{28, 29}. Mediated by ISGs, IFNs generate a variety of cellular and physiological responses, including antiviral, apoptotic, antiproliferative, antitumor, and immunomodulatory effects³⁰. The expression of IFN- α in the duodenum of chickens didn't changed significantly during the observed experimental period.

Several results have shown that some organic acids can affect the expression level of intercellular adhesion³¹. For example, butyric acid, which is one of the main fatty acids, can reduce the expression of E-cadherin in some cells. This may have important consequences for cell adhesion and other processes occurring in intestinal epithelial layer^{32, 33}.

The relationship between the state of the microbiota and the modulation of intestinal functions has attracted the special attention of researchers. The changes in the content of the main microbiota taxa accompanied by the modulation of adhesion proteins expression. In particular, the microbiota effects on the expression of E-cadherin, occludin, and claudin have been reported³⁴. The microbiota involved into the maintenance of the balance between these proteins, which ensures optimal functioning of the intestinal barrier^{35, 36}. The analysis of present results established interdependencies between the modulation of molecular markers and the duodenal microbiome of the broiler chickens where the content of E-cadherin, FN, IFN- α , and IFN- γ are interconnected with the main taxa ratio. Additionally, obtained data have demonstrated correlation links between microbiome community state and the modulation of intercellular adhesion proteins and innate immune molecular markers first time.

²⁸ Molecular Analysis of Chicken Interferon-alpha Inducible Protein 6 Gene and Transcriptional Regulation / J. W. Park et al. *Journal of Animal Science and Technology*. 2023. Vol. 65, No. 1. P. 183–197.

²⁹ The Immune Enhancing Effect of Antimicrobial Peptide LLv on Broilers Chickens / X. Liu et al. *Poultry Science*. 2024. Vol. 103, No. 2. Art. 103235.

³⁰ Parker N., Porter A. C. G. Identification of a Novel Gene Family that Includes the Interferon-Inducible Human Genes 6–16 and ISG12. *BMC Genomics*. 2004. Vol. 5. Art. 8.

³¹ Surface Components and Metabolites of Probiotics for Regulation of Intestinal Epithelial Barrier / Q. Liu et al. *Microbial Cell Factories*. 2020. Vol. 19. Art. 23.

³² Modulation of Apoptosis and Epithelial-Mesenchymal Transition E-cadherin/TGF-beta/Snail/TWIST Pathways by a New Ciprofloxacin Chalcone in Breast Cancer Cells / R. Alaaeldin et al. *Anticancer Research*. 2021. Vol. 41, No. 5. P. 2383–2395.

³³ Fomicheva M., Tross E. M., Macara I. G. Polarity Proteins in Oncogenesis. *Current Opinion in Cell Biology*. 2020. Vol. 62. P. 26–30.

³⁴ Regulation of Intestinal Barrier Function by Microbial Metabolites / S. Ghosh et al. *Cellular and Molecular Gastroenterology and Hepatology*. 2021. Vol. 11, No. 5. P. 1463–1482.

³⁵ The Gut Microbiota in Inflammatory Bowel Disease / P. Qiu et al. *Frontiers in Cellular and Infection Microbiology*. 2022. Vol. 12. Art. 733992.

³⁶ Compound Probiotics Can Improve Intestinal Health by Affecting the Gut Microbiota of Broilers / X. Liu et al. *Journal of Animal Science*. 2023. Vol. 101. Art. skad388.

CONCLUSIONS

The application of SCFA-M feed additive induced an increase in the content of E-cadherin, FN, IFN- α and a decrease in the production of IFN- γ in the duodenum. In particular, the effect of SCFA-M in chickens is characterized by statistically significant increase in the content of E-cadherin, FN, and IFN- α through the all periods of the exposure. Inversely, SCFA-M treatment induced statistically significant decrease in the IFN- γ production in compare with the control group. Present results have demonstrated the effect of SCFA-M supplementation on both microbiome community and intestinal barrier integrity of broiler chickens. Furthermore, SCFA-M blend exhibited the modulatory effect on the bilateral interaction between microflora and intestinal epithelial cells.

SUMMARY

The continuous antibiotics application in the animal farming caused the generation of microbial resistant strains. Multi-drug resistant strains are extremely weighted risk factor for both animals and human. The aim of study was to assess the effect of short chain fatty acids plus monoglycerides (SCFA-M) blend on microbiome community and intestinal barrier integrity of broiler chickens. Control group fed with standard diet and antibiotics supplementation. SCFA-exposed group fed with the same diet without antibiotics and additionally supplemented with SCFA-M in a dose 1% four times for 5 days on 16th, 22th, 29th, and 32nd days of life. The microbiome state was estimated via detection of relative content of principal taxa. Intestinal barrier integrity was assessed through the measuring of E-cadherin and fibronectin contents in gut tissue. Innate immunity was assessed via detection of interferon- α and interferon- γ . The administration of SCFA-M induced upregulation in E-cadherin, fibronectin, and IFN- α content ($P \leq 0.01$) in duodenum of SCFA-M-exposed broilers in compare to control group. Inversely, the level of IFN- γ was significantly decreased ($P \leq 0.01$) in the duodenal intestine of the exposed broilers. The correlative analysis demonstrated the link between molecular markers and the changes of main taxa including *Escherichia coli*, *Staphylococcus* spp., and *Enterococcus* spp. Molecular markers content was correlated with microbiome changes where increase in E-cadherin and fibronectin have a positive correlation with *Escherichia coli* content in 22-day-old broiler chickens ($r = 0.79-0.87$) and negative correlation with *Staphylococcus* spp. content in 45-day-old chickens ($r = -0.83 - -0.51$). The complex correlations of all studied molecular markers expression in the duodenum of broiler chickens significantly varied from 16th to 45th day of life in respect with both magnitude and direction of changes. Thus, the application of SCFA-M to broiler chickens has showed a potential to modulate intestinal barrier integrity and microbiome community via non-linear dependence.

Bibliography

1. Abdelli N., Solà-Oriol D., Pérez J. F. Phytogetic Feed Additives in Poultry: Achievements, Prospective and Challenges. *Animals*. 2021. Vol. 11, No. 12. Art. 3471. DOI: <https://doi.org/10.3390/ani11123471>.
2. Modulation of Apoptosis and Epithelial-Mesenchymal Transition E-cadherin/TGF-beta/Snail/TWIST Pathways by a New Ciprofloxacin Chalcone in Breast Cancer Cells / R. Alaaeldin et al. *Anticancer Research*. 2021. Vol. 41, No. 5. P. 2383–2395. DOI: <https://doi.org/10.21873/anticancer.15013>.
3. Modulation of E-cadherin Monomer Folding by Cooperative Binding of Calcium Ions / O. Courjean et al. *Biochemistry*. 2008. Vol. 47, No. 8. P. 2339–2349. DOI: <https://doi.org/10.1021/bi701340d>.
4. Desbois A. P., Smith V. J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Applied Microbiology and Biotechnology*. 2010. Vol. 85, No. 6. P. 1629–1642. DOI: <https://doi.org/10.1007/s00253-009-2355-3>.
5. Fomicheva M., Tross E. M., Macara I. G. Polarity Proteins in Oncogenesis. *Current Opinion in Cell Biology*. 2020. Vol. 62. P. 26–30. DOI: <https://doi.org/10.1016/j.ceb.2019.07.016>.
6. Regulation of Intestinal Barrier Function by Microbial Metabolites / S. Ghosh et al. *Cellular and Molecular Gastroenterology and Hepatology*. 2021. Vol. 11, No. 5. P. 1463–1482. DOI: <https://doi.org/10.1016/j.jcmgh.2021.02.007>.
7. The Role of the Fibronectin Synergy Site for Skin Wound Healing / I. Gimeno-Lluch et al. *Cells*. 2022. Vol. 11, No. 13. Art. 2100. DOI: <https://doi.org/10.3390/cells11132100>.
8. Kanta J., Zavadakova A. Role of Fibronectin in Chronic Venous Diseases: A Review. *Vascular Medicine*. 2020. Vol. 25, No. 6. P. 588–597. DOI: <https://doi.org/10.1177/1358863X20947789>.
9. Prospects of Organic Acids as Safe Alternative to Antibiotics in Broiler Chickens Diet / R. U. Khan et al. *Environmental Science and Pollution Research International*. 2022. Vol. 29, No. 22. P. 32594–32604. DOI: <https://doi.org/10.1007/s11356-022-19241-8>.
10. Surface Components and Metabolites of Probiotics for Regulation of Intestinal Epithelial Barrier / Q. Liu et al. *Microbial Cell Factories*. 2020. Vol. 19. Art. 23. DOI: <https://doi.org/10.1186/s12934-020-1289-4>.
11. Compound Probiotics Can Improve Intestinal Health by Affecting the Gut Microbiota of Broilers / X. Liu et al. *Journal of Animal Science*. 2023. Vol. 101. Art. skad388. DOI: <https://doi.org/10.1093/jas/skad388>.
12. The Immune Enhancing Effect of Antimicrobial Peptide LLv on Broilers Chickens / X. Liu et al. *Poultry Science*. 2024. Vol. 103, No. 2. Art. 103235. DOI: <https://doi.org/10.1016/j.psj.2023.103235>.

13. Mahfuz S., Shang Q., Piao X. Phenolic compounds as natural feed additives in poultry and swine diets: a review. *Journal of Animal Science and Biotechnology*. 2021. Vol. 12, No. 1. Art. 48. DOI: <https://doi.org/10.1186/s40104-021-00565-3>.
14. In Vitro Investigation of the Antibacterial Activity of Nine Commercial Water Disinfectants, Acidifiers, and Glyceride Blends against the Most Important Poultry Zoonotic Bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381. DOI: <https://doi.org/10.3390/pathogens12030381>.
15. An Isotonic Protein Solution Favorably Modulated the Porcine Intestinal Immune Response and Cellular Adhesion Markers and Reduced PEDV Shedding in Vivo / D. M. Masiuk et al. *Veterinary Immunology and Immunopathology*. 2024. Vol. 271. Art. 110753. DOI: <https://doi.org/10.1016/j.vetimm.2024.110753>.
16. Novel Models for Chronic Intestinal Inflammation in Chickens: Intestinal Inflammation Pattern and Biomarkers / G. C. Dal Pont et al. *Frontiers in Immunology*. 2021. Vol. 12. Art. 676628. DOI: <https://doi.org/10.3389/fimmu.2021.676628>.
17. Effect of prebiotics administered during embryo development on mitochondria in intestinal and immune tissues of adult broiler chickens / A. Dunislawska et al. *Poultry Science*. 2023. Vol. 102, No. 6. Art. 102663. DOI: <https://doi.org/10.1016/j.psj.2023.102663>.
18. Nedzvetsky V. S., Agca C. A., Baydas G. The Peptidoglycan Fraction Enriched with Muramyl Pentapeptide from *Lactobacillus bulgaricus* Inhibits Glioblastoma U373MG Cell Migration Capability and Upregulates PARP1 and NF-kappaB Levels. *Biotechnology Acta*. 2020. Vol. 13, No. 2. P. 65–79. DOI: <https://doi.org/10.15407/biotech13.02.065>.
19. Monobutyryl Reduces Live Cholesterol and Improves Intestinal Barrier Function in Rats Fed High-Fat Diets / T. D. Nguyen et al. *Nutrients*. 2019. Vol. 11, No. 2. Art. 308. DOI: <https://doi.org/10.3390/nu11020308>.
20. Molecular Analysis of Chicken Interferon-alpha Inducible Protein 6 Gene and Transcriptional Regulation / J. W. Park et al. *Journal of Animal Science and Technology*. 2023. Vol. 65, No. 1. P. 183–197. DOI: <https://doi.org/10.5187/jast.2022.e101>.
21. Parker N., Porter A. C. G. Identification of a Novel Gene Family that Includes the Interferon-Inducible Human Genes 6–16 and ISG12. *BMC Genomics*. 2004. Vol. 5. Art. 8. DOI: <https://doi.org/10.1186/1471-2164-5-8>.
22. Use of Short-Chain Fatty Acids for the Recovery of the Intestinal Epithelial Barrier Affected by Bacterial Toxins / D. Pérez-Reytor et al. *Frontiers in Physiology*. 2021. Vol. 12. Art. 650313. DOI: <https://doi.org/10.3390/fphys.2021.650313>.

23. Modulation of E-cadherin Function and Dysfunction by N-glycosylation / S. S. Pinho et al. *Cellular and Molecular Life Sciences*. 2011. Vol. 68, No. 6. P. 1011–1020. DOI: <https://doi.org/10.1007/s00018-010-0595-0>.
24. The Gut Microbiota in Inflammatory Bowel Disease / P. Qiu et al. *Frontiers in Cellular and Infection Microbiology*. 2022. Vol. 12. Art. 733992. DOI: <https://doi.org/10.3389/fcimb.2022.733992>.
25. Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass Yields, Muscle Amino Acid and Fatty Acid Profiles, and Antioxidant Indices of Broilers Supplemented with Synbiotic and/or Organic Acids. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52. DOI: <https://doi.org/10.1111/jpn.12994>.
26. Global Restriction of Using Antibiotic Growth Promoters and Alternative Strategies in Poultry Production / H. M. Salim et al. *Science Progress*. 2018. Vol. 101, No. 1. P. 52–75. DOI: <https://doi.org/10.3184/003685018X15173975498947>.
27. Nutraceuticals Induced Changes in the Broiler Gastrointestinal Tract Microbiota / E. Tolnai et al. *mSystems*. 2021. Vol. 6, No. 1. Art. e01124-20. DOI: <https://doi.org/10.1186/mSystems.01124-20>.
28. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription / H.-B. Wang et al. *Digestive Diseases and Sciences*. 2012. Vol. 57, No. 12. P. 3126–3135. DOI: <https://doi.org/10.1007/s10620-012-2259-4>.
29. Dietary tributyrin improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders / J. Wang et al. *Poultry Science*. 2021. Vol. 100, No. 11. Art. 101429. DOI: <https://doi.org/10.1016/j.psj.2021.101429>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,
Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,

Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

BIOLOGICAL BASIS OF INDUSTRIAL HEMP BREEDING IN THE CONTEXT OF SUSTAINABLE DEVELOPMENT

Mishchenko Serhii

DOI <https://doi.org/10.30525/978-9934-26-714-7-13>

INTRODUCTION

The global agricultural sector's shift towards sustainable development, renewable energy and “green” chemistry has led to a large-scale renaissance of industrial (non-psychotropic) hemp (*Cannabis sativa* L.). As a waste-free crop with high carbon sequestration potential, hemp is a source of high-quality fiber, vegetable protein, valuable oil and specific cannabinoid compounds of a non-psychotropic profile. The high potential for biological productivity combined with low requirements for the use of pesticides and chemical fertilizers makes this crop an important element of environmentally safe agricultural production.

The concept of sustainable development involves a harmonious combination of economic efficiency, environmental safety and social responsibility. Industrial hemp fully meets these principles thanks to its high biomass productivity, phytoremediation ability, low need for pesticides and versatility of product use¹.

The modern agricultural sector requires the creation of hemp varieties with pre-programmed biological properties by combining classical breeding and innovative genomic technologies.

The rapid development of molecular biology has opened up new opportunities for improving the breeding of industrial hemp. Unlike traditional methods based on phenotypic selection, modern biotechnology allows assessing the genotype of a plant regardless of growing conditions and stage of development².

One of the most promising areas is the use of molecular markers. The use of SSR, AFLP, ISSR, and SNP markers has made it possible to assess the level of genetic polymorphism of world cannabis collections,

¹ Amaducci S., Scordia D., Liu F. H., Zhang Q., Guo H., Testa G., Cosentino S. L. Key cultivation techniques for hemp in Europe and China. *Industrial Crops and Products*. 2015. Vol. 68. P. 2–16. DOI: 10.1016/j.indcrop.2014.06.041

² Kovalchuk I., Pellino M., Rigault P. et al. The genomics of *Cannabis* and its close relatives. *Annual Review of Plant Biology*. 2020. Vol. 71. P. 713–739. DOI: 10.1146/annurev-arplant-081519-040203

establish the degree of relatedness between varieties, and identify sources of valuable traits³.

Special attention is paid to the genes of cannabinoid biosynthesis. After genome sequencing, it became possible to create markers closely linked to the CBDAS and THCAS genes, which significantly increased the efficiency of early selection of breeding material⁴.

The use of biotechnological methods in hemp breeding is becoming widespread⁵.

A promising direction is the use of genomic sequencing and transcriptomic analysis. The study of gene expression allows us to establish the mechanisms of resistance to abiotic stresses, growth regulation, cannabinoid accumulation and development of fibrous tissues. At the same time, the use of genetic editing methods (CRISPR/Cas9) in hemp breeding is still at an early stage. The main limitations remain insufficiently developed transformation systems and the complexity of plant regeneration after genome editing.

The success of creating modern high-yielding varieties is based on a deep understanding of the fundamental biological mechanisms of genetic determination of sex and stabilization of the sexual structure of monoecious hemp populations^{6, 7, 8}, the patterns of inheritance of the chemical chemotype and strict control of cannabinoid biosynthesis (with the prevention of exceeding the THC threshold^{9, 10, 11}, the architectonics of fibrous structures, improving the

³ Sawler J., Stout J. M., Gardner K. M. et al. The genetic structure of marijuana and hemp. PLoS ONE. 2015. Vol. 10. Iss. 8. Art. 0133292. DOI: 10.1371/journal.pone.0133292

⁴ Lavery K. U., Stout J. M., Sullivan M. J. et al. A physical and genetic map of *Cannabis sativa* identifies extensive rearrangements at the THC/CBD acid synthase loci. Genome Research. 2019. Vol. 29. Iss. 1. P. 146–156. DOI: 10.1101/gr.242594.118

⁵ Hesami M., Pepe M., Alizadeh M., Rakei A., Baiton A., Jones A. M. P. Recent advances in cannabis biotechnology. *Industrial Crops and Products*. 2020. Vol. 158. Art. 113026. DOI: 10.1016/j.indcrop.2020.113026

⁶ Міщенко С. В. Генетичні детермінанти появи плосконі у статевій структурі популяцій однодомних конопель та основні шляхи її подолання. *Луб'яні та технічні культури*. 2017. Вип. 5 (10). С. 76–90.

⁷ Міщенко С. В., Марченко Т. Ю., Лавриненко Ю. О., Ткаченко С. М. Генетичний контроль ознаки однодомності *Cannabis sativa* L. у процесі інбридингу. *Таврійський науковий вісник. Серія «Сільськогосподарські науки»*. 2022. Вип. 124. С. 85–91. DOI: 10.32851/2226-0099.2022.124.12

⁸ Вировець В. Г., Лайко І. М., Кириченко Г. І. та ін. Ренесанс посівних конопель як результат успішної селекції. *Фактори експериментальної еволюції організмів*. 2017. Т. 20. С. 368–372. DOI: 10.7124/FEEO.v20.796

⁹ Кириченко Г. І., Лайко І. М., Міщенко С. В. Аналіз колекційних зразків *Cannabis sativa* L. за вмістом канабіноїдних сполук і хемотипом. *Генетичні ресурси рослин*. 2019. № 25. С. 115–128. DOI: 10.36814/pgr.2019.25.09.

¹⁰ Міщенко С. В., Лайко І. М. Успадкування ознаки вмісту канабіноїдних сполук гібридами промислових конопель та теорія її генетичної детермінації. *Achievements of Ukraine and the EU in Ecology, Biology, Chemistry, Geography and Agricultural Sciences* : Collective monograph. Riga : Baltija Publishing, 2021. Vol. 2. P. 410–427. DOI: 10.30525/978-9934-26-086-5-32

¹¹ Mishchenko S. V. Phenotypic expression of cannabinoid presence and content upon self-pollination and directional selection in monoecious hemp. *Plant Breeding and Seed Production*. 2020. Vol. 118. P. 58–73. DOI: 10.30835/2413-7510.2020.222333

technological quality of bast fiber and bioenergetic value^{12, 13, 14, 15}, increasing plant tolerance to abiotic and biotic stresses under conditions of global climate change^{16, 17}.

This section of the monograph presents key points of the biological foundations of industrial hemp breeding, a number of fundamental genetic and breeding tasks that have been developed by the author over the last decade at the Institute of Bast Crops of the National Academy of Agrarian Sciences of Ukraine.

1. Genetic and selection control of sexual structure and heterosis

Modern monoecious hemp is characterized by sexual polymorphism – a transitional series of sexual types with masculinized and feminized habit and different ratios of male and female flowers in the inflorescence. The sex of hemp is a complex genetically (sex chromosome and autosome factors act) and epigenetically regulated system. The main problem of breeding monoecious industrial hemp remains genetic instability based on sex and population splitting with the emergence of an undesirable plane (male plants) and a gradual reversal to the evolutionarily formed dioeciousness.

Recently, many studies have been directed at the application of inbreeding methods, the creation of valuable self-pollinated lines on this basis, and the assessment of their combining ability in the topcross system, which allows for significant stabilization of the monoecious trait^{18, 19}. The use of self-pollination

¹² Міщенко С. В., Кмець І. Л. Мінливість анатомічної будови волокнистих структур на поперечному зрізі стебла різних зразків конопель. *Селекція і насінництво*. 2017. Вип. 112. С. 82–93. DOI: 10.30835/2413-7510.2017.120425

¹³ Mishchenko S. Change of cells and layers sizes of hemp (*Cannabis sativa* L.) bast fibers in the synthetic breeding process. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2019. № 3. P. 255–264. DOI: 10.15414/agrobiodiversity.2019.2585-8246.255-264

¹⁴ Міщенко С. В., Кириченко Г. І., Лайко І. М. Новий сорт промислових конопель «Артеміда» універсального напрямку господарського використання з підвищеним умістом олії та поліпшеною якістю волокна. *Plant Varieties Studying and Protection*. 2021. Т. 17. № 1. С. 43–50. DOI: 10.21498/2518-1017.17.1.2021.228208

¹⁵ Вожегова Р. А., Лавриненко Ю. О., Марченко Т. Ю. та ін. Перспективні культури для біоенергетики України. *Аерарні інновації*. 2022. № 11. С. 5–15. DOI: 10.32848/agra.innov.2022.11.1

¹⁶ Міщенко С. В. Кліматично орієнтована селекція промислових конопель. *Climate-Smart Agriculture: Science and Practice* : Scientific monograph. Riga : Baltija Publishing, 2023. P. 455–488. DOI: 10.30525/978-9934-26-389-7-22

¹⁷ Міщенко С. В. Генетичні, селекційні та біотехнологічні аспекти толерантності промислових конопель до абіотичних стресів. *Biological Sciences and Education in the Context of European Integration* : Scientific monograph. Riga : Baltija Publishing, 2024. P. 133–165. DOI: 10.30525/978-9934-26-443-6-7

¹⁸ Міщенко С. В. Ефекти загальної та варіанси специфічної комбінаційної здатності самозапилення ліній і сортів конопель у системі топкросів. *Фактори експериментальної еволюції організмів*. 2017. Т. 21. С. 62–67. DOI: 10.7124/FEEO.v21.808

¹⁹ Mishchenko S. V., Kabanets V. M., Kyrychenko H. I., Marchenko T. Y., Laiko H. M. Combinational ability of varieties and self-pollinated lines of industrial hemp for seed and oil use in the topcross system. *Plant Varieties Studying and Protection*. 2025. Vol. 21. Iss. 1. P. 39–45. DOI: 10.21498/2518-1017.21.1.2025.327500

(incrossing) allows for the elimination of undesirable recessive alleles and the consolidation of valuable economic traits^{20, 21}. Studies of inbreeding systems have shown that despite inbreeding depression for individual morphological traits, the creation of self-pollinated lines is a reliable tool for constructing synthetic populations and interlineage hybrids^{22, 23}. Assessment of the general (GCA) and specific (SCA) combining ability of self-pollinated lines and varieties in the topcross system showed the presence of a high heterosis effect in seed yield, oil yield and total biomass. Interlineal and linear-varietal hybrids demonstrate clear stability in seed and fiber productivity^{24, 25}.

In this case, one should take into account the epigenetic factor (in particular, the photoperiodic response), which is capable of modifying the manifestation of sex even in same-sex hybrids, but this requires strict selection control over phenotypic expression^{26, 27}.

The use of chemical gametocides and simultaneous antioxidant plant protection makes it possible to regulate male sterility, facilitating the production of interline and intervarietal hybrids with a high heterosis effect in terms of stem, fiber, seed yield, and oil content^{28, 29} (Fig. 1).

²⁰ Мигаль М. Д., Міщенко С. В., Лайко І. М. Інцухт і гетерозис конопель : монографія. Суми : ФОП Щербина І. В., 2020. 146 с.

²¹ Міщенко С. В., Кабанець В. М., Кириченко Г. І., Марченко Т. Ю., Мачульський Г. М. Особливості прояву ознак одностатевих конопель у процесі інбридингу та його використання в селекції. *Фактори експериментальної еволюції організмів*. 2025. Т. 37. С. 29–34. DOI: 10.7124/FEE0.v37.1742

²² Mishchenko S. Comparative characteristics of line-variety and intervarietal hemp (*Cannabis sativa* L.) hybrids by productivity. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2017. № 1. P. 324–327. DOI: 10.15414/agrobiodiversity.2017.2585-8246.324-327

²³ Міщенко С. В., Лайко І. М. Можливості використання самозапильних ліній для створення синтетичних популяцій конопель. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 38–46. DOI: 10.48096/btc.2018.6(11).38-46

²⁴ Mishchenko S. Comparative characteristics of line-variety and intervarietal hemp (*Cannabis sativa* L.) hybrids by productivity. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2017. № 1. P. 324–327. DOI: 10.15414/agrobiodiversity.2017.2585-8246.324-327

²⁵ Міщенко С. В., Марченко Т. Ю., Лавриненко Ю. О., Ткаченко С. М. Рівень прояву та усадкування селекційних ознак у міжлінійних гібридів конопель насінневого та волокнистого напрямів використання. *Таврійський науковий вісник. Серія «Сільськогосподарські науки»*. 2022. Вип. 125. С. 84–90. DOI: 10.32851/2226-0099.2022.125.12

²⁶ Міщенко С. В. Епігенетично детермінована зміна статі у одностатевих гібридів конопель під впливом фотоперіоду. *Селекція і насінництво*. 2018. Вип. 113. С. 102–110. DOI: 10.30835/2413-7510.2018.134364

²⁷ Міщенко С. В. Статева структура конвергентних гібридів конопель. *Агробіологія*. 2021. № 1. С. 93–103. DOI: 10.33245/2310-9270-2021-163-1-93-103

²⁸ Міщенко С. В., Лайко І. М. Вплив гаметоцидів на формування чоловічої стерильності та селекційних ознак одностатевих конопель. *Селекція і насінництво*. 2017. Вип. 111. С. 88–97. DOI: 10.30835/2413-7510.2017.104890

²⁹ Міщенко С. В., Кириченко Г. І., Лавриненко Ю. О., Марченко Т. Ю. Антиоксидантний захист *Cannabis sativa* L. від токсичної дії хімічних гаметоцидів. *Таврійський науковий вісник. Серія «Сільськогосподарські науки»*. 2026. Вип. 149. Ч. 2. С. 36–42. DOI: 10.32782/2226-0099.2026.149.2.5

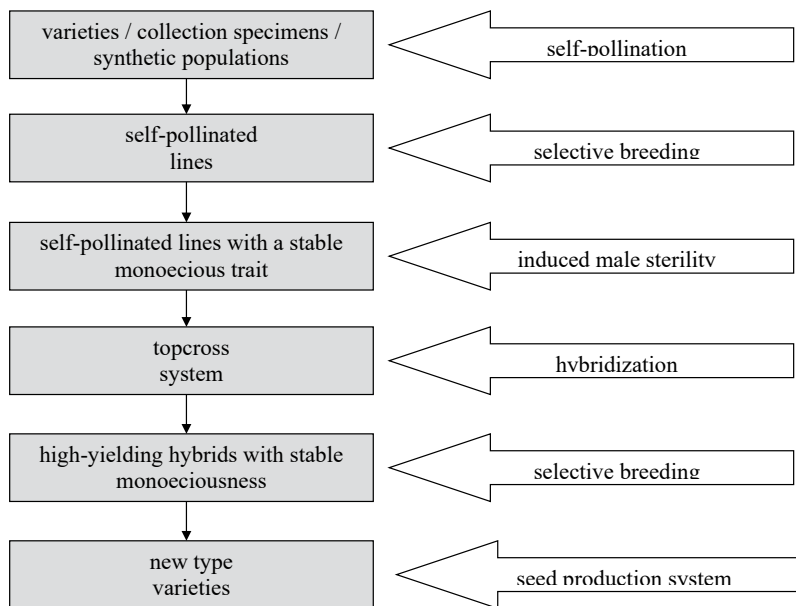


Fig. 1. Principle genetic and breeding model of stabilization of monoeciousness and implementation of heterosis in *Cannabis sativa* L. populations

The use of ascorbic acid and Si compounds between treatments with chemical gametocides contributed to the reduction of their suppressive effect, which was manifested in an increase in the number of formed seeds on mother plants by 17–21% and an increase in the yield of hybrid plants in the phase of biological ripeness by 5–11%. Their action is aimed at protecting the generative sphere of cannabis plants and supporting general metabolism under stressful conditions. The antioxidants used did not affect the level of male sterility, the stability of monoeciousness, the absence of tetrahydrocannabinol and the ratio of non-psychoactive cannabinoids, which confirms the safety of their use in terms of preserving the genetic characteristics of the original breeding material.

2. Genetic and biochemical foundations of breeding for cannabinoid profiles and other biochemical traits

Modern industrial hemp breeding imposes strict requirements on the content of psychotropic tetrahydrocannabinol (according to current Ukrainian legislation, it should not exceed 0.2%). At the same time, there is a growing

demand for varieties with high accumulation of non-psychotropic cannabinoids – cannabidiol (CBD) and cannabigerol (CBG)^{30, 31}.

The development of varieties with a high content of non-narcotic cannabinoids (CBD and CBG) against the background of the absence of Δ^9 -THC is a priority of the breeding direction. It allows obtaining high-value medical and cosmetic raw materials without the risk of improper use of crops.

The expression of the trait of cannabinoid content depends on cytoplasmic factors (to a large extent, high content is transmitted through the maternal line), i.e. they are inherited according to the nuclear-cytoplasmic type. The implementation of the genetic program in the phenotype regarding the synthesis of cannabinoids depends on environmental factors (air temperature, relative humidity, soil moisture and composition, mineral and organic nutrition regime, daylight hours, etc.).

The multi-vector nature of the dominance of the trait of high cannabinoid content (from positive overdominance to negative overdominance) and the continuous, quantitative variability of the trait of cannabinoid compounds content may hypothetically indicate the presence of polygenes that determine its manifestation and act by the type of cumulative polymerization. Despite the fact that the ratio of THC: CBD phenotype is consistent with a simple model of codominant alleles at one locus, it can be assumed that many linked loci for these genes are involved.

Among the common cannabinoids, CBD and THC are strongly correlated, with CBG being the least correlated with other compounds. The level of accumulation of some minor cannabinoids depends on the influence of many genetic and external factors, and different partial and multiple correlations have been established between them: CBDV is most closely related to CBD, CBN is related to THC, and the content of CBG has an impact on the formation of CBD and, especially, THC. It has been hypothesized that the CBG synthase gene is not located in an independent locus, in this case, a more complex genetic mechanism of cannabinoid synthesis determination operates, and the CBG synthase gene, for example, has several linked loci, in particular with the genes of CBDC synthase and, especially, THC synthase³².

³⁰ Міщенко С. В., Лайко І. М. Накопичення канабідіолу в онтогенезі рослин технічних (промислових) конопель. *Plant Varieties Studying and Protection*. 2018. Т. 14. № 4. С. 390–399. DOI: 10.21498/2518-1017.14.4.2018.151902

³¹ Mishchenko S. V., Laiko I. M., Kyrychenko H. I. Breeding of industrial hemp with a high content of cannabigerol by the case of 'Vik 2020' cultivar. *Plant Varieties Studying and Protection*. 2021. Vol. 17. № 2. P. 105–112. DOI: 10.21498/2518-1017.17.2.2021.236514

³² Міщенко С. В., Лайко І. М. Успадкування ознаки вмісту канабіноїдних сполук гібридами промислових конопель та теорія її генетичної детермінації. *Achievements of Ukraine and the EU in Ecology, Biology, Chemistry, Geography and Agricultural Sciences* : Collective monograph. Riga : Baltija Publishing, 2021. Vol. 2. P. 410–427. DOI: 10.30525/978-9934-26-086-5-32

Exogenous factors and growth regulators can modify the quantitative content of cannabinoids, but the qualitative chemotype remains a stable genetic marker³³. In plants of variety USO 31, ascorbic acid as an antioxidant, auxins and GA₃ significantly reduced the content of cannabinoids, whereas nicotinic acid and cytokinins increased it. As a result of triple exposure to nicotinic acid and BAP, changes in the descendants persisted and epigenetic effects were manifested. An additional method to increase the level of non-psychotropic cannabinoids may be treatment of vegetative plants with cytokinin BAP, which, in contrast to high concentrations of nicotinic acid, significantly increased the content of CBD and, to a lesser extent, THC. Such selection traits as stem total length, mass and fiber content, seed productivity and sex underwent the biggest changes. Different genotypes of hemp may have different reactions to phytohormones and their concentrations, which should be established in each case.

Analysis of the accumulation of cannabinoids in ontogenesis indicates the dependence of their biosynthetic activity not only on genetic factors, but also on the stage of growth and development and the anatomical functioning of glandular hairs (or trichomes)³⁴, which should be taken into account in the breeding process.

The biological basis for the selection of drug-free varieties is the study of allelic polymorphism of the cannabidiolic acid synthase (CBDAS) and tetrahydrocannabinolic acid synthase (THCAS) genes and other synthases^{35, 36}.

The final methodological scheme for creating genetically stable breeding material for industrial hemp for medicinal use is as follows (Fig. 2).

In parallel with its cannabinoid profile, hemp is valued as an important oilseed crop. The seeds contain up to 30–35% oil with an ideal ratio of omega-3 to omega-6 polyunsaturated fatty acids for human nutrition. The presence of significant intrapopulation variability allows selection for increased oil content in hemp seeds, an increase in the proportion

³³ Mishchenko S. V., Laiko I. M., Tkachenko S. M., Lavrynenko Y. O., Marchenko T. Y., Piliarska O. O. The influence of exogenous growth regulators on the cannabinoid content and the main selection traits of hemp (*Cannabis sativa* L. SSP. *sativa*). *Journal of Agricultural Sciences (Belgrade)*. 2022. Vol. 67. № 3. P. 237–251. DOI: 10.2298/JAS2203237M

³⁴ Мигаль М. Д., Кмець І. Л., Міщенко С. В. Особливості функціонування секреторних структур конопель, пов'язаних з виділенням канабіноїдів. *Луб'яні та технічні культури*. 2017. Вип. 5 (10). С. 91–101.

³⁵ De Meijer E. P. M., Bagatta M., Carboni A., Crucitti P., Moliterni V. M. C., Ranalli P., Mandolino G. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*. 2003. Vol. 163. № 1. P. 335–346. DOI: 10.1093/genetics/163.1.335

³⁶ Weiblen G. D., Wenger J. P., Craft K. J., ElSohly M. A., Mehmedic Z., Treiber E. L., Marks M. D. Gene duplication and divergence affecting drug content in *Cannabis sativa*. *New Phytologist*. 2015. Vol. 208. № 4. P. 1241–1250. DOI: 10.1111/nph.13562

of a particular polyunsaturated fatty acid, and an optimized Omega-3: Omega-6 ratio^{37, 38}.

3. Anatomical and morphological differentiation of fiber and *in vitro* technology

The basis of hemp breeding for fiber is the stem architecture and the ratio between primary and secondary bast fibers. Primary fibers are characterized by a high cellulose content and low lignin levels, which makes them an ideal raw material for the textile industry, while secondary fibers are shorter and more woody. The fiber: wood ratio is closely related to the length of the plant's growing season. Selection of late-maturing or day-neutral genotypes allows for a longer vegetative phase, which significantly increases bast fiber yield and overall carbon sequestration.

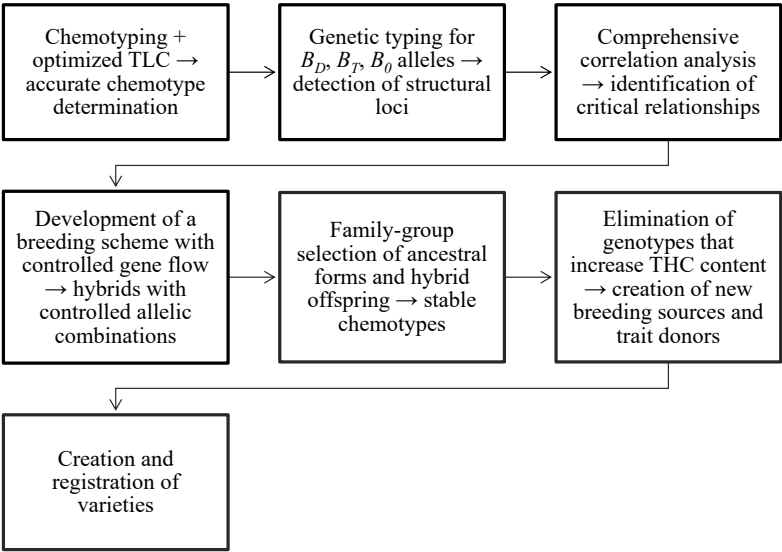


Fig. 2. Methodological scheme for creating genetically stable breeding material of industrial hemp for medicinal use

³⁷ Вировець В. Г., Лайко І. М., Кириченко Г. І. та ін. Збільшення вмісту олії як невідкладне завдання селекції конопель. *Фактори експериментальної еволюції організмів*. 2018. Т. 23. С. 40–45. DOI: 10.7124/FEEO.v23.987

³⁸ Laiko I. M., Kobyzeva L. N., Mishchenko S. V., Kyrychenko H. I. Intra-population variability of oil content and fatty acid composition in modern hemp cultivars. *Plant Breeding and Seed Production*. 2022. Vol. 121. P. 20–27. DOI: 10.30835/2413-7510.2022.260990

The quality of bast fibers is determined by the structure of the elementary bast fiber cells (cell shape, number of layers of secondary sheaths, canal structure, etc.), primary and secondary bundles in the stem^{39, 40}. The selection process is aimed at increasing the size of bast cells and optimizing their location in the stem⁴¹.

An equally important element is the use of ionic forms of silicon, which stimulate the formation of elementary fibrous cells, strengthening the cell wall and increasing the technological properties of the raw material⁴².

Under the influence of Silicon in hemp varieties of different economic uses, the thickness of the layer of bast fibers on the cross-section of the stem, the size of the cells of elementary bast fibers and the thickness of their secondary membranes increased. The thickness of the fiber layer increased mainly due to the secondary fiber, i.e. Silicon activates the activity of the secondary forming tissue – cambium. The size of the cells increased due to the reduction of the channel and the thickening of the secondary membranes. An increase in the proportion of cells of isodiametric and oval shape with a convex contour and cells with a small channel was found, which indicates a structural reorganization of the fiber. To increase the content of total fiber in the stems, Si treatment is advisable to carry out during the period of intensive accumulation of secondary fiber, and to obtain higher-quality fiber – during the period of intensive accumulation of primary fiber.

To accelerate the breeding process, biotechnological methods have also been developed, including modification of nutrient media^{43, 44, 45},

³⁹ Міщенко С. В., Кмець І. Л. Мінливість анатомічної будови волокнистих структур на поперечному зрізі стебла різних зразків конопель. *Селекція і насінництво*. 2017. Вип. 112. С. 82–93. DOI: 10.30835/2413-7510.2017.120425.

⁴⁰ Лайко І. М., Міщенко С. В. Селекційні особливості прояву ознак волокнистості конопель. *Фактори експериментальної еволюції організмів*. 2024. Т. 35. С. 29–34. DOI: 10.7124/FEEO.v34.1654

⁴¹ Mishchenko S. Change of cells and layers sizes of hemp (*Cannabis sativa* L.) bast fibers in the synthetic breeding process. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2019. № 3. P. 255–264. DOI: 10.15414/agrobiodiversity.2019.2585-8246.255-264

⁴² Mishchenko S. V., Lavrynenko Y. O., Marchenko, T. Y., Kyrychenko H. I. The influence of ionic form of silicon on the formation of elementary fiber cells in *Cannabis sativa* L. *Plant Varieties Studying and Protection*. 2026. Vol. 2. Iss. 1. P. 4–10. DOI: 10.21498/2518-1017.22.1.2026.357577

⁴³ Міщенко С. В. Ефективність розмноження *Cannabis sativa* L. з насіння з низькою схожістю та життєздатністю в умовах *in vitro*. *Таврійський науковий вісник*. 2018. Вип. 100. Т. 2. С. 3–8.

⁴⁴ Міщенко С. В. Модифікація живильного середовища для культивування неспихотропних конопель (*Cannabis sativa* L.) середньоєвропейського еколого-географічного типу *in vitro*. *Луб'яні та технічні культури*. 2019. Вип. 7 (12). С. 15–23. DOI: 10.48096/btc.2019.7(12).15-23

⁴⁵ Міщенко С. В. Методичні аспекти культивування конопель посівних *in vitro*: огляд. *Фактори експериментальної еволюції організмів*. 2025. Т. 37. С. 173–178. DOI: 10.7124/FEEO.v37.1767

use of exogenous growth regulators^{46, 47, 48}, and induction of polyploidy⁴⁹ (Fig. 3).

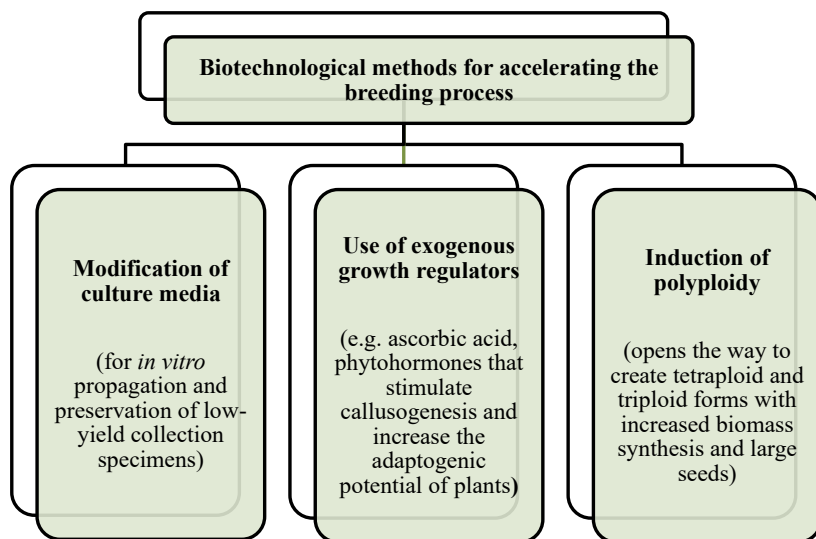


Fig. 3. Biotechnological methods involved in the breeding process with industrial hemp

4. Climate-oriented breeding of industrial hemp

In the context of global climate change, sustainable agricultural development requires the creation of varieties with high tolerance to adverse factors (drought,

⁴⁶ Міщенко С. В. Індукція калусогенезу в технічних (промислових) конопель в умовах *in vitro*. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 21–28. DOI: 10.48096/btc.2018.6(11).21-28

⁴⁷ Міщенко С. В. Вплив аскорбінової кислоти екзогенного походження на *Cannabis sativa* L. в умовах *in vitro* та *in vivo*. *Вісник Центру наукового забезпечення АПВ Харківської області*. 2019. Вип. 26. С. 67–74.

⁴⁸ Mishchenko S. V., Laiko I. M., Tkachenko S. M., Lavrynenko Y. O., Marchenko T. Y. Piliarska O. O. The influence of exogenous growth regulators on the cannabinoid content and the main selection traits of hemp (*Cannabis sativa* L. SSP. *sativa*). *Journal of Agricultural Sciences (Belgrade)*. 2022. Vol. 67. № 3. P. 237–251. DOI: 10.2298/JAS2203237M

⁴⁹ Міщенко С. В. Штучно індукована поліплоїдія у промислових конопель. *Modern Aspects of Natural Science Research in the Context of Sustainable Development of Society* : Scientific monograph. Riga : Baltija Publishing, 2023. P. 2–27. DOI: 10.30525/978-9934-26-395-8-1

salinity, heavy metals, etc.)^{50, 51, 52}. Foreign studies indicate that hemp can be used for phytoremediation of contaminated soils, in particular with heavy metals^{53, 54}.

Selection of genotypes with a developed root system increases the efficiency of nitrogen and phosphorus assimilation, which allows minimizing the use of mineral fertilizers and significantly reduces the carbon footprint when growing this crop.

The approaches developed in recent years at the Institute of Bast Crops of the NAAS include:

1. Salt stress testing.

In vitro modeling of salinity allows for early screening of genotypes for salt tolerance^{55, 56}.

Overcoming abiotic stresses, in particular salt, in agricultural crops can be done in various ways (methods), the leading place among which is occupied by genetic selection. The resistance of plants to adverse environmental factors is genetically determined and manifests itself at different levels of life organization, in particular at the cellular and tissue levels. This makes it possible to use biotechnological methods to select tolerant (resistant) genotypes while reducing material costs in a relatively short period. In the developed method for *in vitro* selection of cannabis genotypes tolerant to salt stress, which includes cultivation of explants under conditions of stress factor directed against normal development and survival of unstable forms, selection of individual genotypes is carried out at the level of somaclones regenerated from calluses with the addition to the nutrient medium, depending on the type of salinity, of 0.25% NaCl or 0.75% MgCl₂ · 6H₂O for chloride salinity, 0.5% MgSO₄ · 7H₂O or 1.0% Na₂SO₄ for sulfate salinity, 0.15% Na₂CO₃ or 0.30% NaHCO₃ for carbonate

⁵⁰ Міщенко С. В. Кліматично орієнтована селекція промислових конопель. *Climate-Smart Agriculture: Science and Practice* : Scientific monograph. Riga : Baltija Publishing, 2023. P. 455–488. DOI: 10.30525/978-9934-26-389-7-22

⁵¹ Міщенко С. В. Генетичні, селекційні та біотехнологічні аспекти толерантності промислових конопель до абіотичних стресів. *Biological Sciences and Education in the Context of European Integration* : Scientific monograph. Riga : Baltija Publishing, 2024. P. 133–165. DOI: 10.30525/978-9934-26-443-6-7

⁵² Кабанець В. М., Михальська Л. М., Швартау В. В. та ін. Фітомеліоративні властивості рослин *Cannabis sativa* L. залежно від соргових особливостей культури. *Plant Varieties Studying and Protection*. 2017. Vol. 13. № 4. P. 423–428. DOI: 10.21498/2518-1017.13.4.2017.117752

⁵³ Linger P., Müssig J., Fischer H., Kobert J. Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. *Industrial Crops and Products*. 2002. Vol. 16. № 1. P. 33–42. DOI: 10.1016/S0926-6690(02)00005-5

⁵⁴ Placido D. F., Lee C. C. Potential of industrial hemp for phytoremediation of heavy metals. *Plants*. 2022. Vol. 11. Iss. 5. Art. 595. DOI: 10.3390/plants11050595

⁵⁵ Міщенко С. В. Подолання сольового стресу *Cannabis sativa* L.: генетико-селекційний аспект. *Зрошуване землеробство*. 2024. Вип. 81. С. 44–49. DOI: 10.32848/0135-2369.2024.81.7

⁵⁶ Міщенко С. В. Схожість насіння *Cannabis sativa* L. за штучно змодельованого сольового стресу в культурі *in vitro*. *Фактори експериментальної еволюції організмів*. 2024. Т. 34. С. 193–198. DOI: 10.7124/FEEO.v34.1639

salinity. The use of the new method allows obtaining tolerant (resistant) to salt stress hemp starting material and accelerating the selection process.

2. Gametophytic selection for heat tolerance.

The dependence of thermotolerance of hybrids on genotype has been established, which allows for the formation of adaptive breeding programs⁵⁷.

The degree of heat resistance of industrial hemp hybrids depended on the genotypic characteristics of the parental forms. The effectiveness of the gametophytic selection method of heat-resistant hemp genotypes was confirmed, according to which the cultivation of mother plants and their pollination is carried out under fabric-film insulators under conditions of elevated air temperature of 40–50°C (selection of female gametes), chemical sterilization of male flowers – by double treatment with a 2.0% suspension of dibutyl phthalate in the BBCH 15 and BBCH 61 phases, pollination – with heated pollen at a temperature of 50–60 °C and exposure for 30–60 min (selection of male gametes), selection of resistant genotypes at the level of the seed embryo to elevated temperature – by warming the seeds at a temperature of 50 °C and exposure for 15–30 min, individual selection in hybrid generations – by a complex of valuable economic traits.

3. Ecological plasticity and phytoremediation.

The study of the soil microbiome during long-term cultivation of hemp monoculture proves the possibility of preserving soil biodiversity under optimal fertilization and agricultural techniques systems^{58, 59}.

CONCLUSIONS

A generalization of the methodological principles of industrial hemp breeding shows that the combination of a scientifically based seed production system aimed at preventing cross-pollination of different varieties and generations, mechanical removal of male plants (flathead) and reducing the frequency of mutation of monoecious alleles in the flathead allele, classical selection and hybridization for combining ability, genetic control of cannabinoids and biotechnological methods ensures the creation of high-yielding varieties with a minimal environmental footprint for bioenergy and industry.

⁵⁷ Міщенко С. В., Кириченко Г. І., Марченко Т. Ю., Кривенко А. І., Пілярська О. О. Залежність жаростійкості гібридів промислових конопель від генотипу. *Аграрні інновації*. 2024. № 23. С. 167–172. DOI: 10.32848/agrar.innov.2024.23.25

⁵⁸ Мигаль М. Д., Лайко І. М., Міщенко С. В., Кириченко Г. І., Лайко Г. М., Бірюкова Т. С. Умови розвитку бур'янів, шкідників і хвороб у посіві монокультури конопель у залежності від удобрення. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 66–80. DOI: 10.48096/btc.2018.6(11).66-80

⁵⁹ Klishchova Z., de Abreu C. G., Laiko H., Primakov O., Mishchenko S., Dias E. S., Kyrylenko S., Pyro V. S. Soil microbiota of industrial hemp (*Cannabis sativa L.*) grown in a long-term monoculture. *Biotechnology for Environment*. 2026. Vol. 3. Article 11. DOI: 10.1186/s44314-026-00045-w

SUMMARY

This section of the monograph presents the key points of the biological foundations of industrial hemp breeding, a number of fundamental genetic and breeding tasks that have been developed in the last decade at the Institute of Bast Crops of the National Academy of Agrarian Sciences of Ukraine. A generalization of the methodological principles of industrial hemp breeding shows that the combination of a scientifically sound seed production system aimed at preventing cross-pollination of different varieties and generations, mechanical removal of male plants (flat plants) and reducing the frequency of mutation of monoecious alleles in the flat plant allele, classical selection and hybridization for combining ability, genetic control of cannabinoids and biotechnological methods ensures the creation of high-yielding varieties.

Bibliography

1. Amaducci S., Scordia D., Liu F. H., Zhang Q., Guo H., Testa G., Cosentino S. L. Key cultivation techniques for hemp in Europe and China. *Industrial Crops and Products*. 2015. Vol. 68. P. 2–16. DOI: 10.1016/j.indcrop.2014.06.041
2. Kovalchuk I., Pellino M., Rigault P. et al. The genomics of *Cannabis* and its close relatives. *Annual Review of Plant Biology*. 2020. Vol. 71. P. 713–739. DOI: 10.1146/annurev-arplant-081519-040203
3. Sawler J., Stout J. M., Gardner K. M. et al. The genetic structure of marijuana and hemp. *PLoS ONE*. 2015. Vol. 10. Iss. 8. Art. 0133292. DOI: 10.1371/journal.pone.0133292
4. Lavery K. U., Stout J. M., Sullivan M. J. et al. A physical and genetic map of *Cannabis sativa* identifies extensive rearrangements at the THC/CBD acid synthase loci. *Genome Research*. 2019. Vol. 29. Iss. 1. P. 146–156. DOI: 10.1101/gr.242594.118
5. Hesami M., Pepe M., Alizadeh M., Rakei A., Baiton A., Jones A. M. P. Recent advances in cannabis biotechnology. *Industrial Crops and Products*. Vol. 158. 2020. Art. 113026. DOI: 10.1016/j.indcrop.2020.113026
6. Міщенко С. В. Генетичні детермінанти появи плосконі у статевій структурі популяцій однодомних конопель та основні шляхи її подолання. *Луб'яні та технічні культури*. 2017. Вип. 5 (10). С. 76–90.
7. Міщенко С. В., Марченко Т. Ю., Лавриненко Ю. О., Ткаченко С. М. Генетичний контроль ознаки однодомності *Cannabis sativa* L. в процесі інбридингу. *Таврійський науковий вісник. Серія «Сільськогосподарські науки»*. 2022. Вип. 124. С. 85–91. DOI: 10.32851/2226-0099.2022.124.12

8. Вировець В. Г., Лайко І. М., Кириченко Г. І. та ін. Ренесанс посівних конопель як результат успішної селекції. *Фактори експериментальної еволюції організмів*. 2017. Т. 20. С. 368–372. DOI: 10.7124/FEEEO.v20.796
9. Кириченко Г. І., Лайко І. М., Міщенко С. В. Аналіз колекційних зразків *Cannabis sativa* L. за вмістом канабіноїдних сполук і хемотипом. *Генетичні ресурси рослин*. 2019. № 25. С. 115–128. DOI: 10.36814/pgr.2019.25.09
10. Міщенко С. В., Лайко І. М. Успадкування ознаки вмісту канабіноїдних сполук гібридами промислових конопель та теорія її генетичної детермінації. *Achievements of Ukraine and the EU in Ecology, Biology, Chemistry, Geography and Agricultural Sciences : Collective monograph*. Riga : Baltija Publishing, 2021. Vol. 2. P. 410–427. DOI: 10.30525/978-9934-26-086-5-32
11. Mishchenko S. V. Phenotypic expression of cannabinoid presence and content upon self-pollination and directional selection in monoecious hemp. *Plant Breeding and Seed Production*. 2020. Vol. 118. P. 58–73. DOI: 10.30835/2413-7510.2020.222333
12. Міщенко С. В., Кмець І. Л. Мінливість анатомічної будови волокнистих структур на поперечному зрізі стебла різних зразків конопель. *Селекція і насінництво*. 2017. Вип. 112. С. 82–93. DOI: 10.30835/2413-7510.2017.120425
13. Mishchenko S. Change of cells and layers sizes of hemp (*Cannabis sativa* L.) bast fibers in the synthetic breeding process. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2019. № 3. P. 255–264. DOI: 10.15414/agrobiodiversity.2019.2585-8246.255-264
14. Міщенко С. В., Кириченко Г. І., Лайко І. М. Новий сорт промислових конопель «Артеміда» універсального напрямку господарського використання з підвищеним умістом олії та поліпшеною якістю волокна. *Plant Varieties Studying and Protection*. 2021. Т. 17. № 1. С. 43–50. DOI: 10.21498/2518-1017.17.1.2021.228208
15. Вожегова Р. А., Лавриненко Ю. О., Марченко Т. Ю. та ін. Перспективні культури для біоенергетики України. *Аграрні інновації*. 2022. № 11. С. 5–15. DOI: 10.32848/agrar.innov.2022.11.1
16. Міщенко С. В. Кліматично орієнтована селекція промислових конопель. *Climate-Smart Agriculture: Science and Practice : Scientific monograph*. Riga : Baltija Publishing, 2023. P. 455–488. DOI: 10.30525/978-9934-26-389-7-22
17. Міщенко С. В. Генетичні, селекційні та біотехнологічні аспекти толерантності промислових конопель до абіотичних стресів. *Biological Sciences and Education in the Context of European Integration :*

Scientific monograph. Riga : Baltija Publishing, 2024. P. 133–165. DOI: 10.30525/978-9934-26-443-6-7

18. Міщенко С. В. Ефекти загальної та варіанси специфічної комбінаційної здатності самозапиленних ліній і сортів конопель у системі топкросів. *Фактори експериментальної еволюції організмів*. 2017. Т. 21. С. 62–67. DOI: 10.7124/FEEO.v21.808

19. Mishchenko S. V., Kabanets V. M., Kyrychenko H. I., Marchenko T. Y., Laiko H. M. Combinational ability of varieties and self-pollinated lines of industrial hemp for seed and oil use in the topcross system. *Plant Varieties Studying and Protection*. 2025. Vol. 21. Iss. 1. P. 39–45. DOI: 10.21498/2518-1017.21.1.2025.327500

20. Мигаль М. Д., Міщенко С. В., Лайко І. М. Інцухт і гетерозис конопель : монографія. Суми : ФОП Щербина І. В., 2020. 146 с.

21. Міщенко С. В., Кабанець В. М., Кириченко Г. І., Марченко Т. Ю., Мачульський Г. М. Особливості прояву ознак однодомних конопель в процесі інбридингу та його використання в селекції. *Фактори експериментальної еволюції організмів*. 2025. Т. 37. С. 29–34. DOI: 10.7124/FEEO.v37.1742

22. Mishchenko S. Comparative characteristics of line-variety and intervarietal hemp (*Cannabis sativa* L.) hybrids by productivity. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. 2017. № 1. P. 324–327. DOI: 10.15414/agrobiodiversity.2017.2585-8246.324-327

23. Міщенко С. В., Лайко І. М. Можливості використання самозапиленних ліній для створення синтетичних популяцій конопель. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 38–46. DOI: 10.48096/btc.2018.6(11).38-46

24. Міщенко С. В., Марченко Т. Ю., Лавриненко Ю. О., Ткаченко С. М. Рівень прояву та успадкування селекційних ознак у міжлінійних гібридів конопель насінневого та волокнистого напрямів використання. *Таврійський науковий вісник*. Серія «Сільськогосподарські науки». 2022. Вип. 125. С. 84–90. DOI: 10.32851/2226-0099.2022.125.12

25. Міщенко С. В. Епігенетично детермінована зміна статі у одностатевих гібридів конопель під впливом фотоперіоду. *Селекція і насінництво*. 2018. Вип. 113. С. 102–110. DOI: 10.30835/2413-7510.2018.134364

26. Міщенко С. В. Статева структура конвергентних гібридів конопель. *Агробіологія*. 2021. № 1. С. 93–103. DOI: 10.33245/2310-9270-2021-163-1-93-103

27. Міщенко С. В., Лайко І. М. Вплив гаметоцидів на формування чоловічої стерильності та селекційних ознак однодомних конопель. *Селекція і насінництво*. 2017. Вип. 111. С. 88–97. DOI: 10.30835/2413-7510.2017.104890

28. Міщенко С. В., Кириченко Г. І., Лавриненко Ю. О., Марченко Т. Ю. Антиоксидантний захист *Cannabis sativa* L. від токсичної дії хімічних гаметоцидів. *Таврійський науковий вісник. Серія «Сільськогосподарські науки»*. 2026. Вип. 149, Ч. 2. С. 36–42. DOI: 10.32782/2226-0099.2026.149.2.5
29. Міщенко С. В., Лайко І. М. Накопичення канабідіолу в онтогенезі рослин технічних (промислових) конопель. *Plant Varieties Studying and Protection*. 2018. Т. 14. № 4. С. 390–399. DOI: 10.21498/2518-1017.14.4.2018.151902
30. Mishchenko S. V., Laiko I. M., Kyrychenko H. I. Breeding of industrial hemp with a high content of cannabigerol by the case of “Vik 2020” cultivar. *Plant Varieties Studying and Protection*. 2021. Vol. 17. № 2. P. 105–112. DOI: 10.21498/2518-1017.17.2.2021.236514
31. Mishchenko S. V., Laiko I. M., Tkachenko S. M., Lavrynenko Y. O., Marchenko T. Y., Piliarska O. O. The influence of exogenous growth regulators on the cannabinoid content and the main selection traits of hemp (*Cannabis sativa* L. SSP. *sativa*). *Journal of Agricultural Sciences (Belgrade)*. 2022. Vol. 67. № 3. P. 237–251. DOI: 10.2298/JAS2203237M
32. Мигаль М. Д., Кмець І. Л., Міщенко С. В. Особливості функціонування секреторних структур конопель, пов’язаних з виділенням канабіноїдів. *Луб’яні та технічні культури*. 2017. Вип. 5 (10). С. 91–101.
33. De Meijer E. P. M., Bagatta M., Carboni A., Crucitti P., Moliterni V. M. C., Ranalli P., Mandolino G. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*. 2003. Vol. 163. № 1. P. 335–346. DOI: 10.1093/genetics/163.1.335
34. Weiblen G. D., Wenger J. P., Craft K. J., ElSohly M. A., Mehmedic Z., Treiber E. L., Marks M. D. Gene duplication and divergence affecting drug content in *Cannabis sativa*. *New Phytologist*. 2015. Vol. 208. № 4. P. 1241–1250. DOI: 10.1111/nph.13562
35. Вировець В. Г., Лайко І. М., Кириченко Г. І. та ін. Збільшення вмісту олії як невідкладне завдання селекції конопель. *Фактори експериментальної еволюції організмів*. 2018. Т. 23. С. 40–45. DOI: 10.7124/FEEO.v23.987
36. Laiko I. M., Kobzyeva L. N., Mishchenko S. V., Kyrychenko H. I. Intra-population variability of oil content and fatty acid composition in modern hemp cultivars. *Plant Breeding and Seed Production*. 2022. Vol. 121. P. 20–27. DOI: 10.30835/2413-7510.2022.260990
37. Лайко І. М., Міщенко С. В. Селекційні особливості прояву ознак волокнистості конопель. *Фактори експериментальної еволюції організмів*. 2024. Т. 35. С. 29–34. DOI: 10.7124/FEEO.v34.1654

38. Mishchenko S. V., Lavrynenko Y. O., Marchenko, T. Y., Kyrychenko H. I. The influence of ionic form of silicon on the formation of elementary fiber cells in *Cannabis sativa* L. *Plant Varieties Studying and Protection*. 2026. Vol. 22. Iss. 1. P. 4–10. DOI: 10.21498/2518-1017.22.1.2026.357577
39. Міщенко С. В. Ефективність розмноження *Cannabis sativa* L. з насіння з низькою схожістю та життєздатністю в умовах *in vitro*. *Таврійський науковий вісник*. 2018. Вип. 100. Т. 2. С. 3–8.
40. Міщенко С. В. Модифікація живильного середовища для культивування непсихотропних конопель (*Cannabis sativa* L.) середньоевропейського еколого-географічного типу *in vitro*. *Луб'яні та технічні культури*. 2019. Вип. 7 (12). С. 15–23. DOI: 10.48096/btc.2019.7(12).15-23
41. Міщенко С. В. Методичні аспекти культивування конопель посівних *in vitro* : огляд. *Фактори експериментальної еволюції організмів*. 2025. Т. 37. С. 173–178. DOI: 10.7124/FEEO.v37.1767
42. Міщенко С. В. Індукція калусогенезу в технічних (промислових) конопель в умовах *in vitro*. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 21–28. DOI: 10.48096/btc.2018.6(11).21-28
43. Міщенко С. В. Вплив аскорбінової кислоти екзогенного походження на *Cannabis sativa* L. в умовах *in vitro* та *in vivo*. *Вісник Центру наукового забезпечення АПВ Харківської області*. 2019. Вип. 26. С. 67–74.
44. Міщенко С. В. Штучно індукована поліплоїдія у промислових конопель. *Modern Aspects of Natural Science Research in the Context of Sustainable Development of Society : Scientific monograph*. Riga : Baltija Publishing, 2023. P. 2–27. DOI: 10.30525/978-9934-26-395-8-1
45. Кабанець В. М., Михальська Л. М., Швартау В. В. та ін. Фітомеліоративні властивості рослин *Cannabis sativa* L. залежно від сортових особливостей культури. *Plant Varieties Studying and Protection*. 2017. Vol. 13. № 4. P. 423–428. DOI: 10.21498/2518-1017.13.4.2017.117752
46. Linger P., Müssig J., Fischer H., Kobert J. Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. *Industrial Crops and Products*. 2002. Vol. 16. № 1. P. 33–42. DOI: 10.1016/S0926-6690(02)00005-5
47. Placido D. F., Lee C. C. Potential of industrial hemp for phytoremediation of heavy metals. *Plants*. 2022. Vol. 11. Iss. 5. Art. 595. DOI: 10.3390/plants11050595
48. Міщенко С. В. Подолання сольового стресу *Cannabis sativa* L.: генетико-селекційний аспект. *Зрошуване землеробство*. 2024. Вип. 81. С. 44–49. DOI: 10.32848/0135-2369.2024.81.7

49. Міщенко С. В. Схожість насіння *Cannabis sativa* L. за штучно змодельованого сольового стресу в культурі *in vitro*. *Фактори експериментальної еволюції організмів*. 2024. Т. 34. С. 193–198. DOI: 10.7124/FEEEO.v34.1639

50. Міщенко С. В., Кириченко Г. І., Марченко Т. Ю., Кривенко А. І., Пілярська О. О. Залежність жаростійкості гібридів промислових конопель від генотипу. *Аграрні інновації*. 2024. № 23. С. 167–172. DOI: 10.32848/ agrar.innov.2024.23.25

51. Мигаль М. Д., Лайко І. М., Міщенко С. В., Кириченко Г. І., Лайко Г. М., Бірюкова Т. С. Умови розвитку бур'янів, шкідників і хвороб у посіві монокультури конопель у залежності від удобрення. *Луб'яні та технічні культури*. 2018. Вип. 6 (11). С. 66–80. DOI: 10.48096/btc.2018.6(11).66-80

52. Klishchova Z., de Abreu C. G., Laiko H., Primakov O., Mishchenko S., Dias E. S., Kyrylenko S., Pylro V. S. Soil microbiota of industrial hemp (*Cannabis sativa* L.) grown in a long-term monoculture. *Biotechnology for Environment*. 2026. Vol. 3. Article 11. <https://doi.org/10.1186/s44314-026-00045-w>

Information about the author:

Mishchenko Serhii,

Doctor of Agricultural Sciences,

Associate Professor at the Department of Biology,

Human Health and Teaching Methods

Oleksandr Dovzhenko Hlukhiv National Pedagogical University

24, Kyivska Street, Hlukhiv, Ukraine;

Chief Researcher at the Department of Hemp Breeding and Seed Growing

Institute of Bast Crops of the National Academy

of Agrarian Sciences of Ukraine

45, Tereshchenkov Street, Hlukhiv, Ukraine

SYSTEM FOR ENSURING VIROLOGICAL PURITY OF VETERINARY IMMUNOBIOLOGICAL DRUGS IN THE CONTEXT OF PESTIVIRUS CONTAMINATION

Napnenko Oleksandr, Solomon Vyacheslav, Midyk Svitlana
DOI <https://doi.org/10.30525/978-9934-26-714-7-14>

INTRODUCTION

Control and prevention of infectious diseases of domestic animals and poultry have historically been a key vector for ensuring epizootic well-being in livestock farming. Significant progress in the elimination and containment of the spread of viral nosoforms has been achieved primarily due to the widespread introduction of live (attenuated) vaccines into the practice of veterinary medicine.

Veterinary immunobiological agents (VIA) produced by the industry from animal raw materials are products of cell life, individual components of endocrine-enzymatic and special raw materials. VIA from animal raw materials differ from each other in the nature of the active base: proteins, peptides, amino acid derivatives, nucleic acids, steroids, etc., which determines different methods of their use. The collection and primary processing of endocrine-enzymatic and special raw materials of animal origin are carried out mainly at meat industry enterprises. The quality of the raw materials at this stage is determined only organoleptically, which does not allow objectively assessing its suitability for the production of therapeutic and prophylactic agents. Therefore, the guarantee of the quality of the raw materials is, first of all, strict compliance with the sanitary requirements for its collection and primary processing.

Biotechnological features of their production are associated with the use of complex biological substrates. As cell systems and components of nutrient media, primary and subcultured mammalian and avian cell cultures, chicken embryos, as well as biological additives of animal origin (in particular, blood serum) are traditionally used. These substrates and components are characterized by a high degree of risk regarding the presence of hidden (latent) pathogens, which defines them as potential sources of biological contamination at all stages

of the production cycle – from the initial biotechnological raw materials to the final immunobiological product^{1, 2}.

A separate type of biological raw material is precisely the strains of microorganisms: bacteria, viruses, mycoplasmas, fungi, etc. Their biological purity is one of the keys to the production of a high-quality, effective drug^{3, 4}.

The absence of contamination of biological means of animal protection is one of the most important indicators of its safety. Despite the use of a number of antimicrobial drugs in the production of biological means of animal protection, contamination of raw materials and products with bacteria, fungi, yeast, mycoplasmas, viruses is quite often found. The production process may also pose a threat of contamination with foreign antigens, nucleic acids.

The most difficult to detect contamination is latent and persistent viruses, which can be unrecognized for a long time in the absence of cytopathic effect (CPA), hemagglutinating and hemadsorbing properties. The ability of viruses to overcome species barriers is associated with their persistence in cell cultures, which in turn can lead to contamination of drugs.

All contaminating viruses can be divided into three groups according to their significance for vaccinated people and animals:

- 1) viruses with established pathogenicity;
- 2) viruses that do not harm the vaccinated population;
- 3) viruses whose pathogenicity has not been proven.

However, the lack of such evidence does not yet indicate their absolute harmlessness to vaccinated people.

Among the possible contaminants of biological raw materials and products, viruses occupy the lion's share. The largest risk group for contamination is cell cultures, especially primary trypsinized ones. Virus contaminants vary greatly in virulence, and under the influence of a biological object and environmental factors, they can mutate and change their biological properties.

¹ Яворський М. І., Коваленко В. І., Литвиненко Л. Ю., Юрченко П. А. Ендокринно-ферментна та спеціальна сировина і лікарські препарати на її основі. Довідник. Асоціація виробників натуральної продукції і лікарських засобів «Вітафарм». Київ : Урожай, 1995. 219 с.

² Напненко О. О. Стандартизація умов розроблення та виробництва ветеринарних імунобіологічних засобів. Матеріали XI міжнародного конгресу спеціалістів ветеринарної медицини (3–4 жовтня 2013 р., м. Київ, Україна). Київ, 2013. С. 95–96.

³ Напненко О. О., Головка А. М., Ареф'єв В. Л., Гордієнко О. І. Створення системи оцінювання ефективності діяльності колекцій штамів мікроорганізмів в Україні. Матеріали Міжнародної науково-практичної конференції «Біотехнологія та її роль в забезпеченні здоров'я людей і тварин». Ніжин : ПП Лисенко М. М., 2023. С. 168–169.

⁴ Напненко О., Головка А., Іванченко П. Система контролю за контамінацією сторонніми агентами ветеринарних імунобіологічних лікарських засобів. Фармаком, 2021. С. 89–91.

The most significant place among viral contaminants is occupied by viruses of the Flaviviridae family of the Pestivirus genus, as evidenced by numerous data from various scientists^{5, 6}.

Of particular interest as contaminants are bovine viruses, which are detected in CK and blood serum used in virological and medical practice. The most common among contaminants is bovine diarrhea virus.

Due to serum contamination, cell culture lines (CK) of various origins were found to be chronically infected with bovine diarrhea virus. These included transfected cell cultures of cattle (lines: MDBK, GBK, BKD, BK4, etc.), horses, pigs, sheep, rabbits, cats and monkeys. The produced series of bovine serum are usually controlled for the absence of viral contamination in primary cell cultures of chicken embryos, calf kidneys and in HeLa cell cultures. In addition to examination under a microscope, cell cultures are checked for hemadsorbing properties with human, chicken and guinea pig erythrocytes.

The fact of the spread of contamination requires the development of modern methods for detecting contaminants, a system for preventing their entry into biological raw materials and finished products.

The provision on the inadmissibility of any foreign agents entering vaccines is generally recognized. By decision of the World Health Organization, the production of medical vaccines on biological systems contaminated with foreign viruses, especially oncogenic ones, is prohibited in all countries^{7, 8}.

Data from literature sources indicate a significant diversity of viral contaminants in biological raw materials and products. There are a large number of methods for detecting contaminating viruses. However, these methods do not always allow us to establish contamination, especially with persistent viruses and in the latent phase. Molecular genetic methods are only being introduced in Ukraine and are used mainly for the purpose of diagnosing animal and poultry diseases, and not for quality control of drugs. Most of the data provided in the literature relate to medical devices, and in the veterinary bioindustry, the introduction of the quality indicator of drugs “absence of contamination with foreign viruses” as a mandatory requirement has only occurred since 2003.

⁵ Напненко О. О. Система запобігання та виявлення контамінації ветеринарних імунобіологічних препаратів пестівірусами : автореф. дис. ... канд. вет. наук : 16.00.03; Одеський державний аграрний університет, 2012. 18 с.

⁶ Test for sterility and freedom from contamination of biological materials. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Publisher: Paris : OIE, World Organisation for Animal Health, 2024. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.09_TESTS_FOR_STERILITY.pdf.

⁷ Державна фармакопея України. Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2-ге видання. Доповнення 3. Харків : Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2018. 416 с.

⁸ European Pharmacopoeia. Sixth Edition. Edition of the Council of Europe, Strasbourg, France, 2022.

Latent and persistent viruses, which are difficult to detect in the material under study, pose a particular danger. During persistence in biological material or in the animal body, these viruses can change their biological properties. It is especially difficult to detect non-cytopathogenic types of viruses.

Virological methods for detecting contaminants require significant time and costs, and also have a low sensitivity threshold. While molecular genetic methods make it possible to detect the presence of proteins, nucleic acids of microorganisms in the material in a short time and in low titers. Therefore, on the way to solving the problem of viral contamination of biological products, the development of a contamination prevention system and diagnostic test systems based on the polymerase chain reaction is an urgent and extremely necessary task.

1. Research Materials and Methods

The study was conducted at the laboratories of the State Scientific and Control Institute of Biotechnology and Strains of Microorganisms (DNKIBSM). Separate studies were performed at the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” and production laboratories of Kherson and Sumy biological factories.

Taking into account the data from literature sources, we identified risk groups for contamination with viruses, which included cell cultures, production strains of microorganisms, preparations from animal blood or with its addition, live culture vaccines, as well as antigens of microorganisms that do not undergo the inactivation stage during the production process.

Veterinary biological preparations for research were selected directly from the warehouses of manufacturers or from the network of pharmacies of the corporation “Ukrzoovetprompostach”, as well as preparations that were received for the purpose of passing state quality control. 119 batches of veterinary immunobiological preparations were studied.

The raw materials from which the preparations are made were selected directly at the production site: cell cultures, embryos, biotechnological sera, strains of microorganisms, biological material from donor animals, trypsin solutions, etc. The following were studied: 18 cell culture lines, 144 strains of production microorganisms, 50 commercial batches of bovine blood serum.

The research methods met the requirements of the European Pharmacopoeia, the recommendations of the International Epizootic Bureau and the regulatory documents in force in Ukraine: standards, technical conditions, technological regulations, etc. Each preparation, biological material was studied using three to four methods, in particular in cell culture, on laboratory animals, by hemagglutination reaction (HAR), hemadsorption reaction (HAR),

immunofluorescence reaction (IFR), neutralization reaction (NR) and polymerase chain reaction (PCR)⁹.

Cell culture studies were performed to detect contamination of cell cultures themselves and contaminants of various products. Results were recorded based on the intensity of cytopathogenic effect (CPA), immunofluorescence reaction results, and hemadsorption reaction^{10, 11}.

Control of manufactured virus vaccines for contamination by foreign viruses began with determining the neutralization of the vaccine virus by specific serum. Based on data on the virus titer in the biological product and the neutralizing titer of the specific serum, the corresponding volumes of the biological product and specific serum were mixed. Cell cultures were contaminated with a sample of the product after exposure for 45–60 min at a temperature of 37 °C.

The viral suspension of the main seed culture in an amount of 1.0 cm³ was distributed into monolayers with a growth area of 75 cm² cells. At least two monolayers used in the test were maintained as uninfected controls.

The cell culture (experimental and control) was cultivated for 21 days (3 subcultures) at a temperature of (37 ± 0.5) °C, controlling under a microscope for the presence of cytopathic changes. At the end of the observation period, the culture was tested in the hemadsorption and immunofluorescence reactions, as indicated above. The material was considered to have passed the control if there were no cytopathic changes in the experimental and control cultures and a negative hemadsorption result. If there were cytopathic changes and (or) hemadsorption in the control cultures, the experiment was repeated on another batch of cell cultures. For the study, samples of cell cultures obtained from various enterprises of the biological industry of Ukraine and the DNAIBSM cell culture bank were selected. Before the study, the cell cultures were stored in liquid nitrogen¹².

Studies on the detection of possible viral contaminants were performed by conducting three consecutive subcultures and by reverse transcriptase polymerase chain reaction, since it is the most sensitive method for detecting bovine diarrhea virus.

⁹ Напненко О., Головка А., Безвін Є. Організація серійного контролю якості ветеринарних імунобіологічних засобів в Україні. *Біологія тварин*, 2024, Т. 26, № 3. С. 96.

¹⁰ Блоцька О., Головка А., Кузьмич Г., Напненко О. Біотехнологія. Методи попередження контамінації культур клітин : ДСТУ 8115:2015. Введено в дію 01.01.2017. Київ : ДП «УкрНДНЦ», 2018. 9 с.

¹¹ Code of Federal Regulations. Animal and Animals Products. Title 9, Parts 1.199. The Office of the Federal Register, National Archives and Records Administration: US Government Printing Office, Washington DC, USA, 2024. <https://www.govinfo.gov/content/pkg/CFR-2024-title9-vol1/pdf/CFR-2024-title9-vol1.pdf>.

¹² Напненко О. О., Головка А. М., Ареф'єв В. Л., Гордієнко О. І. Створення системи оцінювання ефективності діяльності колекцій штамів мікроорганізмів в Україні. Матеріали Міжнародної науково-практичної конференції «Біотехнологія та її роль в забезпеченні здоров'я людей і тварин». Ніжин : ПП Лисенко М. М., 2023. С. 168–169.

During the work, 18 cell culture lines of more than 50 different passages were studied: SNEV (SPEV), VNK-21, FLK, HeLa, A-72, CV-1, KST, Marc, RK-13, PK-15, Na, McCoy, FEK, Vero, PO-2, CHO-KI, KF, TrT¹³.

Laboratory animals were used to detect virus contamination and monitor the activity of control virus strains: white mice weighing from 16 g to 20 g; in the amount of 1750 heads; rabbits weighing from 1.5 kg to 2.5 kg; in the amount of 12 heads; guinea pigs weighing 40–50 kg, in the amount of 18 heads.

In order to study the spread of viral diarrhea among cattle, seromonitoring of this disease was carried out in different regions of Ukraine. The neutralization reaction was performed according to the classical method in test tubes and using the micromethod in culture plates.

Theoretical calculation of primers during the development of a diagnostic kit for the detection of pestivirus RNA by PCR was carried out by analyzing nucleotide sequences published in GeneBank, EMBL, DDBJ¹⁴.

The obtained research results were statistically processed using digital indicators of the Microsoft Excel for Windows XP program on a personal computer.

Interlaboratory commission tests were conducted with the participation of representatives of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” (NSC “IEKVM”) and the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise.

2. Analysis of Contamination Risk Groups

In order to develop a system for preventing and detecting contamination of VIP with pestiviruses, viral contamination risk groups were identified. The following risk groups were identified:

- animal blood serum;
- cell culture production lines;
- virus production strains;
- live viral vaccines, primarily cultured;
- therapeutic and prophylactic animal blood serums;
- diagnostic antigen and serum kits.

¹³ Barone P., Wiebe M. E., Leung J. C., Hussein I. T. M., Keumurian F. J., Bouressa J., Brussel A., Chen D., Chong M., Dehghani H., Gerentes L., Gilbert J., Gold D., Kiss R., Kreil T. R., Labatut R., Li Y., Müllberg J., Mallet L., Menzel C., Moody M., Monpocho S., Murphy M., Plavsky M., Roth N. J., Roush D., Ruffing M., Schicho R., Snyder R., Stark D., Zhang C., Wolfrum J., Sinskey A. J., & Springs S. L. Viral contamination in biologic manufacture and implications for emerging therapies. *Nat. Biotechnol.* 2020. Vol. 38. P. 563–572.

¹⁴ U.S. Dept. of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications; Availability. Federal Register. 2010. Vol. 75, No. 42. <https://www.govinfo.gov/content/pkg/FR-2010-03-04/pdf/2010-4553.pdf>.

3. Development of a Test System for Detecting Contamination

At the time of the beginning of this dissertation work, there were no registered domestic or imported diagnostic kits in Ukraine aimed at detecting the genome of pestiviruses¹⁵.

The theoretical basis for the development of such a diagnostic kit was literature data and the results of our own research, which were conducted using imported test systems for the detection of pestiviruses, which indicate the prospects of PCR for the detection of pathogens of the genus Pestivirus¹⁶.

The first and main stage in the development of a diagnostic kit is the selection of sequences for oligonucleotide primers. In order to solve the problem, a large number of nucleotide sequences of pestivirus genomes were analyzed: classical swine fever virus (CSFV), bovine diarrhea virus (BDV), sheep border disease virus, published as a web page on the database server of the European Molecular Biology Library (EMBL), the Japanese Database of Nucleotide Sequences (DDBJ) and PDB sequences.

Determination of optimal conditions for PCR. One of the factors affecting the results of PCR is the creation of optimal temperature conditions for primer annealing. Experimental verification of the effect of temperature was carried out using three annealing temperature regimes, namely 58 °C, 60 °C and 62 °C¹⁷.

According to the results of the detection of the reaction products, which was carried out by electrophoresis in a 1.5% agarose gel stained with ethidium bromide, using Tris-borate buffer with a voltage gradient of 10 V/cm and was evaluated by taking into account the results after electrophoresis using a transilluminator under UV light for the presence (or absence) of red-orange DNA fragments. The first pair of primers reacted weakly with the CSF virus strain "K" (ASV) at temperature regimes of 58 and 62 °C, a barely noticeable amplification product was observed at a temperature of 60 °C. Clear luminescence of the amplification product on the transilluminator was established with the cattle diarrhea virus strain Oregon C24V at temperature regimes of 58, 60, 62 °C (Fig. 1).

In the second pair of primers PestF2 and PestR2, at annealing temperatures of 58 °C, 60 °C and 62 °C, clear specific reaction bands were detected in PCR with both CSF virus and bovine diarrhea virus, which indicates the specificity

¹⁵ Стегній Б. Т., Герілович А. П., Лиманська О. Ю. Полімеразна ланцюгова реакція у практиці ветеринарної медицини. Харків : ННЦ «ІЕКВМ». 2006. 110 с.

¹⁶ Levings R. L., Wessman S. J. Bovine viral diarrhea virus contamination of nutrient serum, cell cultures and viral vaccines. *Dev Biol Stand*, 1991. Vol. 75. P. 177–81.

¹⁷ Erickson G. A., Bolin S. R., Landgraf J. G. Viral contamination of fetal bovine serum used for tissue culture: risks and concerns. *Dev Biol Stand.*, 1991. Vol.75. P. 173–175.

and sensitivity of the developed primers. This pair of primers detected both pestivirus genotypes (Fig. 2).

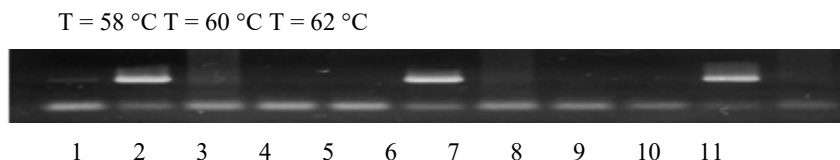


Fig. 1. Results of amplification with primers PestF1 and PestR1 obtained at different annealing temperatures in the range of 58–62 °C
 (1, 5, 9 – blood of rabbits infected with CSF virus strain “K” (ASV);
 2, 6, 10 – culture of TrT cells infected with cattle diarrhea virus strain Oregon C24V; 3, 7, 11 – culture of TrT cells; 4, 8 – negative reaction control)

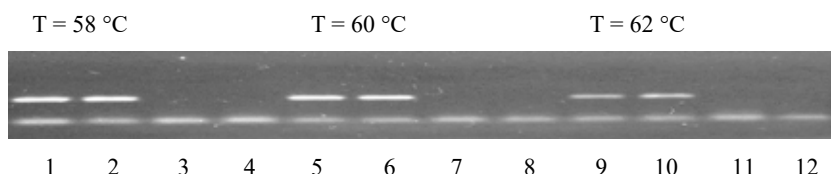


Fig. 2. Results of amplification with primers PestF2 and PestR2 obtained at different annealing temperatures in the range of 58–62 °C
 (1, 5, 9 – blood of rabbits infected with CSF virus strain “K” (ASV);
 2, 6, 10 – culture of TrT cells infected with cattle diarrhea virus strain Oregon C24V; 3, 7, 11 – culture of TrT cells; 4, 8, 12 – negative reaction control)

The control primer pair, which was taken from the article by Barbara Wirz, showed clear specific reaction bands with both CSF virus and bovine diarrhea virus in PCR, which indicates the specificity and sensitivity of the designed primers. This primer pair detected both pestivirus genotypes (Fig. 3).

The obtained results give grounds to conclude that the first pair of primers is specific to cattle diarrhea virus, and the second pair of primers is specific to both representatives of the pestivirus family. The control pair of primers also reacted with both representatives of the pestivirus genus.

Taking into account the obtained research results, the optimal annealing temperature of the primers was determined to be 60 °C, at which amplification products were formed. At temperatures of 58 and 62 °C, amplicons were formed in small quantities or were not detected at all. Of the two developed primer

pairs, the second pair PestF2 and PestR2 was chosen for the diagnostic kit – with a number of 310 nucleotide pairs, since it turned out to be more sensitive and specific compared to the others during the detection of pestivirus RNA.

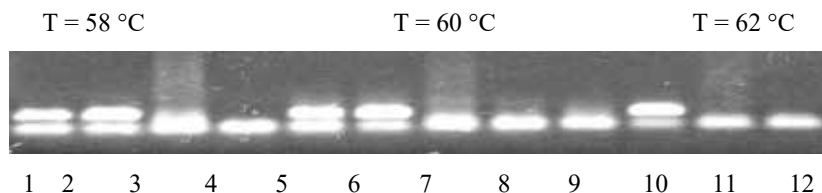


Fig. 3. Results of amplification with Barbara Wirz primers obtained at different annealing temperatures in the range of 58–62 °C
(1, 5, 9 – blood of rabbits infected with CSFV strain “K” (ASV);
2, 6, 10 – culture of TrT cells infected with cattle diarrhea virus strain Oregon C24V; 3, 7, 11 – culture of TrT cells; 4, 8, 12 – negative reaction control)

4. Determination of Sensitivity and Specificity of the Test System

In the process of studying the sensitivity and specificity of the “PCR Pestivir” test system, RNA of bovine diarrhea virus and CSF virus, DNA and RNA of viruses of other families, bacteria, cell cultures, as well as samples of biological material from patients with CSF were studied.

In the process of determining the specificity, reference strains of diarrhea virus “Oregon C24V”, “VK-1”, CSF virus “K”, “Shi-Min” and “Washington” were analyzed in a comparative aspect with strains of viruses causing Aujeszky's disease, parainfluenza-3 of cattle and enteroviruses, as well as pathogens of escherichiosis, pasteurellosis of pigs and erysipelas; pathological material from a pig sick with CSF, TrT cell culture.

The choice of these samples was due to the fact that pestivirus infections quite often occur in association with other diseases and have similar clinical signs.

A characteristic amplification product was observed in positive samples of reference strains of diarrhea virus “Oregon C24V”, “VK-1”, CSF virus “K”, “Shi-Min” and “Washington” and material from a pig sick with CSF. At the same time, in other samples corresponding to bacterial and heterogeneous virus strains, TrT cell culture, the amplification product was absent, which indicated the specificity of the developed set. The sensitivity of the primers was determined by studying the classical swine fever virus strain “Shi-Min” and the cattle diarrhea virus strain “Oregon C24V” in different dilutions with

a step of 10. The initial titer of the viruses was: 6 lg LD₅₀/cm³ – strain of CSF “Shi-Min” and 6.5 lg TCD₅₀/cm³ – strain of cattle FD “Oregon C24V”. It was found that in the studied dilution ranges of CSF virus from 10⁻¹ to 10⁻⁹ and for bovine diarrhea virus from 10⁻¹ to 10⁻⁸, RNA amplification products were detected. Clear specific bands on the trans illuminator were detected at the level of CSF virus from 10⁻¹ to 10⁻⁶, and for bovine diarrhea virus – at the level of 10⁻¹ to 10⁻⁷. Barely noticeable bands were at the level of other dilutions. The results obtained indicate high sensitivity of the primers (Table 1).

The results of the study showed that the use of the drug makes it possible to detect pestivirus in a titer of 0.1 lg, which is sufficient for quality control of the drugs.

The positive result of the dossier examination for the developed diagnostic kit gave the right to conduct interdepartmental commission tests of the “Diagnostic kit for the detection of pestiviruses PCR-PESTIVIR”. The tests were conducted at the DNAIBSM with the participation of employees of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” and the Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise. The results of interdepartmental commission tests confirmed the high sensitivity and specificity of the developed diagnostic kit.

5. Comparative Study of the Effectiveness of the Methods

In order to determine the effectiveness of the polymerase chain reaction, it was studied in comparison with the neutralization reaction (RN) of the virus in cell culture and the immunofluorescence reaction (IFR).

For this purpose, bovine blood serum was taken and artificially contaminated with the bovine diarrhea virus cytopathogenic strain “Oregon C24 V”. Lyophilized virus with an activity of 6.5 lg TCD₅₀/cm³ was dissolved in sterile phosphate buffer solution to the original volume (2 cm³) and tenfold dilutions were prepared in the same solution. To contaminate serum samples, the virus was added in dilutions of 10⁻² (4.5 lg TCD₅₀ of the virus), 10⁻⁴ (2.5 lg) and 10⁻⁵ (1.5 lg). To each serum sample of 4.5 cm³, 0.5 cm³ of the appropriate virus dilution was added. In this way, we kept serum samples containing 3.5 lg, 1.5 lg and 0.5 lg of virus, respectively.

The obtained serum samples were examined using a transfected culture of calf tracheal cells (TpT). For the study of each serum sample, eight tubes with cell culture were taken and eight tubes were left intact. After 15 minutes of contact of the samples with cells, 4 tubes from each group of tubes were examined in the neutralization reaction and 4 tubes in the RIF. Control cell cultures were contacted with blood serum without the addition of virus.

At the same time, serum samples containing different amounts of virus were examined by the reverse transcriptase polymerase chain reaction method. Each sample was examined in four replicates.

To avoid false negative and false positive PCR results, four controls were set. As a positive control, a virus-containing culture fluid containing the bovine diarrhea virus Oregon C 24 V strain undiluted with an activity of 6.5 lg TCD₅₀/cm³ and the same fluid in a dilution of 10⁻⁶ were used. As a negative control, we used bovine blood serum free from virus contamination, and we also set a blank control, which is a reaction mixture containing all reacting components, except nucleic acid.

Studies have shown that the polymerase chain reaction has a sensitivity higher than the neutralization reaction and the immunofluorescence reaction, which makes it possible to use it in further work as a promising reaction for quality control of VIP.

The reliability of the result obtained by PCR is confirmed by reaction controls. Negative results of the blank control indicate the purity of the reaction mixture.

The results of the study are shown in the table.

Table 1

**Efficiency of Different Methods for Detecting Pestiviruses
in Bovine Serum**

Serum virus titer	Samples examined	Of them responded positively					
		Number				%	
		neutralization reaction (PH)		immunofluorescence reaction (IFR)		polymerase chain reaction (PCR)	
3.5 lg	4	4	100.0	4	100.0	4	100.0
1.5 lg	4	4	100.0	4	100.0	4	100.0
0.5 lg	4	1	25.0	2	50.0	4	100.0

Studies have shown that PCR is the most effective and sensitive method for determining serum contamination with pestiviruses.

When comparing the effectiveness of PCR and IFR for detecting possible contamination of biological systems when cultivating production strains of different types of viruses, we conducted an experiment on artificial contamination of cell culture in which the bovine parainfluenza-3 virus was cultivated with the bovine diarrhea virus of the Oregon C24V strain in different titers. The studies were accompanied by the establishment of a negative control – uncontaminated cell culture and a positive control – bovine diarrhea virus.

The results of the study showed that the contaminant virus in the biological system of the production strain of parainfluenza-3 virus was detected by PCR

in titers of 3.5 lg, 1.5 lg and 0.5 lg of the virus in 100.0%, while in IFR – in titer of 0.5 lg in 50.0%, which confirms the superiority of PCR for detecting contaminant pestiviruses.

6. Research on Pestivirus Contamination of Biological Raw Materials and Products

The research of various raw materials and products made from them was carried out using the developed PCR test system and generally accepted methods.

One of the main types of raw materials used in the production of VIP is animal blood serum, most often cattle. In Ukraine, most biological industry enterprises do not have specialized animal husbandry farms to meet production needs. Therefore, the source of blood serum is commodity farms, which do not always control the infection of the organism with pestiviruses^{18, 19}.

This prompted us to study the spread of the diarrhea virus among cattle in different regions of Ukraine. This work was carried out in close cooperation with the staff of the National Center “IEKVM”. Seromonitoring of the presence of specific antibodies in the serum to this pathogen in the RN was carried out. Blood was collected from cows that were not vaccinated against viral diarrhea of cattle.

We established the presence of antibodies to the diarrhea virus in 23.41% of animals, which indicates a significant spread of the virus in the cattle population.

During the work, blood donor cows belonging to the Kherson State Enterprise – Biological Factory, Zhytomyr National Agroecological University and cows (village Kalynivka), which are blood donors for the needs of the company “Ukrvak”, were examined. Blood serum samples were examined in PCR by the nested method using the PCR-Pestivir kit. The level of contamination of blood serum from donors was 10%.

50 batches of native bovine blood serum without preservatives were tested by PCR, 7 (14.0%) were positive for pestivirus contamination. In order to confirm the PCR results, samples from seven batches suspected of contamination were tested at the IFR – of these, 5 (10.0%) confirmed contamination with diarrhea virus. Contaminated batches were discarded.

¹⁸ European Commission (2015). The Rules Governing Medicinal Products in the European Union. Eudralex. Vol. 4–9. European Commission, DG Health and Food Safety, Public health, EU Pharmaceutical information, Eudralex. Available online at: http://ec.europa.eu/health/documents/eudralex/index_en.htm (accessed 25 July 2023).

¹⁹ World Health Organization (WHO). 2012. WHO Expert Committee on Biological Standardization. World Health Organization Technical Report Series, Report No. 964. World Health Organization, Geneva, Switzerland. Available online at: https://apps.who.int/iris/bitstream/handle/10665/89142/9789241209779_eng.pdf (Accessed 25 July 2023).

7. Study of Pestivirus Contamination of Cell Cultures

We studied samples of different QC lines that were in the biological materials bank of the National Center for Microorganism Strains DNAIBSM.

QC studies in PCR were performed using two kits: “Diagnostic kit for the detection of pestiviruses PCR-Pestivir” and “Test system for the detection of RNA of bovine diarrhea virus “BOVI-RNA-TEST-BVDV”.

By virological research methods: by the manifestation of CPD and RGAd, contamination with viruses was not detected, although in several cases the result was questionable. In the latter case, a delay in cell proliferation was observed, filling the monolayer by 60–75%.

IFR was performed using a kit for the diagnosis of viral diarrhea in cattle. Positive PCR results were confirmed by positive IFR results.

Of the 18 studied cell culture lines, 7 (36.0%) were found to be contaminated with pestiviruses. Of the 26 isolates, only 3 (11.5%) had a cytopathogenic effect in cell cultures.

Research on pestivirus contamination of production virus strains in cell cultures

For the study, samples of strains stored in the depository of the National Center for Microorganism Strains DNAIBSM and samples of strains received for the purpose of deposit were selected, special attention was paid to those virus strains for the cultivation of which cell cultures are used.

We studied 144 virus strains, most of which were found to be uncontaminated by foreign hemadsorbing, hemagglutinating and cytopathogenic viruses. As a result of the studies, the following strains were found to be contaminated: attenuated strains of porcine enteroviruses PS 27 (SMEDI A) serogroup 8; PS 026 (SMEDI B) serogroup 3; PS 37 (SMEDI E); Bereznyansky 652; Teshen disease virus Perechensky 642, Chernihiv 2372; Aujeszky's disease virus strains “Arsky” and “18b UNDIEV”; bovine coronavirus VS-1; bovine parainfluenza virus-3: “ZKSM” and infectious rhinotracheitis “Moldavian”, bovine rotavirus “Tiverval”. When studying the above strains, signs of CPD of viruses were revealed, which were manifested by rounding of cells, their destruction, or delayed proliferation.

The above virus strains were additionally examined in PCR using commercial kits for the detection of pestiviruses, in particular the diagnostic kit for the detection of pestiviruses “PCR-Pestivir” and the kit developed at the NSC IEKVM “Test systems for the detection of bovine diarrhea virus RNA “BOVI-RNA-TEST-BVDV”. The PCR results were confirmed by setting the IFR.

The results of the studies showed that the level of contamination of the broodstock of production strains of viruses used in the production of VIP for the pig industry ranges from 22.0 to 45.0%.

The level of contamination of the broodstock of production strains of viruses used in the production of VIP for the cattle industry ranges from 33.0 to 50.0%. The production strains of bovine coronavirus were contaminated in 100.0%.

Pestivirus contamination study of diagnostic kits

Among the diagnostic kits that were tested for contamination with foreign viruses, the majority were kits for diagnosing bovine leukemia in the immunodiffusion reaction.

21 series of antigen specific to bovine leukemia virus and 14 series of specific sera were tested, produced by the following companies: NPP “Leukonad”, Poltava; SE “Veterinary Medicine”, Kharkiv and the Institute of Epizootology of the NAAS of Ukraine, Rivne.

The absence of contamination of sera and antigens with pestiviruses was confirmed by IFR and PCR. It was found that 52.0% of antigen series and 50.0% of blood serum series were contaminated.

Among other virological kits, in particular for diagnosing Aujeszky's disease, coronavirus infection of cattle, and enterovirus infections of pigs, no contaminated series were detected. With a small sample of VIP samples (1–2 series), it cannot be stated that the production technology provides a 100.0% guarantee of the release of uncontaminated products.

Pestivirus contamination studies of live viral vaccines

For the study, vaccines from various manufacturers, both from Ukraine and abroad, were selected, in particular, Kherson State Enterprise – Biological Factory, Sumy State Biological Factory, Pokrovsky Plant of Biological Products, Stavropol Biological Factory, Bio-Test Laboratory, Hipra Laboratory, Intervet International.

Vaccine series not contaminated with bacteria and fungi were taken into the study, which was confirmed by studies in accordance with DSTU 4483.

The absence of contamination of vaccines with pestiviruses, in particular with the cattle diarrhea virus, was studied by PCR, special attention was focused on the study of culture vaccines.

Among the vaccines studied, live culture vaccines against infectious diseases of pigs and cattle prevailed, since pestiviruses pose a threat to them. The study found that the degree of contamination of culture vaccines varied from 2.0 to 60.0% in different groups of vaccines. We found that no contamination was found in drugs imported from countries where the GMP system has been implemented in the production of VIP. Contaminated VIP batches were found in batches of drugs manufactured in Ukraine.

Development of a system for preventing and detecting contamination with VIP viruses

The search for the causes and sources of contamination of finished products turned out to be difficult and in many cases ineffective. An obstacle to resolving this issue was the lack of requirements regulated by law for the registration of production process protocols. Thus, only in 40.0% of cases of contamination of cell cultures were the causes established.

Our studies confirm that the main source of contamination of veterinary biological products with pestiviruses is cattle blood serum.

Taking into account the results we obtained, we developed a system for preventing and detecting contamination with VIP viruses. This system includes not only control of raw materials and products, but also control of production conditions, control of the human factor. The basis of this system was the scheme for controlling medicines, approved by the World Health Organization and interpreted for use in the practice of manufacturing veterinary drugs.

The developed system provides for measures to prevent contamination and clear documentation of all actions and their results; self-control during research; research of the limits of permissible deviations and double control of each sample at each stage of production.

The result of work in this direction was the development and implementation of state standards of Ukraine: DSTU 4517:2010 and DSTU 5832:2008.

The implementation of the developed regulatory documents and the implementation of the developed system for the prevention and detection of viral contamination of veterinary immunobiological preparations in the practice of VIP production allowed to significantly reduce the level of contamination. Thus, during 2004–2005, the level of contamination of commercial batches of blood serum reached 62.0%, cell cultures – 36.0%, passages of individual lines of cell cultures had a significantly higher level of contamination: SNEV – 75.0%, TrT – 100.0%. While in 2011 the level of contamination of cattle blood serum decreased to 10.0%, and cell cultures – to 8.0–12.0%. Due to the fact that uncontaminated passages of cell cultures were taken to obtain production virus broodstocks, uncontaminated production virus broodstocks were obtained. Due to the provision of uncontaminated raw materials to the enterprises of the biological industry of Ukraine over the past two years, no contaminated batches of VIP were detected.

CONCLUSIONS

Based on the conducted virological and molecular biological, serological studies, the situation regarding the contamination of veterinary immunobiological preparations with pestiviruses was studied, a diagnostic kit was developed

for their detection and identification by PCR, and a system of measures was proposed to detect and prevent contamination of VIP with foreign viruses.

Based on the results of bioinformatic analysis of the gene sequences of pestiviruses, in particular, the bovine diarrhea virus and the classical swine fever virus, the nucleotide sequence of two pairs of primers Pest1F and Pest1R and Pest2F and Pest2R was calculated, which in PCR (33 amplification cycles, 2.0 mM Mg²⁺, 2 U of polymerase, annealing temperature 60 °C) form amplification products with a length of 310 and 350 bp, respectively.

A diagnostic kit for the detection of pestiviruses based on PCR using genus-specific primers has been developed and implemented, which allows for reliable detection of the virus in biological material at a minimum titer of 0.1 lg.

The main contaminant of veterinary immunobiological preparations is pestiviruses and, in particular, bovine diarrhea virus, which contaminated up to 50.0% of the studied series of VIP.

Isolates of bovine diarrhea virus, which contaminated cell cultures only in 11.0% of cases showed CPD.

The main factor of VIP contamination is bovine blood serum, which is used in the production of cell cultures and virus strains. The level of contamination of commercial series of bovine blood serum is on average 10%.

At enterprises with a low level of implementation of measures to prevent contamination of veterinary immunobiological drugs with viruses, its level can reach 60.0–80.0%.

The main reason for contamination of finished products is improper control of input raw materials used for the production of drugs. Before the introduction of the system for preventing and detecting contamination with pestiviruses of VIP, its level reached 62.0% of the studied series of blood serum of cattle, up to 36.0% – passages of cell culture lines, up to 30.0% – broodstock of production strains of viruses.

During the period of implementation of the system for preventing and detecting contamination of VIP with pestiviruses, the level of contamination decreased in cell cultures from 36.0% in 2004 to 12.0% in 2011, blood serum for the production of VIP from 62% to 10%; The use of uncontaminated cell cultures and blood serum allows the risk of contamination of production virus broodstock to be reduced to zero.

SUMMARY

Situations were studied in relation to distribution of the phenomenon of contamination of veterinary immunobiological preparations of Pestivirus; it is set that basic contaminant is virus of Bovine Virus Diarrhea. A diagnostic set is worked out for an exposure of the Pestivirus method of polymerase chain

reaction (PCR). The basic source of contamination is blood sera of Bovine, which is used for the production of veterinary immunobiological preparations. It is set that most isolates which contaminant preparations were does not own a cytopathic effect that testifies to absence for them of potential pathogenicity. The system of measures, which allows substantially bringing down the risk of contamination the viruses of products, is worked out.

Bibliography

1. Яворський М. І., Коваленко В. І., Литвиненко Л. Ю., Юрченко П. А. Ендокринно-ферментна та спеціальна сировина і лікарські препарати на її основі. Довідник. Асоціація виробників натуральної продукції і лікарських засобів «Вітафарм». Київ : Урожай, 1995. 219 с.
2. Напненко О. О. Стандартизація умов розроблення та виробництва ветеринарних імунобіологічних засобів. Матеріали XI міжнародного конгресу спеціалістів ветеринарної медицини (3–4 жовтня 2013 р., м. Київ, Україна). Київ, 2013. С. 95–96.
3. Напненко О. О., Головка А. М., Ареф'єв В. Л., Гордієнко О. І. Створення системи оцінювання ефективності діяльності колекцій штамів мікроорганізмів в Україні. Матеріали Міжнародної науково-практичної конференції «Біотехнологія та її роль в забезпеченні здоров'я людей і тварин». Ніжин: ПП Лисенко М. М., 2023. С. 168–169.
4. Напненко О., Головка А., Іванченко П. Система контролю за контамінацією сторонніми агентами ветеринарних імунобіологічних лікарських засобів. *Фармаком*, 2021. С. 89–91.
5. Напненко О. О. Система запобігання та виявлення контамінації ветеринарних імунобіологічних препаратів пестівірусами : автореф. дис. ... канд. вет. наук : 16.00.03; Одеський державний аграрний університет, 2012. 18 с.
6. Test for sterility and freedom from contamination of biological materials. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Publisher: Paris: OIE, World Organisation for Animal Health, 2024. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.09_TESTS_FOR_STERILITY.pdf.
7. Державна фармакопея України. Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2-ге видання. Доповнення 3. Харків : Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2018. 416 с.
8. European Pharmacopoeia. Sixth Edition. Edition of the Council of Europe, Strasbourg, France, 2022.

9. Напненко О., Головка А., Безвін Є. Організація серійного контролю якості ветеринарних імунобіологічних засобів в Україні. *Біологія тварин*, 2024, Т. 26, № 3. С. 96.

10. Блоцька О., Головка А., Кузьмич Г., Напненко О. Біотехнологія. Методи попередження контамінації культур клітин : ДСТУ 8115:2015. Введено в дію 01.01.2017. К.: ДП «УкрНДНЦ», 2018. 9 с.

11. Code of Federal Regulations. Animal and Animals Products. Title 9, Parts 1.199. The Office of the Federal Register, National Archives and Records Administration: US Government Printing Office, Washington DC, USA, 2024. <https://www.govinfo.gov/content/pkg/CFR-2024-title9-vol1/pdf/CFR-2024-title9-vol1.pdf>.

12. Напненко О. О., Головка А. М., Ареф'єв В. Л., Гордієнко О. І. Створення системи оцінювання ефективності діяльності колекцій штамів мікроорганізмів в Україні. Матеріали Міжнародної науково-практичної конференції «Біотехнологія та її роль в забезпеченні здоров'я людей і тварин». Ніжин: ПП Лисенко М. М., 2023. С. 168–169.

13. Barone P., Wiebe M. E., Leung J. C., Hussein I. T. M., Keumurian F. J., Bouressa J., Brussel A., Chen D., Chong M., Dehghani H., Gerentes L., Gilbert J., Gold D., Kiss R., Kreil T. R., Labatut R., Li Y., Müllberg J., Mallet L., Menzel C., Moody M., Monpoeho S., Murphy M., Plavsic M., Roth N. J., Roush D., Ruffing M., Schicho R., Snyder R., Stark D., Zhang C., Wolfrum J., Sinskey A. J., & Springs S. L. Viral contamination in biologic manufacture and implications for emerging therapies. *Nat. Biotechnol.* 2020. Vol. 38. P. 563–572.

14. U.S. Dept. of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications; Availability. Federal Register. 2010. Vol. 75, No. 42. <https://www.govinfo.gov/content/pkg/FR-2010-03-04/pdf/2010-4553.pdf>.

15. Стегній Б. Т., Герілович А. П., Лиманська О. Ю. Полімеразна ланцюгова реакція у практиці ветеринарної медицини. Харків, ННЦ «ІЕКВМ». 2006. 110 с.

16. Levings R. L., Wessman S. J. Bovine viral diarrhea virus contamination of nutrient serum, cell cultures and viral vaccines. *Dev Biol Stand*, 1991. Vol. 75. P. 177–81.

17. Harasawa R., Mizusawa H. Detection of Pestiviruses from Mammalian Cell Cultures by the Polymerase Chain Reaction. Proceedings of 3rd Internet World Congress on Biomedical Sciences, 1996. Vol. 12. P. 9–20.

18. Erickson G. A., Bolin S. R., Landgraf J. G. Viral contamination of fetal bovine serum used for tissue culture: risks and concerns. *Dev Biol Stand.*, 1991. Vol. 75. P. 173–175.

19. European Commission (2015). The Rules Governing Medicinal Products in the European Union. Eudralex. Vol. 4–9. European Commission, DG Health and Food Safety, Public health, EU Pharmaceutical information, Eudralex. Available online at: http://ec.europa.eu/health/documents/eudralex/index_en.htm (accessed 25 July 2023).

20. World Health Organization (WHO). 2012. WHO Expert Committee on Biological Standardization. World Health Organization Technical Report Series, Report No. 964. World Health Organization, Geneva, Switzerland. Available online at: https://apps.who.int/iris/bitstream/handle/10665/89142/9789241209779_eng.pdf (Accessed 25 July 2023).

Information about the authors:

Napnenko Oleksandr,

Candidate of Veterinary Sciences, Senior Research Fellow,
Associate Professor at the Department of Veterinary Hygiene
named after A. K. Skorokhodko
National University of Life and Environmental Sciences of Ukraine
15, Heroyiv Oborony Street, Kyiv, Ukraine

Solomon Vyacheslav,

Candidate of Veterinary Sciences,
Head of Department of Veterinary Hygiene
named after A. K. Skorokhodko
National University of Life and Environmental Sciences of Ukraine
15, Heroyiv Oborony Street, Kyiv, Ukraine

Midyk Svitlana,

Candidate of Veterinary Sciences, Senior Researcher,
Associate Professor at the Department of Veterinary Hygiene
named after A. K. Skorokhodko
National University of Life and Environmental Sciences of Ukraine
15, Heroyiv Oborony Street, Kyiv, Ukraine

**ATMOSPHERIC AIR: GLOBAL TRENDS,
MONITORING, AND CLIMATE CHALLENGES**

Pytuliak Myroslava, Pytuliak Mykola

DOI <https://doi.org/10.30525/978-9934-26-714-7-15>

INTRODUCTION

Atmospheric air is one of the most important components of the natural environment, and its condition directly affects public health, the functioning of natural ecosystems, and the prospects for sustainable socio-economic development. The atmosphere facilitates gas exchange among the components of the biosphere, contributes to climate formation, participates in the cycling of matter and energy, and helps maintain ecological balance. At the same time, the intensive development of industry, energy, transport, agriculture, and urbanization processes has led to a significant increase in anthropogenic pressure on the atmosphere, making air pollution a global environmental problem.

The main anthropogenic sources of atmospheric air pollution continue to include industrial and energy facilities, road transport, heating systems, fossil fuel and biomass combustion, agricultural production, and waste management. A wide range of pollutants is released into the atmosphere, among which particulate matter (PM_{2.5} and PM₁₀), nitrogen and sulfur oxides, carbon monoxide, volatile organic compounds, heavy metals, black carbon, and other components are of particular concern. Their accumulation adversely affects human health, the condition of natural ecosystems, and the dynamics of climate processes.

A distinctive feature of contemporary atmospheric pollution is its transboundary nature. The movement of air masses facilitates the transport of gaseous pollutants and aerosols over considerable distances from their sources. As a result, industrial emissions, smoke from wildfires, dust, and other pollutants can affect air quality far beyond the boundaries of individual regions and countries. This highlights the need to strengthen international cooperation, improve monitoring systems, and ensure the timely exchange of environmental information.

The issue of atmospheric air quality is closely linked to global climate change. On the one hand, certain atmospheric pollutants affect the Earth's radiation balance and the climate system; on the other hand, rising air temperatures, the increasing frequency of droughts, heat waves, and large-scale wildfires alter

the conditions governing the formation, transformation, and dispersion of pollutants. Therefore, atmospheric air protection and climate policy should be regarded as interconnected areas of environmental security.

These issues are particularly relevant to Ukraine. The full-scale war has significantly transformed the structure of anthropogenic pressure on atmospheric air. The destruction of industrial and energy facilities, fires, explosions, damage to infrastructure, changes in transport flows, the relocation of enterprises, and the widespread use of autonomous energy supply systems are creating new environmental risks. At the same time, hostilities complicate systematic observations and the collection of complete and reliable information on atmospheric air quality throughout the country. Under these conditions, the modernization of the national environmental monitoring system is becoming particularly important.

Modern atmospheric air monitoring is gradually evolving from a traditional network of stationary monitoring stations into an integrated digital system. Satellite observations and European information systems play an important role in this transformation, expanding the possibilities for spatial analysis of atmospheric conditions and forecasting the dispersion of pollutants.

For Ukraine, the modernization of the monitoring system is also of particular importance in the context of European integration and post-war recovery. Harmonization of national environmental policy with the approaches adopted by the European Union requires the improvement of air quality assessment methods, enhanced reliability and accessibility of data, automation of data transmission, and open public access to environmental information. At the same time, the reconstruction of the country's industrial and energy potential provides an opportunity to introduce modern low-carbon technologies and advanced emission control systems.

The aim of the study is to provide a comprehensive synthesis of contemporary scientific approaches to assessing the state of atmospheric air, to analyze global and national trends in air pollution, to examine modern monitoring systems, and to identify the interrelationships between air quality, anthropogenic pressure, and climate change.

The practical significance of the study lies in the potential application of its findings to improve the atmospheric air monitoring system in Ukraine, substantiate environmental protection measures, develop digital monitoring infrastructure, and enhance the effectiveness of environmental management decisions. The comprehensive integration of traditional observation methods with satellite-based, sensor, geoinformation, and intelligent technologies should become one of the key directions in developing a modern environmental security system in Ukraine and ensuring its sustainable post-war development.

1. Contemporary Sources and Chemical Mechanisms of Technogenic Atmospheric Air Pollution

In recent years, the international scientific community has increasingly regarded atmospheric air as a key link in the interactions among natural ecosystems, public health, and socio-economic development. Within these conceptual frameworks, atmospheric air quality is no longer viewed exclusively as an environmental characteristic but has become one of the fundamental indicators of sustainable development, environmental security, and the quality of life of the population. This approach makes it possible to assess atmospheric processes not in isolation but as an integral component of the complex functioning of natural and anthropogenic systems.

At the same time, the rapid increase in technogenic pressure significantly exceeds the capacity of the atmosphere's natural self-purification mechanisms. While natural processes are capable of maintaining a relative equilibrium in atmospheric gas composition under conditions of moderate pollutant inputs, the current scale of industrial emissions, transport-related emissions, fossil fuel combustion, and urbanization processes increasingly exceeds the assimilative capacity of the atmospheric system. This is reflected in the increasing frequency of episodes of severe air pollution, the prolonged duration of smog events, the intensification of transboundary pollutant transport, and the emergence of new environmental risks to human populations and natural ecosystems.

Under current conditions, the impact of military activities on atmospheric air quality also deserves particular attention. The destruction of industrial enterprises, oil depots, energy facilities, transport infrastructure, and residential buildings is accompanied by the release of a wide range of toxic substances into the atmosphere, including nitrogen oxides, sulfur oxides, fine particulate matter, polycyclic aromatic hydrocarbons, dioxins, heavy metals, and other hazardous compounds. For Ukraine, this problem is not only of environmental but also of strategic importance, as the consequences of wartime atmospheric pollution may persist for a considerable period, affecting public health, ecosystem productivity, and post-war territorial recovery processes.

Thus, atmospheric air constitutes an extremely complex, multi-level system whose functioning is determined by the interaction of natural and anthropogenic processes. Increasing technogenic pressure, the intensification of photochemical transformations, ongoing global climate change, and the growing transboundary transport of pollutants necessitate a transition from the traditional monitoring of individual pollutant concentrations to integrated air quality management. This approach forms the methodological basis of contemporary environmental policy, international monitoring systems, and further research into the sources and chemical characteristics of technogenic atmospheric pollution.

The current stage of civilization development is characterized by an unprecedented increase in anthropogenic pressure on atmospheric air, which is a natural consequence of industrialization, urbanization, the intensification of transport flows, economic globalization, and growing global demand for energy resources. Over the past half-century, the scale and structure of technogenic emissions have undergone substantial transformations in both quantitative and qualitative terms. Whereas in the mid-twentieth century large industrial enterprises were the predominant sources of atmospheric pollution, today a complex, multicomponent emission system has emerged in which transport, energy production, the agro-industrial sector, housing and municipal services, construction, extractive industries, and other types of economic activity play significant roles.

Recent studies indicate that the structure of anthropogenic sources of atmospheric air pollution is constantly changing under the influence of technological development, national environmental policies, the transition towards a low-carbon economy, and global socio-economic transformations. In the European Union, emissions of sulfur dioxide and heavy metals have decreased substantially over recent decades due to the modernization of the energy sector, the implementation of best available techniques, stringent environmental legislation, and the development of renewable energy sources. At the same time, so-called diffuse sources of pollution are becoming increasingly important. These comprise millions of relatively small individual sources, including motor vehicles, private heating systems, small enterprises, construction sites, and agricultural holdings. The cumulative impact of such sources often exceeds the contribution of individual large industrial facilities, consi

The Energy Sector as a Dominant Source of Technogenic Atmospheric Pollution

Despite the rapid development of renewable energy, the energy sector remains one of the largest anthropogenic sources of atmospheric air pollution worldwide. The generation of electricity and heat from fossil fuels is accompanied by substantial emissions of carbon dioxide, sulfur oxides, nitrogen oxides, carbon monoxide, particulate matter, volatile organic compounds, and heavy metals. The magnitude of these emissions depends on the type of fuel used, the technological level of the equipment, the efficiency of flue-gas treatment systems, and the environmental standards in force in a particular country.

Hard coal and lignite remain among the most environmentally hazardous types of fuel.

Significant changes in the structure of energy-related emissions have occurred as a result of the development of renewable energy. The large-scale deployment of solar and wind power plants, along with the increasing share of hydropower and bioenergy, contributes to reducing the consumption of

fossil fuels. Particular attention should be paid to bioenergy, which is often regarded as a climate-neutral energy source. However, biomass combustion may be accompanied by substantial emissions of fine particulate matter, carbon monoxide, organic aerosols, and polycyclic aromatic hydrocarbons. Therefore, the environmental performance of bioenergy technologies largely depends on the type of biofuel used, combustion conditions, and the efficiency of flue-gas treatment systems¹.

Thus, the energy sector remains a key factor influencing the chemical composition of atmospheric air on a global scale. Further reduction of its adverse environmental impact requires comprehensive modernization of energy infrastructure, the development of zero-carbon technologies, improvements in energy efficiency, and the widespread implementation of industrial emission control and treatment systems. At the same time, assessments of the environmental safety of the energy sector should take into account not only direct atmospheric emissions but also the entire life cycle of energy technologies, in accordance with contemporary principles of sustainable development and the circular economy.

Industry as a Complex Source of Technogenic Atmospheric Air Pollution

The industrial sector has traditionally been one of the most significant sources of anthropogenic pressure on atmospheric air. Despite the substantial modernization of production processes in most economically developed countries, industry continues to account for a considerable share of global emissions of gaseous and particulate pollutants. Industrial emissions are characterized by their high concentrations, diverse chemical composition, and capacity to form complex multicomponent mixtures that undergo intensive physicochemical transformations after being released into the atmosphere.

Unlike the transport or residential sectors, where fuel combustion products predominate, industrial enterprises generate a wide range of specific substances whose composition is determined by production technology, the type of raw materials used, operating conditions of the equipment, and the level of environmental modernization. Consequently, industry is a source not only of conventional air pollutants, such as nitrogen oxides, sulfur oxides, carbon monoxide, and particulate matter, but also of numerous toxic trace components, including heavy metals, polycyclic aromatic hydrocarbons, dioxins, furans, benzene, formaldehyde, persistent organic pollutants, and other hazardous substances.

Large industrial agglomerations, where metallurgical enterprises, thermal power plants, cement plants, chemical facilities, and oil refineries operate simultaneously, pose a particular environmental hazard.

¹ Боголюбов В. М., Клименко М. О., Мокін В. Б. та ін. Моніторинг довкілля : підручник. Херсон : Грінь Д. С., 2011. 530 с.

The metallurgical industry is among the most resource-intensive and environmentally hazardous industrial sectors. Virtually all technological stages involved in the production of ferrous and non-ferrous metals are accompanied by atmospheric emissions of varying composition and intensity. Sinter production, coke-chemical processes, blast-furnace smelting, basic oxygen steelmaking, electric steelmaking, and rolling processes generate a specific spectrum of pollutants dominated by nitrogen oxides, sulfur oxides, carbon monoxide, particulate matter, heavy metals, and organic toxicants.

Particular attention should be paid to particulate emissions from metallurgical enterprises. Unlike natural dust, metallurgical aerosols contain significant concentrations of iron, manganese, zinc, cadmium, chromium, nickel, lead, and other metals characterized by high biological activity.

Chemical industry enterprises represent one of the most complex sources of atmospheric pollution, as they handle a wide range of organic and inorganic substances. A distinctive feature of this sector is the considerable diversity of technological processes, which results in a complex and variable composition of atmospheric emissions.

The principal pollutants include volatile organic compounds (VOCs), ammonia, chlorine, hydrogen fluoride, hydrogen sulfide, formaldehyde, benzene, toluene, xylene, phenols, and other toxic substances. A significant proportion of these compounds act as precursors of photochemical smog and secondary organic aerosols. Once released into the atmosphere, they actively interact with nitrogen oxides under the influence of solar radiation, contributing to the formation of ground-level ozone, one of the most hazardous secondary pollutants in the modern atmosphere. Accidental releases from chemical industry facilities pose a particularly serious environmental hazard.

Cement production is one of the most energy-intensive industrial processes and, at the same time, an important source of atmospheric emissions. Cement plants generate large amounts of carbon dioxide, particulate matter, nitrogen oxides, sulfur dioxide, and fine particles of mineral origin.

Oil refineries generate a specific spectrum of atmospheric pollutants, including hydrogen sulfide, volatile organic compounds, benzene, polycyclic aromatic hydrocarbons, nitrogen oxides, and sulfur dioxide. A significant proportion of these emissions is associated not only with technological processes but also with the evaporation of crude oil and petroleum products during transportation and storage.

The Transport Sector as a Dynamic Source of Atmospheric Air Pollution

At the beginning of the twenty-first century, the transport sector emerged as one of the most dynamic factors influencing atmospheric air quality. The rapid growth in motor vehicle use, the globalization of international trade, the expansion of air transportation, and the intensification of logistics flows have substantially altered

the structure of atmospheric emissions. Whereas during the second half of the twentieth century large stationary industrial facilities were the primary contributors to atmospheric pollution, mobile emission sources are now playing an increasingly important role, with their number continuing to grow worldwide each year.

A distinctive feature of transport-related pollution is its spatial concentration directly within populated areas. Unlike industrial enterprises, which are often located outside residential zones, transport infrastructure passes through densely populated urban areas, resulting in continuous exposure to air pollutants within the human breathing zone.

The main components of transport emissions include nitrogen oxides (NO_x), carbon monoxide (CO), carbon dioxide (CO_2), volatile organic compounds (VOCs), fine particulate matter ($\text{PM}_{2.5}$ and PM_{10}), black carbon, polycyclic aromatic hydrocarbons, aldehydes, and numerous ultrafine particles. Their relative proportions depend on the type of vehicle, engine technology, fuel quality, operating conditions, and the level of vehicle maintenance.

Contemporary research pays particular attention to non-exhaust emissions, whose contribution to air pollution has long been underestimated. These include particles generated by the wear of tires, brake pads, and road surfaces, as well as the resuspension of road dust by air currents. In large cities, such sources may account for a significant proportion of the total PM_{10} mass. With the increasing adoption of electric vehicles, their relative importance is expected to grow further, since the absence of exhaust emissions does not eliminate the mechanical generation of particulate matter.

Road transport is a major source of nitrogen oxide emissions in urban ecosystems. In the presence of solar radiation, these compounds actively participate in photochemical reactions leading to the formation of ground-level ozone and secondary organic aerosols. Thus, transport not only directly deteriorates atmospheric air quality but also triggers the formation of secondary pollution, the magnitude of which often exceeds the impact of primary emissions².

Diesel-powered transport remains a significant environmental challenge.

A distinct area of contemporary research focuses on the impact of transport electrification on atmospheric air quality. The large-scale adoption of electric vehicles undoubtedly contributes to reducing local emissions of nitrogen oxides, carbon monoxide, and volatile organic compounds. At the same time, assessing the environmental performance of electric transport requires consideration of its entire life cycle, including battery production, electricity generation, and the disposal or recycling of components at the end of their service life.

² Защепкіна Н., Рудницький Р. Аналіз основних чинників, які впливають на рівень забруднення атмосферного повітря. *Вимірювальна та обчислювальна техніка в технологічних процесах*. 2026. № 1. С. 21–27. URL: <https://doi.org/10.31891/2219-9365-2026-85-3>.

Table 1

Comparative Characteristics of Major Anthropogenic Sources of Air Pollution, Their Chemical Composition, and Environmental Impacts^{3, 4, 5, 6}

Pollution Source	Key Pollutants	Emissions Formation Features	Impact Scale	Major Environmental Impacts	Priority Environmental Measures
Thermal power engineering	CO ₂ , SO ₂ , NO _x , PM _{2.5} , PM ₁₀ , CO, Hg	Combustion of hard coal, lignite, fuel oil, and natural gas	Global, regional	Acid precipitation, smog, climate change, ecosystem degradation	Transition to RES, BAT, electrostatic precipitators, desulfurization, carbon capture and storage (CCUS)
Metallurgy	PM _{2.5} , PM ₁₀ , Fe, Mn, Zn, Pb, Cd, SO ₂ , NO _x , CO	Coking, sintering, blast furnace, and steelmaking operations	Local, regional	Air and soil pollution, accumulation of heavy metals, carcinogenic risk	Gas cleaning plants, sealing of technological processes, closed-loop production cycles
Chemical industry	VOC, NH ₃ , Cl ₂ , H ₂ S, HF, formaldehyde, benzene, toluene	Chemical synthesis, production of fertilizers, polymers, and acids	Local	Photochemical smog, toxic effects, formation of secondary aerosols	LDAR, catalysts, equipment sealing, automated monitoring
Cement industry	CO ₂ , PM ₁₀ , PM _{2.5} , NO _x , SO ₂	Clinker burning, raw material grinding, material transportation	Local, regional	Dust pollution, contribution to global CO ₂ emissions	Bag filters, alternative fuels, low-carbon cements, CCUS
Oil refining industry	VOC, benzene, PAHs, SO ₂ , H ₂ S, NO _x	Oil processing, storage, and transportation of petroleum products	Regional	Ozone formation, carcinogenic impact, toxic air pollution	LDAR systems, storage tank sealing, vapor recovery units

³ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

⁴ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

⁵ European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

⁶ European Commission. Zero Pollution Action Plan: Towards Zero Pollution for Air, Water and Soil. Brussels : European Commission, 2021.

Table 1 (Continued)

Pollution Source	Key Pollutants	Emissions Formation Features	Impact Scale	Major Environmental Impacts	Priority Environmental Measures
Road transport	NO _x , CO, CO ₂ , VOC, PM _{2.5} , black carbon	Operation of internal combustion engines, wear of tires and road surface	Local, global	Urban smog, noise pollution, respiratory diseases	Electric vehicles, catalytic converters, Euro standards, public transport development
Aviation transport	CO ₂ , NO _x , water vapor, ultrafine particles	Operation of aircraft engines at high altitudes	Global	Intensification of the greenhouse effect, contrail formation	SAF fuels, route optimization, new engine designs
Maritime transport	SO ₂ , NO _x , PM, CO ₂	Use of heavy fuel oil	Global	Coastal area pollution, acid rain	Low-sulfur fuels, LNG, port electrification
Agriculture	NH ₃ , CH ₄ , N ₂ O, PM	Livestock farming, fertilizer application, soil tillage	Regional	Secondary fine particulate matter, eutrophication, climate change	Precision agriculture, fertilizer application optimization, biogas plants
Housing and communal sector	PM _{2.5} , CO, NO _x , SO ₂ , benzo(a)pyrene	Residential heating, combustion of solid fuels and waste	Local	Cold-season smog, increased risk of respiratory diseases	Building energy efficiency, district heating, ban on waste burning
Construction sector	PM ₁₀ , PM _{2.5} , SiO ₂ , cement dust	Demolition, earthworks, material transportation	Local	Dust accumulation in areas, deterioration of urban air quality	Construction site watering/irrigation, dust control screens, modern construction techniques
Mining industry	PM, CH ₄ , SO ₂ , NO _x	Open-pit quarries, underground mines, blasting operations	Local, regional	Dust load, landscape degradation, methane emissions	Dust suppression, mine degasification, land reclamation
Military activities	PM _{2.5} , PM ₁₀ , CO, NO _x , SO ₂ , PAHs, dioxins, heavy metals	Fires, explosions, destruction of industrial facilities, combustion of fuels and lubricants	Local, regional, transboundary	Complex toxic air pollution, transboundary transport of aerosols, long-term public health impacts	Operational monitoring systems, satellite surveillance, post-war environmental reclamation

Aviation and maritime transport are equally important, as they support the functioning of the global economy while simultaneously creating specific environmental challenges. Aviation is a source of nitrogen oxides, water vapour, soot, and aerosol emissions in the upper troposphere, affecting the formation of condensation trails (contrails) and altering the atmospheric radiation balance. Maritime transport, particularly vessels using heavy fuel oils, remains a significant source of sulfur dioxide, nitrogen oxides, and fine particulate matter. In response to these challenges, the International Maritime Organization (IMO) has introduced stricter requirements for the sulfur content of marine fuels, which have already demonstrated positive effects in reducing atmospheric emissions in coastal regions.

In the future, the key directions for improving the environmental sustainability of the transport sector include the development of public transport, electrification of the vehicle fleet, the adoption of hydrogen technologies, improvements in urban mobility, digital management of traffic flows, and the integration of transport policy with Smart City concepts. At the same time, achieving a substantial environmental effect will only be possible through a comprehensive approach combining technological innovation, environmental regulation, and changes in the transport behaviour of the population.

Agriculture as a Specific Source of Atmospheric Pollution

For a long time, the agricultural sector was regarded primarily as an economic activity with a relatively minor impact on atmospheric air quality. However, contemporary research indicates that the intensification of agricultural production has substantially altered the nature of interactions between agroecosystems and the atmosphere. According to estimates by international environmental organizations, agriculture is currently one of the leading anthropogenic sources of ammonia (NH_3), methane (CH_4), nitrous oxide (N_2O), as well as secondary fine particulate matter formed through atmospheric chemical reactions.

A distinctive feature of agricultural emissions is that most of them are diffuse in nature, which significantly complicates their monitoring, quantitative assessment, and regulation. For this reason, the agricultural sector is increasingly regarded as one of the most challenging areas of contemporary environmental policy.

Livestock farming makes the largest contribution to agricultural air pollution. The raising of cattle, pigs, and poultry is accompanied by intensive emissions of ammonia, methane, hydrogen sulfide, and other gaseous compounds.

Another important factor is the application of mineral fertilizers. Improper fertilizer application practices, excessive use of nitrogen compounds, and adverse weather conditions lead to intensive ammonia volatilization and the formation of nitrous oxide, one of the most potent greenhouse gases. In addition

to their direct impact on atmospheric air quality, these processes contribute to global climate change, further emphasizing the inseparable relationship between air quality and the Earth's climate system⁷.

An additional source of atmospheric pollution is the mechanical cultivation of soils. During ploughing, cultivation, harvesting, and the transportation of agricultural products, considerable amounts of mineral dust are released into the atmosphere. Under arid climatic conditions or where soil degradation occurs, the scale of such dust emissions may increase substantially. Dust originating from degraded arable land poses a particular hazard, as it often contains residues of agrochemicals, heavy metals, and microorganisms.

The contemporary concept of sustainable agriculture involves a gradual transition towards precision farming technologies, digital management of fertilizer application, the use of biogas facilities, improvements in organic waste management systems, and optimization of land-use patterns. Taken together, these measures can significantly reduce atmospheric emissions without compromising agricultural productivity.

The Residential and Municipal Sector and the Urban Environment

The residential and municipal sector occupies a significant place in the contemporary structure of anthropogenic emissions, with its impact being particularly pronounced in densely populated urban agglomerations. Unlike industry, where pollution is generated by a limited number of large enterprises, the residential and municipal sector comprises numerous small emission sources that collectively exert considerable technogenic pressure on atmospheric air.

The main sources of pollution include individual heating systems, boiler plants, solid fuel combustion, the use of generators, cooking, household waste burning, and construction activities. In many Central and Eastern European countries, residential heating with wood and coal remains one of the major sources of winter smog.

Uncontrolled burning of household waste poses a particular environmental hazard. The combustion of polymeric materials, rubber, paints and coatings, and other synthetic components results in the formation of dioxins, furans, polycyclic aromatic hydrocarbons, chlorinated organic compounds, and heavy metals. Most of these substances are characterized by high toxicity, resistance to biodegradation, and the ability to bioaccumulate.

In modern cities, construction activities represent an additional source of atmospheric pollution. Building demolition, earthworks, and the production and transportation of construction materials are accompanied by the generation of

⁷ Боголюбов В. М., Клименко М. О., Мокін В. Б. та ін. Моніторинг довкілля : підручник. Херсон : Грінь Д. С., 2011. 530 с.

substantial amounts of PM₁₀ and PM_{2.5}. Where dust suppression systems are not applied, particulate matter concentrations in adjacent areas may exceed recommended limits severalfold.

At the same time, urbanized areas create specific microclimatic conditions known as the urban heat island effect. Elevated air temperatures, reduced wind speeds, and high building density contribute to the accumulation of pollutants in the near-surface atmospheric layer and intensify photochemical reactions. This results in more intensive formation of ground-level ozone and secondary aerosols, particularly during the summer period.

Military Activities as an Emerging Factor of Technogenic Atmospheric Air Pollution

One of the most pressing environmental issues of the twenty-first century is the impact of armed conflicts on atmospheric air quality. Unlike conventional sources of technogenic pressure, military activities are characterized by the extreme intensity, unpredictability, and complexity of pollution processes. The destruction of industrial enterprises, energy infrastructure, fuel and lubricant storage facilities, residential buildings, and transport hubs is accompanied by the large-scale release of a wide range of toxic substances into the atmosphere.

A distinctive feature of wartime pollution is the simultaneous generation of large quantities of both primary and secondary pollutants. Fires and explosions release nitrogen oxides, sulfur oxides, carbon monoxide, fine particulate matter, black carbon, polycyclic aromatic hydrocarbons, dioxins, heavy metals, and numerous organic toxicants into the atmosphere. The destruction of buildings is accompanied by the generation of mineral dust, which may contain cement, asbestos, quartz, lead, residues of paints and coatings, and other hazardous components.

For Ukraine, this problem has become particularly acute. The full-scale war has placed unprecedented pressure on atmospheric air as a result of fires at oil depots, the destruction of industrial facilities, explosions at ammunition depots, wildfires, attacks on energy infrastructure, and the intensive use of military equipment. It is important to emphasize that the consequences of such events are not confined to the areas in which they occur. Due to atmospheric circulation, fine particulate aerosols and gaseous pollutants can be transported over hundreds or even thousands of kilometres, creating transboundary environmental risks^{8, 9}.

⁸ Скок А., Хрутьба В. Якість атмосферного повітря в Україні до і під час повномасштабного вторгнення. Міжнародний фонд відродження. Київ, 2023. С. 5–24.

⁹ Хондак І., Стищенко Т. Забруднення атмосферного повітря в умовах війни в Україні. Біологічні, хімічні та екологічні загрози під час війни : колективна монографія / за заг. ред. В. В. Поповича, В. О. Сергієнка, Н. О. Іванченко. Львів : ЛДУБЖД, 2025. С. 391–399.

An analysis of the contemporary structure of anthropogenic sources of atmospheric air pollution indicates that technogenic pressure results from the interaction of numerous economic sectors, each characterized by a specific spectrum of emissions and mechanisms of atmospheric transformation. Whereas in the second half of the twentieth century large stationary industrial enterprises played the dominant role, the current period is characterized by a transition towards a multicomponent pollution model in which diffuse sources, urbanized environments, transport, the agricultural sector, and military activities are becoming increasingly important. This transformation requires a revision of traditional approaches to atmospheric air monitoring, a transition towards integrated air quality management systems, and consideration of the interrelationships among local emission sources, the transboundary transport of pollutants, and global climate processes.

Volatile Organic Compounds (VOCs) as Precursors of Secondary Atmospheric Pollution

Among the wide range of anthropogenic atmospheric pollutants, volatile organic compounds (VOCs) occupy a special place, representing one of the largest and most diverse groups of organic substances present in the atmosphere. To date, more than 10,000 organic compounds capable of existing in the gaseous phase of the atmosphere have been identified; however, only a small proportion of them are systematically monitored within national monitoring programmes¹⁰.

A distinctive feature of VOCs is that most of them are not among the most toxic components of atmospheric air in their primary form. Their environmental hazard is primarily determined by their high photochemical reactivity and their ability to participate in complex atmospheric oxidation reactions, resulting in the formation of ground-level ozone, peroxyacetyl nitrate (PAN), secondary organic aerosols (SOAs), organic peroxides, and other oxygen-containing organic compounds¹¹.

Photochemical Processes and the Formation of Secondary Organic Aerosols

Over the past two decades, the understanding of atmospheric chemistry has changed substantially. Whereas aerosol particles were previously regarded primarily as products of the mechanical fragmentation of materials or combustion processes, contemporary research demonstrates that a significant proportion of atmospheric aerosols is formed directly within the atmosphere as a result of photochemical reactions¹².

¹⁰ Seinfeld J. H., Pandis S. N. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change. 3rd ed. Hoboken : John Wiley & Sons, 2016. 1152 p.

¹¹ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

¹² Seinfeld J. H., Pandis S. N. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change. 3rd ed. Hoboken : John Wiley & Sons, 2016. 1152 p.

In recent years, it has been established that secondary organic aerosols not only deteriorate atmospheric air quality but also actively influence the Earth's climate system. They alter the optical properties of the atmosphere, scatter and absorb solar radiation, act as cloud condensation nuclei, and modify precipitation formation processes.

Despite significant progress in the study of atmospheric chemistry, the mechanisms underlying the formation of secondary organic aerosols remain among the most complex issues in contemporary atmospheric environmental science. The quantitative contributions of anthropogenic and biogenic VOCs to SOA formation, the role of multiphase reactions within aerosol droplets, the influence of climate change on the rates of photochemical processes, and the interactions between organic aerosols and black carbon remain subjects of scientific debate. For this reason, international research programmes and initiatives such as ACTRIS, the Copernicus Atmosphere Monitoring Service (CAMS), and the Global Atmosphere Watch (GAW/WMO) place particular emphasis on improving atmospheric chemistry models and integrating ground-based and satellite observations^{13 14 15}.

Heavy Metals in Atmospheric Air: Sources, Chemical Forms, Atmospheric Transport, and Environmental Risks

Heavy metals are among the most hazardous components of technogenic atmospheric air pollution because they combine high toxicity, environmental persistence, the ability to bioaccumulate, and a virtually unlimited persistence within natural biogeochemical cycles. Unlike most gaseous pollutants, which are gradually degraded or transformed in the atmosphere, metal atoms are not subject to biochemical degradation. Only their physicochemical forms, oxidation states, solubility, and bioavailability change, largely determining their toxicological properties and environmental hazards¹⁶.

In contemporary atmospheric environmental science, the concept of potentially toxic elements (PTEs) is increasingly used as a broader term than the traditional designation "heavy metals". This is because the environmental hazard posed by a substance is determined not only by its atomic mass but primarily by its toxicity, bioavailability, ability to accumulate in living organisms, and involvement in biogeochemical processes. This group includes lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), nickel

¹³ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

¹⁴ European Environment Agency. *Air Quality Status Report 2025*. Luxembourg : Publications Office of the European Union, 2025.

¹⁵ European Commission. *Zero Pollution Action Plan: Towards Zero Pollution for Air, Water and Soil*. Brussels : European Commission, 2021.

¹⁶ European Environment Agency. *Air Quality Status Report 2025*. Luxembourg : Publications Office of the European Union, 2025.

(Ni), cobalt (Co), copper (Cu), zinc (Zn), vanadium (V), manganese (Mn), and other elements that enter the atmosphere predominantly as a result of anthropogenic activities¹⁷.

The principal sources of atmospheric heavy metal emissions remain thermal power generation, metallurgical production, non-ferrous metallurgy, the cement industry, waste incineration, mining and mineral processing, transport, and certain technological processes within the chemical industry.

One of the most important findings of contemporary atmospheric ecotoxicology has been the recognition that the greatest hazard is posed not by metals alone, but by their association with fine particulate aerosols. PM_{2.5} particles have an exceptionally large specific surface area onto which lead, cadmium, nickel, arsenic, and other elements can be adsorbed. As a result, multicomponent aerosols are formed that simultaneously combine the physical effects of fine particulate matter with the toxic effects of adsorbed substances.

2. Fine Particulate Matter (PM₁₀, PM_{2.5}) and Its Transboundary Transport

Over the past two decades, the problem of fine particulate aerosol pollution of atmospheric air has become one of the leading global environmental challenges. Whereas in the second half of the twentieth century scientific attention was focused primarily on the study of sulfur oxides, nitrogen oxides, and carbon monoxide, contemporary research increasingly emphasizes atmospheric particulate matter with small aerodynamic diameters. This is primarily because fine particulate matter, particularly PM₁₀ and PM_{2.5}, is associated with some of the most pronounced adverse effects on public health, the functioning of natural ecosystems, regional climate processes, and the economic development of countries^{18, 19}.

Fine atmospheric particulate matter is among the most compositionally complex components of atmospheric air. Unlike gaseous pollutants, solid aerosol particles are not individual chemical substances but rather multicomponent mixtures of mineral, organic, and biological constituents. Their relative proportions are determined by the nature of emission sources, meteorological conditions, atmospheric photochemical processes, and the residence time of particles in the atmosphere.

¹⁷ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

¹⁸ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

¹⁹ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

According to the recommendations of the World Health Organization and the European Environment Agency, atmospheric aerosols are classified primarily according to their aerodynamic diameter, which determines their atmospheric transport characteristics, deposition rates, and depth of penetration into the human body^{20, 21}.

The PM₁₀ category includes particles with an aerodynamic diameter of up to 10 µm. These particles are predominantly deposited in the upper respiratory tract, although their finer fractions may penetrate into the bronchi and bronchioles. PM_{2.5} particles, with an aerodynamic diameter not exceeding 2.5 µm, are capable of reaching the pulmonary alveoli, crossing the alveolar–capillary barrier, and entering the systemic circulation. For this reason, PM_{2.5} is considered one of the most hazardous environmental risk factors for human health.

In recent years, increasing attention has been devoted to the study of ultrafine particles (UFPs, PM_{2.5}), with diameters of less than 100 nm. Despite their relatively minor contribution to the total mass of atmospheric aerosols, they are characterized by an exceptionally large specific surface area and high chemical reactivity. Such particles are capable of penetrating not only into the circulatory system but also into the central nervous system via the olfactory nerve, a process that has been associated with the development of neurodegenerative diseases.

Chemical Composition of Fine Particulate Matter

Contemporary studies demonstrate that the chemical composition of PM_{2.5} and PM₁₀ varies substantially depending on the region, season, and predominant emission sources. Nevertheless, in most urbanized areas, fine particulate matter typically contains the following components: sulfates (SO₄²⁻); nitrates (NO₃⁻); ammonium (NH₄⁺); organic carbon (OC); black carbon (BC); mineral components (Si, Al, Ca, Fe); heavy metals (Pb, Cd, Hg, As, Ni, Cr); polycyclic aromatic hydrocarbons; and biological components (pollen, bacteria, and fungal spores).

The relative proportions of these components represent a distinctive “chemical fingerprint” of pollution sources. For example, elevated concentrations of black carbon and organic carbon are characteristic of transport emissions and combustion processes, whereas high concentrations of calcium, silicon, and aluminium generally indicate contributions from soil dust or construction activities. The presence of vanadium and nickel may serve as an indicator of

²⁰ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

²¹ European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

heavy fuel oil combustion, while lead, cadmium, or zinc may indicate emissions from metallurgical production or waste incineration²².

One of the most important concepts in contemporary atmospheric chemistry is the classification of aerosols into primary and secondary aerosols.

Primary aerosols are emitted directly into the atmosphere from emission sources. These include dust generated during industrial processes, transport activities, construction, mining, biomass combustion, and fossil fuel combustion. Primary aerosols may also originate from natural sources, including sea spray aerosols, dust storms, and volcanic ash.

Secondary aerosols are formed directly in the atmosphere as a result of chemical reactions involving gaseous precursors, including sulfur oxides, nitrogen oxides, ammonia, and volatile organic compounds. They often account for a substantial proportion of the PM₁₀ mass in European cities. This fact is of fundamental importance for environmental policy, since effectively reducing PM_{2.5} concentrations requires not only the reduction of direct particulate emissions but also the mitigation of emissions of gaseous precursors.

Transboundary Transport of Fine Particulate Matter: Mechanisms, Modelling, and Environmental Consequences²²

One of the most important characteristics of fine particulate matter, including PM₁₀ and PM_{2.5}, is its ability to be transported over considerable distances by atmospheric airflows. Whereas larger aerosol particles generally settle relatively close to their emission sources, fine particulate matter may remain suspended in the atmosphere for several days or even weeks, travelling hundreds or thousands of kilometres. As a result, atmospheric pollution is no longer merely a local or even national problem but acquires a transboundary dimension, requiring international coordination of environmental policies^{23, 24}.

In contemporary atmospheric science, transboundary transport is defined as the movement of gaseous and aerosol pollutants across national borders under the influence of large-scale atmospheric circulation. The intensity of this process depends on the physicochemical properties of the particles, the altitude at which they enter the atmosphere, wind speed and direction, atmospheric temperature stratification, turbulent mixing, precipitation levels, and the atmospheric residence time of aerosols.

²² Seinfeld J. H., Pandis S. N. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change. 3rd ed. Hoboken : John Wiley & Sons, 2016. 1152 p.

²³ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

²⁴ European Commission. Zero Pollution Action Plan: Towards Zero Pollution for Air, Water and Soil. Brussels : European Commission, 2021.

PM_{2.5} particles are characterized by considerably greater mobility than PM₁₀ particles. Due to their small aerodynamic diameter, they have a low gravitational settling velocity, which enables them to remain suspended in the atmosphere for extended periods. Consequently, fine particulate matter can be transported over long distances even in the absence of major emission sources in regions where elevated concentrations are recorded. This characteristic explains why atmospheric air quality in one country may depend to a considerable extent on emission levels in neighbouring countries.

The transport of atmospheric aerosols results from the simultaneous action of several physical processes. Advection, turbulent diffusion, convective mixing, and gravitational settling play the most important roles.

Meteorological Factors Influencing Transboundary Transport

The efficiency of fine particulate matter transport is largely determined by meteorological conditions. The most important factors include wind speed and direction, the height of the planetary boundary layer, air temperature, relative humidity, the intensity of solar radiation, and precipitation.

The height of the atmospheric boundary layer plays a particularly important role. During the daytime, when the boundary layer becomes deeper, pollutants are actively mixed and dispersed. At night or under anticyclonic conditions, the boundary layer becomes shallower, resulting in the accumulation of aerosols near the Earth's surface and a marked deterioration in air quality.

Climate change is another important factor. According to the IPCC, rising temperatures, the increasing frequency of prolonged droughts, and large-scale wildfires can substantially alter the spatial distribution of atmospheric aerosols. Furthermore, climate change affects the rates of photochemical reactions responsible for the formation of secondary PM_{2.5}²⁵.

For Ukraine, the issue of transboundary transport is of particular importance due to the country's geographical location in the centre of Europe and the presence of major industrial regions in neighbouring countries. Scientific studies conducted by the Ukrainian Hydrometeorological Institute of the State Emergency Service of Ukraine and the National Academy of Sciences of Ukraine, the Borys Sreznevskyi Central Geophysical Observatory, as well as within the framework of cooperation with the EMEP programme, confirm that fine particulate matter concentrations in Ukraine are influenced by both domestic emission sources and atmospheric transport from the territories of other countries²⁶.

²⁵ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

²⁶ Огляд стану забруднення навколишнього природного середовища на території України за даними мережі моніторингу гідрометеорологічних організацій у 2024 році.

URL: <http://cgo-sreznevskyi.kyiv.ua/uk/diialnist/khimichne-zabrudnennia/8-materialy-na-glavnoj/171-stan-zabrudnennya-prirodnogo-seredovishcha-na-teritoriji-ukrajini>.

Since 2022, the nature of transboundary transport has become considerably more complex due to large-scale fires, the destruction of industrial enterprises, fuel and lubricant storage facilities, and energy infrastructure. Satellite observations conducted by Copernicus and NASA have repeatedly recorded the transport of aerosol plumes generated by fires beyond the borders of Ukraine. This highlights the need to integrate the national monitoring system with pan-European observation networks and to develop joint programmes for modelling atmospheric processes.

3. Environmental Monitoring of Atmospheric Air

Effective air quality management is impossible without a modern environmental monitoring system capable of providing reliable, timely, and spatially representative information on the state of the atmospheric environment. Over recent decades, the concept of monitoring has undergone substantial transformation. Whereas in the past its primary objective was the periodic measurement of concentrations of individual pollutants, the contemporary approach is based on the integration of continuous measurements, mathematical modelling, Earth remote sensing, geoinformation technologies, and tools for analysing large datasets^{27, 28}.

According to the definition provided by the United Nations Environment Programme (UNEP), environmental monitoring of atmospheric air is a comprehensive system of regular observations, analysis, assessment, and forecasting of atmospheric conditions aimed at the timely identification of adverse trends, assessment of environmental risks, and provision of information to support environmental policy. Thus, contemporary monitoring performs not only an informational function but also predictive and management functions.

In accordance with the requirements of Directive 2008/50/EC of the European Parliament and of the Council on ambient air quality and cleaner air for Europe, monitoring systems should ensure the measurement of concentrations of major atmospheric pollutants, including PM_{2.5}, PM₁₀, nitrogen dioxide (NO₂), sulfur dioxide (SO₂), ground-level ozone (O₃), carbon monoxide (CO), benzene (C₆H₆), lead, and other toxic components. Particular attention is paid to the spatial representativeness of observations, the standardization of measurement methods, and ensuring the comparability of results across different countries²⁹.

²⁷ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

²⁸ European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

²⁹ Directive (EU) 2024/2881 of the European Parliament and of the Council of 23 October 2024 on ambient air quality and cleaner air for Europe (recast). *Official Journal of the European Union*. 2024.

International Atmospheric Air Monitoring Systems

Several global observation systems currently operate worldwide, providing the information base required for assessing atmospheric air quality.

The World Meteorological Organization coordinates the Global Atmosphere Watch (GAW) programme, which integrates hundreds of ground-based observation stations across different regions of the world. The programme provides long-term measurements of greenhouse gas concentrations, aerosols, ozone, and other atmospheric constituents³⁰.

Within the European Union, the Copernicus Atmosphere Monitoring Service (CAMS) plays a central role, integrating data from satellite observations, ground-based monitoring, and numerical modelling. CAMS provides daily air quality forecasts, assesses the transboundary transport of pollutants, and analyses the impacts of large-scale natural and anthropogenic events³¹.

The EMEP programme also plays an important role, providing the scientific basis for the implementation of the Convention on Long-range Transboundary Air Pollution (CLRTAP). Its primary objectives are to assess the flows of atmospheric pollutants between countries, analyse the effectiveness of environmental protection measures, and support environmental policy development in European countries.

Ukrainian Atmospheric Air Monitoring System

In Ukraine, state monitoring of atmospheric air is carried out in accordance with the Law of Ukraine “On Atmospheric Air Protection,” the Law of Ukraine “On Environmental Protection,” and the Procedure for State Monitoring in the Field of Atmospheric Air Protection. An important role in the development of the national monitoring system is played by the Ukrainian Hydrometeorological Center, the Ukrainian Hydrometeorological Institute of the State Emergency Service of Ukraine and the National Academy of Sciences of Ukraine, the Borys Sreznevskiy Central Geophysical Observatory, as well as regional monitoring laboratories^{32, 33, 34, 35}.

³⁰ World Meteorological Organization. State of Global Air Quality and Climate. Geneva : WMO, 2024.

³¹ European Centre for Medium-Range Weather Forecasts. Copernicus Atmosphere Monitoring Service (CAMS): Global and Regional Air Quality Products. Reading: ECMWF, 2024.

³² Про Основні засади (стратегію) державної екологічної політики України на період до 2030 року : Закон України від 28 лютого 2019 року № 2697-VIII. URL: <https://zakon.rada.gov.ua/go/2697-19>.

³³ Огляд стану забруднення навколишнього природного середовища на території України за даними мережі моніторингу гідрометеорологічних організацій у 2024 році. URL: <http://cgo-sreznevskiy.kyiv.ua/uk/diialnist/khimichne-zabrudnennia/8-materialy-na-glavnoj/171-stan-zabrudnennya-prirodnogo-seredovishcha-na-teritoriji-ukrajini>.

³⁴ Про Основні засади (стратегію) державної екологічної політики України на період до 2030 року : Закон України від 28 лютого 2019 року № 2697-VIII. URL: <https://zakon.rada.gov.ua/go/2697-19>.

³⁵ Про охорону атмосферного повітря : Закон України від 16 жовтня 1992 р. № 2707-XII. *Відомості Верховної Ради України*. 1992. № 50. С. 678.

In recent years, automated observation networks, municipal air quality monitoring systems, and community-based initiatives using low-cost sensors have been actively developing in Ukraine. At the same time, one of the major challenges remains the harmonization of the national monitoring system with European standards, the expansion of the network of automatic monitoring stations, the provision of open access to environmental information, and the integration of Ukrainian data into international information platforms.

Environmental monitoring of atmospheric air has evolved from a system of local observations into a complex digital network that combines automated measurements, satellite data, mathematical modelling, and geoinformation technologies. The integration of heterogeneous data sources provides a contemporary framework for air quality assessment and creates the necessary conditions for a transition from responding to existing pollution to forecasting and preventing it.

Automated Air Quality Monitoring Systems and Sensor Networks

The rapid development of information technologies, the automation of measurement processes, and the digitalization of environmental information have contributed to the emergence of a new generation of atmospheric air monitoring systems. Whereas traditional environmental monitoring methods involved periodic sampling followed by laboratory analysis, modern automated systems provide continuous real-time measurements of pollutant concentrations, rapid data transmission, and their integration into unified information and analytical platforms^{36, 37}.

Modern automated environmental monitoring stations are sophisticated technological systems equipped with gas analysers, particulate matter monitors, meteorological sensors, automatic calibration systems, and telemetry equipment. The principal advantage of such stations is their ability to continuously monitor changes in atmospheric pollutant concentrations, making it possible to detect episodes of high pollution in a timely manner, analyse their causes, and forecast the subsequent development of air quality conditions.

In European Union countries, automated monitoring networks operate in accordance with the requirements of Directive 2008/50/EC and the harmonized EN standards. This ensures the comparability of results across countries, high measurement accuracy, and the possibility of integrating data into the European Environment Information and Observation Network (Eionet)³⁸.

³⁶ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

³⁷ European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

³⁸ Directive (EU) 2008/50 of the European Parliament and of the Council of 23 October 2008 on ambient air quality and cleaner air for Europe (recast). *Official Journal of the European Union*. 2008.

Reference Methods for Measuring Atmospheric Air Quality

One of the fundamental requirements of contemporary environmental monitoring is the use of reference measurement methods that ensure high accuracy, reproducibility, and metrological traceability of results.

One of the most important trends of the past decade has been the widespread adoption of low-cost sensors, which have substantially expanded the capabilities of environmental monitoring. The cost of such devices is many times lower than that of professional reference monitoring stations, making it possible to establish dense observation networks within cities, industrial zones, and transport corridors.

Sensor networks are primarily based on laser optical sensors that determine aerosol particle concentrations by measuring the intensity of light scattering. They may additionally be equipped with electrochemical sensors for measuring NO₂, SO₂, CO, and O₃, as well as sensors for temperature, humidity, atmospheric pressure, and wind speed.

Alongside the development of stationary networks, mobile monitoring systems are becoming increasingly important. Measurement equipment is installed on cars, public transport vehicles, bicycles, and even personal devices, making it possible to obtain highly detailed spatial maps of air pollution.

A particularly promising area is the use of unmanned aerial vehicles (UAVs). Equipped with compact gas analysers and particulate matter monitors, drones can perform measurements at different altitudes and investigate areas surrounding industrial facilities, waste disposal sites, fire-affected areas, or accident sites. For Ukraine, this approach is becoming particularly relevant due to the need for rapid environmental assessment of territories affected by military activities.

In Ukraine, the modernization of atmospheric air monitoring systems is gradually gaining momentum. Alongside the state observation network, municipal automated monitoring stations, regional information systems, and community-based sensor networks are being actively developed. Local authorities, universities, research institutions, and non-governmental organizations make a significant contribution to the implementation of modern monitoring technologies.

At the same time, a number of challenges remain, including the insufficient density of automatic monitoring stations, limited funding, varying levels of metrological support, and the need to harmonize measurement methodologies with European standards. The further development of Ukraine's monitoring system should involve the integration of state, municipal, and community-based networks, the establishment of a unified digital platform for environmental data, and the expanded use of modern information technologies.

Automated atmospheric air monitoring systems form the foundation of contemporary environmental management. The integration of reference monitoring stations, sensor networks, Internet of Things (IoT) technologies, mobile platforms, and unmanned aerial vehicles creates a multi-level observation system capable of ensuring the rapid detection of pollution, forecasting its development, and providing information support for environmental management decisions. For Ukraine, the modernization of such systems is an important prerequisite for alignment with European standards, improving the effectiveness of environmental monitoring and control, and supporting post-war environmental recovery³⁹.

Conceptual Framework for Remote Monitoring of Atmospheric Air

The rapid development of space technologies over the past two decades has substantially transformed approaches to atmospheric air quality assessment. Whereas traditional monitoring systems are primarily based on networks of stationary ground-based observation stations, modern satellite systems make it possible to obtain information on the spatial distribution of atmospheric pollutants on an almost global scale. The integration of ground-based measurements with remote sensing data provides a fundamentally new level of environmental monitoring, which is particularly important for assessing the transboundary transport of pollutants and analysing large-scale wildfires, dust storms, volcanic eruptions, and other major natural and anthropogenic events^{40, 41}.

The principal advantage of satellite monitoring is its ability to simultaneously observe vast areas, including remote and difficult-to-access regions, marine areas, and border zones. This makes it possible to obtain a comprehensive picture of atmospheric conditions that cannot be provided solely by ground-based measurements. Furthermore, satellite observations are particularly important for countries with insufficiently developed networks of automated monitoring stations, as they can partially compensate for the lack of ground-based data.

At the same time, the contemporary scientific community emphasizes that satellite data cannot fully replace ground-based measurements. Satellite observations characterize the integrated state of the atmospheric column or individual atmospheric layers and therefore require validation against ground-based monitoring data. For this reason, an integrated approach combining

³⁹ Моніторинг забруднення повітря на території України. URL: <https://www.saveecobot.com/maps/kharkiv>).

⁴⁰ European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

⁴¹ World Meteorological Organization. State of Global Air Quality and Climate. Geneva : WMO, 2024.

remote sensing data, automated monitoring stations, mathematical modelling, and geoinformation analysis is considered the most effective.

Integration of Satellite and Ground-Based Observations

One of the key trends in contemporary atmospheric science is the integration of satellite information with data obtained from automated ground-based monitoring stations. This approach makes it possible to compensate for the limitations inherent in each method when used separately. Ground-based measurements provide high accuracy in determining pollutant concentrations in the near-surface atmospheric layer, whereas satellite observations enable the analysis of their spatial distribution on regional and global scales.

To integrate heterogeneous sources of information, geographic information systems (GIS), spatial interpolation methods, data assimilation techniques, and machine-learning algorithms are being increasingly applied. These approaches make it possible to generate highly detailed maps of $PM_{2.5}$, PM_{10} , and other pollutant concentrations even in regions with an insufficient number of ground-based monitoring stations.

Satellite Monitoring under Wartime Conditions

For Ukraine, remote sensing of the atmosphere has acquired particular importance since 2022. Data from Sentinel-5P, MODIS, and VIIRS satellite instruments are extensively used to detect large-scale fires, assess the dispersion of smoke plumes, and analyse the consequences of damage to industrial facilities, oil depots, and energy infrastructure. The integration of satellite data with atmospheric transport models makes it possible to assess potential pollutant dispersion pathways and forecast their impacts on the territory of Ukraine and neighbouring countries.

Furthermore, remote sensing is an important tool for post-war environmental recovery. Satellite observations can be used to assess long-term changes in atmospheric air quality, monitor the effectiveness of environmental protection measures, and support the development of environmental policy at the national level.

The most advanced monitoring systems are gradually evolving from separate information platforms into integrated environmental ecosystems.

Their structure includes automated monitoring stations; networks of low-cost sensors; unmanned aerial vehicles (UAVs); Copernicus and NASA satellite systems; atmospheric mathematical models; geographic information systems (GIS); artificial intelligence algorithms; digital twins of territories; and cloud-based data processing services.

It is precisely the integration of all these components that enables the transition from passive observation to proactive environmental management, in which the system not only detects pollution but also forecasts its development,

proposes potential response scenarios, and assesses the effectiveness of management decisions.

For Ukraine, the implementation of artificial intelligence, Big Data, and digital twin technologies represents one of the most promising directions for modernizing the national environmental monitoring system. This is particularly relevant in the context of post-war recovery, when there is a need to rapidly assess atmospheric air quality, monitor the environmental consequences of damage to industrial facilities, model the dispersion of pollutants, and provide a scientific basis for environmental protection measures.

Promising directions for further development include the integration of Ukrainian monitoring systems with the Copernicus and CAMS platforms; the application of artificial intelligence algorithms for forecasting PM_{2.5} concentrations; the development of digital twins for Ukraine's largest cities; the establishment of a national open environmental data platform; and the use of unmanned aerial vehicles for rapid environmental monitoring^{42, 43}.

Global Trends in Atmospheric Air Pollution and the Development of Monitoring Systems

According to research conducted by IQAir, an international company that annually assesses atmospheric air quality using data from a global network of monitoring stations, air pollution remains one of the most serious environmental threats of the modern era. Analysis of data from more than 30,000 monitoring stations located in thousands of settlements across different countries indicates systematic exceedances of the guideline levels for fine particulate matter (PM_{2.5}) recommended by the World Health Organization (WHO).

The analysis presented in the 2025 World Air Quality Report confirms the persistence of adverse trends. The report was based on data from monitoring stations located in 9,446 cities across 143 countries, territories, and regions worldwide, providing the broadest geographical coverage achieved over the entire period of such assessments. Compared with the previous year, the monitoring network was expanded to include additional countries and territories, thereby improving the representativeness of the global assessment of atmospheric air quality.

The results demonstrate that only 14% of cities worldwide met the WHO guideline for annual mean PM_{2.5} concentrations, while 91% of countries and territories exceeded the recommended level. These findings highlight the global

⁴² Моніторинг забруднення повітря на території України. URL: <https://www.saveecobot.com/maps/kharkiv>).

⁴³ Огляд стану забруднення навколишнього природного середовища на території України за даними мережі моніторингу гідрометеорологічних організацій у 2024 році. URL: <http://cgo-sreznvskiy.kyiv.ua/uk/diialnist/khimichne-zabrudnennia/8-materialy-na-glavnoj/171-stan-zabrudnennya-prirodnoho-seredovishcha-na-teritoriji-ukrajini>.

nature of the problem and the need for further improvement of environmental policies at the international level⁴⁴.

The countries with the highest levels of atmospheric air pollution include Pakistan, Bangladesh, Tajikistan, Chad, and the Democratic Republic of the Congo, where annual mean PM_{2.5} concentrations exceed WHO recommendations severalfold. Loni, India, was identified as the world's most polluted city, with an annual mean PM_{2.5} concentration of 112.5 µg/m³, more than twenty times higher than the recommended level. In contrast, the lowest concentration of fine particulate matter was recorded in Nieuwoudtville, South Africa, where the annual mean PM_{2.5} concentration was only 1.0 µg/m³.

A distinctive feature of 2025 was that all twenty-five of the world's most polluted cities were located in India, Pakistan, and China, confirming the exceptionally high concentration of environmental risks in South and East Asia. At the same time, for the second consecutive year, no city in East Asia met the WHO guideline for annual mean PM_{2.5} concentrations.

Regional analysis revealed considerable variation in air quality trends. In Europe, annual mean PM_{2.5} concentrations increased in 23 countries in 2025, while decreases were recorded in 18 countries. Seasonal deterioration in air quality was significantly influenced by residential wood burning for heating, the transboundary transport of smoke from large-scale wildfires in Canada, and the transport of dust masses from the Sahara Desert.

Countries in Latin America and the Caribbean generally demonstrated a positive trend, with annual mean PM_{2.5} concentrations decreasing in most cities. Oceania remained one of the cleanest regions in the world, with more than 60% of cities meeting WHO recommendations, although certain climatic events caused short-term seasonal deterioration in air quality.

One of the major factors contributing to the deterioration of atmospheric air quality in 2025 was the occurrence of large-scale wildfires, the increasing intensity of which is closely associated with climate change. Substantial emissions from biomass burning resulted in the transboundary transport of pollutants and the deterioration of atmospheric air quality across extensive areas of North America and Europe. In particular, smoke from wildfires in Canada adversely affected air quality not only within Canada itself but also in the United States and several European countries.

The report also places particular emphasis on the development of global atmospheric air monitoring systems. The discontinuation in 2025 of the air quality monitoring programme supported by the U.S. Department of State

⁴⁴ Звіт про якість повітря IQAir у світі за 2025 рік. URL: <https://www.iqair.com/newsroom/waqr-2025-pr>.

through its network of embassies and consulates resulted in a reduction in the availability of open environmental data in many countries. According to estimates by the Centre for Research on Energy and Clean Air (CREA), monitoring capacity was weakened in 44 countries, while six countries were temporarily left without functioning air quality monitoring networks.

These trends confirm that effective atmospheric air quality management is impossible without the development of a modern environmental monitoring system. Particular importance is attached to the implementation of automated observation networks, the use of affordable sensor technologies, digital platforms for processing environmental information, geographic information systems, and artificial intelligence tools. The expansion of monitoring networks enables the timely acquisition of reliable information on atmospheric air quality, facilitates the early identification of environmental risks, enhances the effectiveness of environmental policy, and provides a scientific basis for informed management decisions aimed at reducing the adverse impacts of atmospheric pollution on public health and the environment⁴⁵.

Atmospheric Air Pollution in Ukraine: Current Challenges and Mitigation Strategies

The main anthropogenic sources of atmospheric air pollution in Ukraine are stationary and mobile sources. Stationary sources include industrial enterprises, thermal power plants, boiler facilities, production complexes, and other facilities whose activities are associated with substantial volumes of emissions. Mobile sources are represented primarily by road transport, which in many cities accounts for a major share of atmospheric air pollution and significantly affects the quality of life of the population.

Statistical data indicate that between 2020 and 2025, substantial changes occurred in both the structure and total volume of emissions of pollutants and greenhouse gases in Ukraine. The analysis demonstrates the clear impact of wartime events, the structural transformation of the economy, and changes in the intensity of industrial activity⁴⁶.

In 2020, the total volume of emissions amounted to 3,675.3 thousand tonnes, representing one of the highest values recorded during the study period. In 2021, emissions from stationary sources decreased to 2,239.8 thousand tonnes, representing a reduction of approximately 39.1% compared with 2020 (3,675.3 thousand tonnes). This indicates that industrial pressure had already begun to decline before the sharp decrease in economic activity observed in 2022.

⁴⁵ Звіт про якість повітря IQAir у світі за 2025 рік. URL: <https://www.iqair.com/newsroom/waqr-2025-pr>.

⁴⁶ Державна служба статистики України. Викиди забруднюючих речовин і парникових газів у атмосферне повітря. URL: <https://www.ukrstat.gov.ua/>.

The most substantial changes occurred in 2022. Emissions from stationary sources decreased to 1,190.9 thousand tonnes, representing a reduction of almost half (–46.8%) compared with 2021. This decline can be attributed to the full-scale war, the loss of control over parts of major industrial regions, the destruction of production infrastructure, reductions in metallurgical and energy production, and decreased transport activity.

In 2023, emissions increased slightly to 1,225.9 thousand tonnes, which may be associated with the gradual adaptation of the economy to wartime conditions and the partial resumption of operations at certain enterprises.

In 2024, emissions decreased again to 997.8 thousand tonnes, after which they remained virtually stable at 998.4 thousand tonnes in 2025. The minimal difference between the 2024 and 2025 values indicates the emergence of a new, relatively stable level of anthropogenic pressure.

According to [1], the total volume of emissions from all sources amounted to 3,677.7 thousand tonnes in 2021. In 2022, it decreased to 2,990.6 thousand tonnes, followed by an increase to 3,053.1 thousand tonnes in 2023. In 2024, the indicator declined again to 2,845.5 thousand tonnes, before increasing slightly to 2,876.5 thousand tonnes in 2025.

Overall, over the five-year period, the total volume of emissions decreased by approximately 21.8%, indicating a substantial reduction in anthropogenic pressure on atmospheric air.

Specific emissions from stationary pollution sources characterize the intensity of environmental pressure per unit area of the country.

In 2021, this indicator amounted to 3,884.4 kg/km², whereas in 2022 it decreased to 2,065.4 kg/km², representing a reduction of almost 46.8%. In 2023, a slight increase to 2,126.1 kg/km² was recorded, followed by a decline to 1,730.5 kg/km² in 2024. The indicator remained virtually unchanged in 2025, amounting to 1,731.6 kg/km² (Table 2).

A similar trend can be observed in total specific emissions per unit area. While in 2021 they amounted to 6,378.2 kg/km², in 2022 they decreased to 5,186.5 kg/km². In 2023, a slight increase to 5,294.8 kg/km² was recorded; however, in 2024 the indicator reached its lowest value during the study period, at 4,934.9 kg/km². In 2025, only a minor increase to 4,988.6 kg/km² was observed (Table 2).

The results indicate that a new trend in atmospheric pollution dynamics emerged in Ukraine during 2020–2025. Following the sharp reduction in emissions in 2022, the indicators gradually stabilized at a lower level; however, this should not be interpreted as evidence of a substantial improvement in the environmental situation. The primary reason for the decline in emissions was not the widespread implementation of environmentally friendly technologies

or digital monitoring systems, but rather the reduction in industrial production, the destruction of production facilities, and structural changes in the economy resulting from the war.

At the same time, the relative stabilization of indicators in 2024–2025 creates preconditions for the transition towards a new model of environmental management. In the context of post-war recovery, the implementation of digital atmospheric air monitoring systems, automated observation networks, geographic information technologies, Earth remote sensing, and artificial intelligence tools is becoming particularly important. Their application will not only improve the timeliness and efficiency of atmospheric air quality monitoring but will also facilitate scientifically informed management decisions in the field of environmental protection and support the alignment of Ukraine’s environmental policy with European Union standards.

Prior to the onset of the full-scale war, the main hotspots of atmospheric air pollution in Ukraine were major industrial centres, including Mariupol, Kryvyi Rih, Zaporizhzhia, and Dnipro, where the predominant share of emissions originated from metallurgical, energy, and chemical industries. In Kyiv, the capital of Ukraine, road transport remained the principal source of atmospheric air pollution, contributing to high concentrations of nitrogen oxides, carbon monoxide, and particulate matter⁴⁷.

Table 2

Dynamics of Pollutant and Greenhouse Gas Emissions in Ukraine⁴⁸

Baseline Statistical Indicator	2021	2022	2023	2024	2025
Amount of pollutant and greenhouse gas emissions per unit area (stationary sources of pollution, kg/km ²)	3884.4	2065.4	2126.1	1730.5	1731.6
Amount of pollutant and greenhouse gas emissions per unit area (total, kg/km ²)	6378.2	5186.5	5294.8	4934.9	4988.6

The full-scale military aggression has caused substantial changes in the territorial distribution of industrial production. A considerable number of enterprises have been relocated to safer regions of the country; however, assessing their spatial distribution and the scale of their environmental impact is complicated by restricted access to certain state registers. As a result, environmental pressure is gradually shifting towards areas that were previously characterized by relatively favourable atmospheric air quality. These regions

⁴⁷ Хондак І., Стищенко Т. Забруднення атмосферного повітря в умовах війни в Україні. Біологічні, хімічні та екологічні загрози під час війни : колективна монографія / за заг. ред. В. В. Поповича, В. О. Сергієнка, Н. О. Іванченко. Львів : ЛДУБЖД, 2025. С. 391–399.

⁴⁸ Державна служба статистики України. Викиди забруднюючих речовин і парникових газів у атмосферне повітря. URL: <https://www.ukrstat.gov.ua/>.

include Ternopil, Lviv, and Volyn oblasts, where increasing industrial activity is accompanied by a growing potential risk of atmospheric pollution.

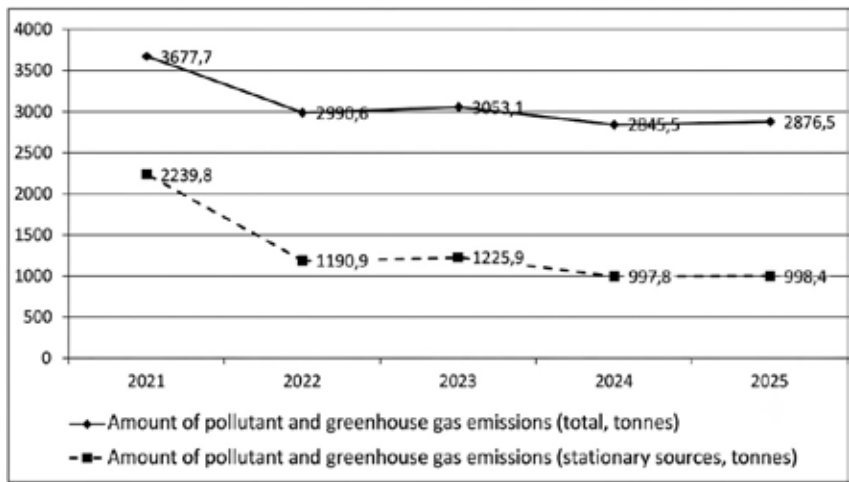


Fig. 1. Dynamics of pollutant and greenhouse gas emissions in Ukraine

Despite the challenging socio-economic and security conditions, atmospheric air pollution remains one of the most pressing environmental problems in Ukraine. Addressing this issue requires the implementation of a comprehensive set of organizational, technical, and management measures. For stationary pollution sources, priority measures include the modernization of technological processes, the introduction of highly efficient gas-cleaning systems, automated emission monitoring systems and digital environmental monitoring platforms, as well as improvements in the reporting system for pollutant emissions.

Reducing the adverse impact of mobile pollution sources can be achieved through the development of environmentally sustainable transport, an increase in the share of electric vehicles and low-emission vehicles, improvements in transport infrastructure, and incentives for the use of alternative fuels.

Environmental awareness among the population also plays an important role in ensuring environmental safety. Although individuals cannot substantially influence the scale of industrial emissions, they can monitor air quality in residential and occupational environments, use modern air purification technologies, and comply with safety requirements for the operation of autonomous energy supply systems. This issue has become particularly relevant due to the widespread use of generators, battery storage systems, and inverters,

the improper operation of which may lead to the accumulation of hazardous concentrations of combustion products and pose a threat to human health⁴⁹.

4. Atmospheric Pollution and Climate Change

For a long time, the problems of atmospheric air pollution and global climate change were regarded as two relatively independent areas of environmental research. Atmospheric pollution was traditionally associated primarily with local or regional impacts on public health, ecosystem functioning, and the state of the natural environment, whereas climate change was interpreted as a global process driven by the accumulation of greenhouse gases in the atmosphere. However, the findings of fundamental research conducted over recent decades have convincingly demonstrated that these processes are interconnected components of the Earth's integrated climate-atmosphere system^{50, 51}.

The development of the contemporary scientific concept of the relationship between atmospheric air quality and climate has been made possible by advances in atmospheric physics, atmospheric chemistry, climate modelling, and satellite monitoring. The reports of the Intergovernmental Panel on Climate Change (IPCC) have played a particularly important role, demonstrating that numerous atmospheric pollutants simultaneously affect both air quality and the energy balance of the climate system. This applies primarily to black carbon, tropospheric ozone, methane, and secondary aerosols, which contribute to radiative forcing and alter the properties of the atmosphere.

Contemporary environmental science considers the atmosphere as a single dynamic system in which physical, chemical, and biogeochemical processes are in constant interaction. Pollutants released into the atmosphere as a result of human activities not only deteriorate air quality but also alter atmospheric circulation patterns, cloud formation processes, the albedo of the Earth's surface, the intensity of solar radiation, and the global water cycle. At the same time, climate change affects the rates of photochemical reactions, the frequency of extreme weather events, the scale of wildfires, dust storms, and other processes that determine atmospheric pollutant concentrations. Thus, a complex bidirectional mechanism of interaction between atmospheric pollution and climate change is formed.

Contemporary International Scientific Framework

In the twenty-first century, international organizations have increasingly adopted an integrated approach according to which policies aimed at improving

⁴⁹ Новіков М. А., Хондак І. І. Проблема забруднення атмосферного повітря в Україні. *Радіоелектроніка та молодь у XXI столітті* : матеріали 29-го Міжнар. молодіж. форуму, 16–19 квітня 2025 р. Харків : ХНУРЕ, 2025. Т. 2. С. 132–134.

⁵⁰ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

⁵¹ World Meteorological Organization. *State of Global Air Quality and Climate*. Geneva : WMO, 2024.

atmospheric air quality and mitigating climate change should be regarded as complementary components of sustainable development.

The World Health Organization emphasizes that reducing emissions of fine particulate matter, nitrogen oxides, black carbon, and other atmospheric pollutants simultaneously contributes to improvements in public health and reductions in climate-related pressures. According to WHO estimates, the implementation of integrated clean-air policies has the potential to substantially reduce premature mortality associated with atmospheric pollution while simultaneously contributing to the fulfilment of international climate commitments.

Documents issued by the United Nations Environment Programme (UNEP) and the World Meteorological Organization (WMO) emphasize that combating atmospheric air pollution is one of the most effective approaches to simultaneously achieving the environmental, social, and economic objectives of sustainable development. Particular importance is attached to reducing emissions of short-lived climate pollutants (SLCPs), among which black carbon, methane, and tropospheric ozone play a leading role^{52, 53}.

An important milestone was the publication of the IPCC Sixth Assessment Report (AR6), which confirmed that anthropogenic influence is the dominant driver of contemporary global warming. The report emphasizes that effectively limiting the increase in global mean temperature is impossible without comprehensive reductions in both greenhouse gas emissions and a range of atmospheric pollutants that directly affect the Earth's radiative balance.

The Ukrainian scientific community also devotes considerable attention to the interaction between atmospheric pollution and climate change. Ukrainian research institutions, including the Ukrainian Hydrometeorological Institute of the State Emergency Service of Ukraine and the National Academy of Sciences of Ukraine, the Borys Sreznevskyi Central Geophysical Observatory, and specialized universities, are actively developing research on atmospheric pollution assessment, climate modelling, the analysis of extreme weather events, and climate change adaptation. These studies have become particularly relevant under wartime conditions, which have substantially altered the structure of atmospheric pollution sources and created new challenges for environmental monitoring.

One of the most important trends in contemporary environmental policy is the transition towards integrated atmospheric environmental management. This approach implies that decisions concerning emission reductions, transport

⁵² World Meteorological Organization. State of Global Air Quality and Climate. Geneva : WMO, 2024.

⁵³ United Nations Environment Programme. Global Environment Outlook 6: Healthy Planet, Healthy People. Cambridge : Cambridge University Press, 2019. 708 p.

development, energy systems, industry, land use, and urban planning should simultaneously take into account their impacts on both air quality and the climate system.

The practical implementation of this concept is reflected in the European Green Deal, which aims to achieve climate neutrality by 2050, promote clean energy, reduce greenhouse gas emissions, modernize the transport system, and enhance the environmental resilience of cities. For Ukraine, integration into European environmental policy provides opportunities to modernize the atmospheric air monitoring system, develop a low-carbon economy, and implement contemporary nature-based solutions (NbS)⁵⁴.

An analysis of contemporary international and Ukrainian research indicates that atmospheric air pollution and global climate change should be considered interconnected processes within a single natural–anthropogenic system. Atmospheric pollutants can influence climatic conditions by altering the radiative balance, cloud formation, and biogeochemical cycles, while climate change modifies the conditions governing the formation, transformation, and dispersion of pollutants. Therefore, effective environmental policy in the twenty-first century should be based on an integrated approach combining measures to improve air quality, mitigate climate change, and adapt society to emerging environmental challenges.

Climate-Forming Role of Atmospheric Pollutants

The Earth's atmosphere is a complex physicochemical system in which energy transfer, substance transformation, and interactions among gaseous components, aerosols, clouds, and the underlying surface occur continuously. The release of anthropogenic pollutants into the atmosphere substantially alters these processes, affecting the radiative balance, atmospheric circulation, cloud formation, and the global water cycle. For this reason, contemporary climatology considers atmospheric pollutants not only as factors contributing to the deterioration of air quality but also as major drivers of transformations within the Earth's climate system⁵⁵.

The key mechanism underlying this influence is radiative forcing, which refers to a change in the balance between the energy received from the Sun and the energy emitted by the Earth back into space. If a particular substance increases the retention of thermal radiation in the atmosphere, it produces positive radiative forcing and contributes to warming. Conversely, if it reflects or scatters incoming solar radiation, negative radiative forcing occurs, producing a cooling effect. At the same time, the overall climatic effect is determined by

⁵⁴ European Commission. The European Green Deal. Brussels : European Commission, 2019.

⁵⁵ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

complex interactions among different groups of atmospheric constituents, their concentrations, atmospheric lifetimes, and spatial distribution patterns.

According to current IPCC estimates, anthropogenic activities have already caused an increase in global mean temperature of approximately 1.1°C compared with the pre-industrial period (1850–1900), with rising greenhouse gas concentrations being the primary driver of this process. However, short-lived climate pollutants (SLCPs), aerosols, and products of photochemical reactions also play a significant role in shaping the contemporary climate.

Greenhouse Gases as the Main Driver of Contemporary Global Warming

Greenhouse gases constitute the principal group of atmospheric components determining the current pattern of climate change. Their ability to absorb longwave infrared radiation emitted by the Earth's surface produces the natural greenhouse effect, without which the Earth's average temperature would be approximately 33°C lower. However, the sharp increase in the concentrations of these gases as a result of anthropogenic activities has disrupted the natural energy balance of the atmosphere.

Carbon dioxide (CO₂) makes the largest contribution to contemporary global warming. Its atmospheric concentration has increased from approximately 280 ppm during the pre-industrial period to more than 420 ppm in the twenty-first century. The main sources include fossil fuel combustion, cement production, land-use change, and deforestation. A distinctive feature of CO₂ is its long persistence in the atmosphere, with its climatic influence extending from decades to centuries.

Methane (CH₄) is the second most important greenhouse gas. Although its atmospheric concentration is substantially lower than that of CO₂, methane has a considerably greater global warming potential over the first decades following its emission. An important characteristic of methane is its involvement in the photochemical formation of tropospheric ozone, which enhances its indirect climatic effect.

Nitrous oxide (N₂O) is another important greenhouse gas, with its main sources including the application of nitrogen fertilizers, agricultural production, and certain industrial processes.

Fluorinated gases (HFCs, PFCs, SF₆, and others) constitute a separate group and are used in refrigeration equipment, the electrical industry, and the manufacture of specialized equipment. Their atmospheric concentrations remain relatively low; however, the global warming potential of some compounds within this group is thousands of times greater than that of carbon dioxide.

In addition to conventional greenhouse gases, increasing attention is being paid to short-lived climate pollutants, which remain in the atmosphere for periods ranging from several days to several decades but exert significant

effects on both the climate system and public health. This group includes black carbon, tropospheric ozone, methane, and certain organic aerosols.

A distinctive feature of these substances is that reducing their emissions can produce rapid benefits both for improving atmospheric air quality and for slowing the rate of global warming. For this reason, international organizations regard the control of SLCP emissions as one of the most effective approaches in contemporary climate policy⁵⁶.

Interactions Among Different Groups of Atmospheric Pollutants

The climatic effects of individual atmospheric pollutants cannot be considered in isolation. Under real atmospheric conditions, pollutants interact with one another, participate in photochemical reactions, modify aerosol properties, and influence cloud formation processes.

It is precisely the multicomponent nature of atmospheric processes that necessitates a comprehensive approach to assessing the impacts of different pollutants on the climate system. Contemporary climate models increasingly integrate atmospheric physics, chemistry, biogeochemical cycles, and socio-economic development scenarios, enabling more scientifically robust predictions of the consequences of different environmental policy options.

An analysis of contemporary research indicates that the climatic role of atmospheric pollutants is determined not only by the magnitude of their emissions but also by their physicochemical properties, atmospheric lifetime, and interactions with other components of the climate system. Whereas the traditional approach focused primarily on reducing CO₂ emissions, contemporary international practice is increasingly oriented towards the integrated management of different groups of pollutants. For Ukraine, this implies the need to integrate decarbonization policies with programmes aimed at improving atmospheric air quality, enhancing energy efficiency, modernizing industry, and adapting to climate change.

Aerosols as Regulators of the Earth's Radiative Balance and Climate System

Aerosols are among the most important atmospheric components influencing the current state of the climate system. Unlike greenhouse gases, which primarily alter the thermal balance by absorbing longwave radiation, aerosol particles interact simultaneously with both shortwave solar radiation and longwave thermal radiation. Their effects depend on particle size, chemical composition, shape, colour, concentration, and altitude in the atmosphere. This multifactorial nature makes the quantitative assessment of aerosol impacts on climate particularly complex and explains why aerosols remain one of the major sources of uncertainty in contemporary climate models.

⁵⁶ Jacob D. J. Introduction to Atmospheric Chemistry. Princeton: Princeton University Press, 1999. 266 p.

According to their origin, atmospheric aerosols are classified as natural or anthropogenic. Natural aerosols include sea-salt aerosols, desert dust, volcanic ash, biological particles, and products of naturally occurring fires. Anthropogenic aerosols are generated through fossil fuel combustion, industrial activities, transport, biomass burning, and agricultural production. In contemporary urbanized regions, anthropogenic sources account for a predominant share of fine particulate matter (PM_{2.5}), which has particularly significant impacts on both public health and climate processes⁵⁷.

A distinctive feature of aerosols is their ability to exert both cooling and warming effects on the atmosphere. Light-coloured particles, particularly sulfate aerosols, efficiently scatter incoming solar radiation back into space, thereby increasing the albedo of the atmosphere and the Earth's surface. This produces negative radiative forcing, which partially offsets the warming caused by the accumulation of greenhouse gases. Consequently, during the second half of the twentieth century, intensive industrial sulfate pollution to some extent masked the magnitude of global warming.

In contrast, black carbon has an exceptionally high capacity to absorb solar radiation. Once released into the atmosphere, black carbon particles rapidly heat up and transfer heat to the surrounding air, causing localized atmospheric warming. Furthermore, when deposited on snow or ice surfaces, black carbon substantially reduces surface albedo, thereby accelerating the melting of glaciers and seasonal snow cover. This mechanism is particularly pronounced in high-altitude regions and the Arctic, where even a relatively small reduction in surface reflectivity can result in a substantial increase in the absorption of solar energy.

The most extensively studied mechanism through which aerosols interact with the climate system is their direct effect on the atmospheric radiative balance. This effect involves changes in the amount of solar energy reaching the Earth's surface as a result of the scattering or absorption of radiation by aerosol particles.

Sulfate, nitrate, and certain organic aerosols have high reflective properties and produce a cooling effect. In contrast, black carbon actively absorbs solar energy, thereby warming the atmosphere and altering the vertical temperature distribution. Mineral dust occupies an intermediate position, as its radiative properties depend largely on its mineralogical composition and particle size.

The overall direct effect of aerosols is determined not only by their concentration but also by the relative proportions of different particle types. In

⁵⁷ World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

industrial regions, the cooling effect of sulfate aerosols tends to predominate, whereas in areas characterized by intensive biomass burning and extensive use of diesel fuels, the contribution of black carbon becomes considerably more significant.

Equally important is the indirect effect of aerosols, which occurs through their involvement in cloud formation. Most atmospheric aerosols act as cloud condensation nuclei around which water vapour condenses to form cloud droplets. As the number of such nuclei increases, a greater number of smaller droplets are formed, enhancing cloud reflectivity and increasing the amount of incoming solar radiation reflected back into space.

In addition to modifying the optical properties of clouds, aerosols also influence cloud lifetime. Smaller droplets coalesce more slowly, which delays precipitation formation and prolongs the lifetime of cloud systems.

Through the combined effects of direct and indirect interactions, aerosols are capable of altering not only air temperature but also precipitation patterns, monsoon characteristics, cyclone trajectories, and even the frequency of certain extreme weather events⁵⁸.

Despite substantial advances in climate modelling, aerosols remain one of the major sources of uncertainty in projections of global climate change. The principal reasons include difficulties in determining their chemical composition, rapid transformation processes within the atmosphere, heterogeneous spatial distribution, and complex interactions with water vapour, clouds, and natural biogeochemical cycles.

The IPCC Sixth Assessment Report emphasizes that improving scientific understanding of aerosol processes is one of the key directions in the development of contemporary climatology. Particular attention is devoted to the integration of satellite observations, ground-based monitoring, and numerical atmospheric models, which makes it possible to reduce uncertainty in projections of future climate scenarios.

The analysis conducted indicates that aerosols act as distinctive “regulators” of the climate system, capable of both amplifying and mitigating the effects of global warming. Unlike greenhouse gases, their influence is not unidirectional and is determined by a complex combination of physical, chemical, and meteorological factors. Therefore, contemporary environmental policy cannot be based solely on reducing the concentrations of individual pollutants. It must take into account the complex interactions between aerosol particles and the climate system, the specific characteristics of regional atmospheric pollution, and the potential secondary effects of environmental protection measures⁵⁹.

⁵⁸ World Meteorological Organization. State of Global Air Quality and Climate. Geneva : WMO, 2024.

⁵⁹ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

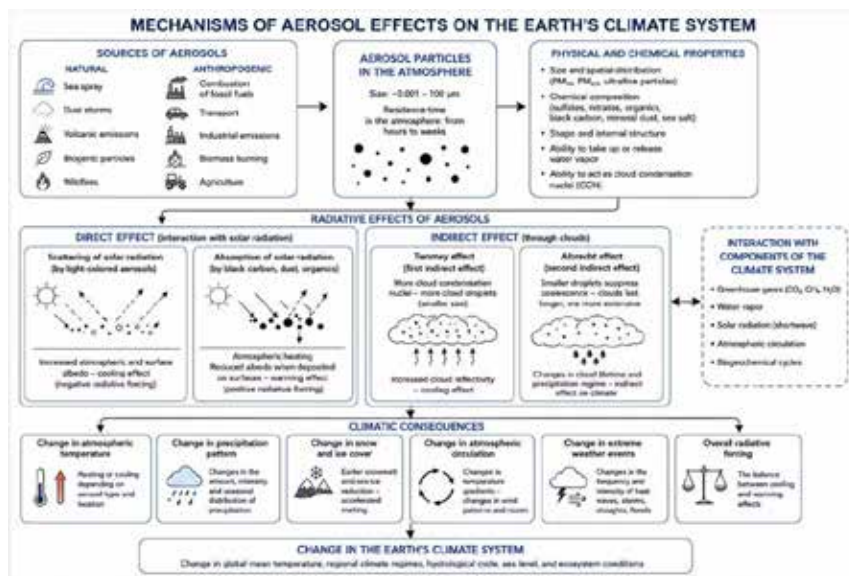


Fig. 2. Mechanisms of Aerosol Effects on the Earth's Climate System^{60, 61, 62}

Black Carbon as a Short-Lived Climate Pollutant

Among all atmospheric aerosols, black carbon (BC) occupies a particularly important position and is classified by contemporary international organizations as a short-lived climate pollutant (SLCP). Unlike most light-scattering aerosols, black carbon actively absorbs solar radiation, causing atmospheric warming and altering the atmospheric energy balance. According to estimates by the Intergovernmental Panel on Climate Change (IPCC), black carbon is among the most significant anthropogenic contributors to positive radiative forcing after carbon dioxide, although its atmospheric lifetime typically ranges from only a few days to approximately two weeks⁶³.

The main sources of black carbon include the incomplete combustion of fossil fuels, diesel engines, thermal power generation, residential heating with solid fuels, the burning of agricultural residues, wildfires, and other biomass combustion processes. In highly urbanized countries, road transport accounts for a substantial share of black carbon emissions, whereas in regions where

⁶⁰ Vallero D. A. Fundamentals of Air Pollution. 5th ed. Amsterdam : Elsevier, 2014. 996 p.

⁶¹ Jacob D. J. Introduction to Atmospheric Chemistry. Princeton : Princeton University Press, 1999. 266 p.

⁶² Möller D. Chemistry of the Climate System. Berlin; Boston : De Gruyter, 2014. 786 p.

⁶³ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

wood and coal are traditionally used for household purposes, residential and communal sources tend to predominate.

A distinctive feature of black carbon is its exceptionally high optical activity.

For Ukraine, the issue of black carbon has become particularly relevant in the context of the full-scale war. Large-scale fires resulting from hostilities, the destruction of industrial enterprises, oil depots, energy infrastructure facilities, and residential buildings are accompanied by significant emissions of incomplete combustion products, among which black carbon occupies a prominent place. In addition to directly deteriorating air quality, these emissions may potentially affect the regional radiation balance and climate processes, highlighting the need for their systematic monitoring and integration into post-war environmental recovery programs.

The analysis demonstrates that black carbon is a unique atmospheric pollutant that simultaneously acts as a toxic component of fine particulate matter and one of the most powerful short-lived climate forcers. Its impact extends far beyond local air pollution and manifests itself through changes in the radiation balance, snow-cover albedo, cloud microphysics, and regional atmospheric circulation. For Ukraine, particularly during the war and post-war periods, controlling black carbon emissions should become one of the priorities of environmental and climate policy, as it provides a dual benefit: rapid improvement in atmospheric air quality and a contribution to mitigating the effects of global climate change^{64, 65}.

Sulfate and Organic Aerosols as Drivers of Climate-System Cooling

Among atmospheric aerosols, sulfate and organic aerosols occupy a special place because, unlike black carbon, they predominantly produce negative radiative forcing and temporarily mitigate the effects of global warming. Their climatic effect is determined by their strong ability to scatter shortwave solar radiation, thereby increasing atmospheric albedo and reducing the amount of energy reaching the Earth's surface. Therefore, these aerosols are regarded as one of the natural and anthropogenic mechanisms that partially offset the warming caused by the accumulation of greenhouse gases.

Sulfate aerosols are formed primarily through the oxidation of sulfur dioxide (SO₂), which is released into the atmosphere during the combustion of coal, fuel oil, and petroleum products, as well as from metallurgical production and certain chemical processes. In addition to anthropogenic sources, volcanic eruptions make a significant contribution to the formation of sulfate aerosols.

⁶⁴ Моніторинг забруднення повітря на території України. URL: <https://www.saveecobot.com/maps/kharkiv>).

⁶⁵ Скок А., Хрутьба В. Якість атмосферного повітря в Україні до і під час повномасштабного вторгнення. Міжнародний фонд відродження. Київ, 2023. С. 5–24.

A distinctive feature of sulfate aerosols is their high efficiency in scattering solar radiation. They reflect part of the incoming energy back into space, thereby reducing the warming of the Earth's surface. According to IPCC estimates, sulfate aerosols provide the largest cooling contribution among anthropogenic aerosols. However, this effect is short-lived, since the average atmospheric lifetime of such particles is only a few days to several weeks⁶⁶.

Along with their direct radiative effect, sulfate aerosols significantly influence cloud formation processes. Due to their high hygroscopicity, they readily act as cloud condensation nuclei, increasing the number of small droplets in clouds. As a result, cloud reflectivity increases, cloud lifetime is prolonged, and the spatial distribution of precipitation is altered. According to IPCC assessments, this indirect effect through cloud modification remains one of the major sources of uncertainty in contemporary climate projections⁶⁷.

Organic aerosols constitute an extremely diverse group of atmospheric particles composed of hundreds of organic compounds of different origins. They are commonly divided into primary organic aerosols (POA), which are emitted directly into the atmosphere, and secondary organic aerosols (SOA), which are formed through photochemical reactions involving volatile organic compounds.

Sources of organic aerosols include transport, industry, biomass combustion, forest fires, agriculture, as well as natural biogenic processes. A significant proportion of secondary organic aerosols is formed through the oxidation of isoprene and monoterpenes emitted by vegetation. Thus, even natural ecosystems contribute to the formation of the background concentration of organic aerosols in the atmosphere.

The climatic effect of organic aerosols is more complex than that of sulfate aerosols. Most organic particles scatter solar radiation and thereby contribute to atmospheric cooling. At the same time, a specific group of organic aerosols, known as brown carbon, is capable of absorbing part of the incoming solar radiation, particularly in the ultraviolet and blue spectral ranges, thereby producing a localized warming effect. Therefore, the overall climatic impact of organic aerosols is determined by the balance between light-scattering and light-absorbing components⁶⁸.

Another important aspect is the interaction of organic aerosols with water vapor. Some organic compounds increase the hygroscopicity of aerosol particles, facilitating the formation of cloud droplets. Others, by contrast, reduce their

⁶⁶ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

⁶⁷ IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

⁶⁸ Jacob D. J. *Introduction to Atmospheric Chemistry*. Princeton : Princeton University Press, 1999. 266 p.

ability to act as condensation nuclei, thereby altering cloud microphysics. This demonstrates the complex nature of interactions between organic matter and atmospheric processes and explains why its role in the climate system continues to be actively investigated.

One of the most important features of contemporary climate policy is the so-called aerosol paradox. Measures aimed at reducing emissions of sulfur dioxide and fine particulate aerosols undoubtedly improve atmospheric air quality and reduce morbidity and premature mortality among the population. However, at the same time, they diminish the cooling effect of atmospheric aerosols, which has partially offset the warming caused by greenhouse gases.

Therefore, contemporary international policy is increasingly focused on an integrated approach that involves the simultaneous reduction of greenhouse gas emissions, black carbon, and other atmospheric pollutants. Only such an integrated strategy can prevent undesirable climatic consequences and ensure the achievement of the objectives of the European Green Deal and the Paris Agreement^{69, 70}.

Climate Change as a Factor Contributing to the Deterioration of Atmospheric Air Quality

For a long time, atmospheric air pollution was regarded primarily as a driver of global climate change. However, contemporary research demonstrates the existence of a reverse cause-and-effect relationship: climate change itself is becoming an important factor in the transformation of the atmospheric environment and the deterioration of air quality. Therefore, in recent years, the concept of “Climate–Air Quality Feedback” has been increasingly used in the international scientific literature.

The increase in global mean temperature contributes to the intensification of photochemical reactions in the troposphere, resulting in enhanced formation of ground-level ozone and secondary organic aerosols. Rising temperatures also increase the evaporation and emission of volatile organic compounds from natural and anthropogenic sources, creating additional conditions conducive to the formation of photochemical smog.

At the same time, the increasing frequency of heat waves, droughts, large-scale forest fires, and dust storms is accompanied by a sharp increase in the concentrations of fine particulate matter (PM_{2.5}), black carbon, and toxic combustion products. Such phenomena are increasingly transboundary in nature and are capable of affecting air quality far beyond the regions in which they originate.

⁶⁹ European Commission. The European Green Deal. Brussels : European Commission, 2019.

⁷⁰ Paris Agreement. URL: <https://unfccc.int/process-and-meetings/the-paris-agreement>.

For Ukraine, risks associated with longer periods of drought, increased wildfire risk in natural ecosystems, degradation of soil cover, and changes in atmospheric circulation patterns are becoming particularly relevant. The combined effects of climate change, anthropogenic pressure, and the consequences of military hostilities create new challenges for environmental monitoring systems and atmospheric air quality management.

Thus, contemporary environmental policy should take into account the bidirectional nature of the interaction between climate change and atmospheric pollution, since the effectiveness of emission reduction measures directly depends on future climatic conditions.

Adaptation Strategies for Atmospheric Air Quality Management under Climate Change

Climate change adaptation is one of the key areas of contemporary environmental policy. Unlike climate change mitigation measures aimed at reducing anthropogenic emissions, adaptation strategies are designed to enhance the resilience of natural and socio-economic systems to climate transformations that have already become unavoidable.

In the field of atmospheric air protection, priority areas of adaptation include the development of integrated monitoring systems, modernization of automated observation station networks, widespread use of satellite-based Earth remote sensing technologies, implementation of mathematical forecasting models, and application of artificial intelligence algorithms for the rapid assessment of environmental risks.

Equally important are Nature-based Solutions, which involve expanding urban green spaces, creating ecological corridors, developing green roofs and vertical greening systems, restoring wetlands, and optimizing the spatial structure of urban areas. Such measures not only improve atmospheric air quality but also contribute to reducing urban temperatures, enhancing carbon dioxide sequestration, and increasing the resilience of urbanized areas to extreme weather events^{71, 72, 73}.

Of particular importance for Ukraine is the integration of climate policy into post-war recovery programs. The reconstruction of industry, transport infrastructure, housing, and the energy sector should be carried out in accordance with the principles of low-carbon development, resource efficiency, and the European Green Deal. Such an approach creates the prerequisites for simultaneously enhancing environmental security, improving atmospheric air quality, and strengthening the country's climate resilience.

⁷¹ European Commission. The European Green Deal. Brussels : European Commission, 2019.

⁷² Paris Agreement. URL: <https://unfccc.int/process-and-meetings/the-paris-agreement>.

⁷³ European Centre for Medium-Range Weather Forecasts. Copernicus Atmosphere Monitoring Service (CAMS): Global and Regional Air Quality Products. Reading: ECMWF, 2024.

The analysis has demonstrated that atmospheric pollution and global climate change are interdependent processes that form an integrated natural–anthropogenic system. Greenhouse gases, aerosols, short-lived climate pollutants, and secondary products of atmospheric chemistry affect the Earth's radiation balance in different ways; however, their cumulative effect is manifested in the transformation of climatic conditions, changes in atmospheric circulation patterns, and an increasing frequency of extreme natural events.

It has been established that climate change, in turn, exacerbates atmospheric air quality problems by intensifying photochemical processes, increasing the scale of wildfires and dust storms, and enhancing secondary aerosol formation. This confirms the existence of a persistent bidirectional relationship between atmospheric pollution and the climate system.

The transition to an integrated model of atmospheric air quality management that combines modern monitoring systems, mathematical modelling, geoinformation technologies, satellite observations, and artificial intelligence tools has been substantiated. Such an approach ensures the timely identification of environmental risks, enhances the effectiveness of environmental protection policies, and facilitates adaptation to climate change.

For Ukraine, the integration of environmental policy with post-war recovery and European integration processes is of particular importance. The implementation of the principles of low-carbon development, the digital transformation of environmental monitoring, and the introduction of Nature-based Solutions create the necessary prerequisites for ensuring environmental security, improving public health, and achieving the Sustainable Development Goals.

CONCLUSIONS

The study has demonstrated that atmospheric air pollution remains one of the most significant environmental problems of the present day, characterized by a pronounced global and transboundary nature and directly affecting public health, the functioning of natural ecosystems, and climate processes. Increasing anthropogenic pressure, urbanization, the development of industry, energy production and transport, the use of fossil fuels, as well as the growing frequency of natural and anthropogenic emergencies necessitate further improvement of atmospheric air protection mechanisms.

It has been established that the current structure of atmospheric pollution is shaped by both stationary and mobile sources, whose contributions vary considerably depending on the level of industrialization, the structure of the energy sector, transport intensity, and the natural and climatic characteristics of a particular area. Among the priority pollutants, particulate matter (PM_{2.5}

and PM₁₀), nitrogen and sulfur oxides, ground-level ozone, carbon monoxide, volatile organic compounds, heavy metals, and black carbon pose particular environmental hazards. A significant proportion of these components are characterized not only by their toxic effects on the human body but also by their ability to participate in complex physicochemical processes in the atmosphere.

The problem of fine particulate matter, particularly PM_{2.5} and PM₁₀, is of particular importance. The small size of these particles contributes to their prolonged residence in the atmosphere, long-range transport, and penetration into the human respiratory system. An analysis of global trends confirms that PM_{2.5} concentrations in a considerable number of countries and cities remain above the levels recommended by the World Health Organization. This necessitates strengthened control over sources of particulate matter emissions and the expansion of continuous monitoring networks.

Black carbon is an important component of atmospheric aerosols, combining the properties of an air pollutant and a short-lived climate forcer. Its formation is primarily associated with the incomplete combustion of fossil fuels and biomass. Reducing black carbon emissions can provide a dual benefit: improving atmospheric air quality and reducing the adverse anthropogenic impact on the climate system.

The study has also confirmed the close relationship between atmospheric air pollution and climate change. Rising temperatures, changes in atmospheric circulation patterns, droughts, heat waves, dust events, and the increasing scale of wildfires can contribute to the accumulation and transport of pollutants. At the same time, certain atmospheric components affect the Earth's radiation balance and climate processes. This substantiates the need to integrate air quality management policies with climate change mitigation and adaptation measures.

The analysis of the situation in Ukraine has revealed a substantial transformation in the structure of anthropogenic pressure on atmospheric air under the conditions of the full-scale war. Statistical data demonstrate an overall reduction in emissions compared with the pre-war period. However, this trend should not be interpreted unequivocally as an indication of improved environmental efficiency of the economy. To a considerable extent, it is associated with declining industrial production, the destruction or shutdown of enterprises, changes in the structure of energy consumption, the occupation of parts of the country's territory, and incomplete statistical reporting. At the same time, military hostilities have created new sources of pollution associated with fires, explosions, the destruction of industrial and energy infrastructure, and the use of autonomous energy supply systems.

It has been established that one of the key areas for improving atmospheric air protection is the modernization of the environmental monitoring system.

The traditional model, based predominantly on stationary observation stations, no longer fully meets the requirements of operational air quality management. An integrated model combining reference and automated monitoring stations, sensor networks, mobile measurement systems, satellite observations, geographic information systems, and mathematical modelling represents a promising approach.

For Ukraine, the modernization of the atmospheric air monitoring system should be carried out in accordance with European principles and standards, ensuring the compatibility of national information systems with European platforms and making extensive use of the capabilities provided by Copernicus and the Copernicus Atmosphere Monitoring Service (CAMS). Important prerequisites for the effectiveness of such a system include open access to environmental information, spatial expansion of the monitoring network, automation of data transmission, and ensuring data accessibility for public authorities, researchers, and the general public.

Thus, a prospective model of atmospheric air protection should be based on a comprehensive combination of emission reduction measures, the development of a modern monitoring system, the digitalization of environmental information, and the integration of climate and environmental protection policies. For Ukraine, the implementation of such an approach is particularly important in the context of European integration and post-war recovery. The reconstruction of industry, the energy sector, and transport infrastructure should be based on the principles of environmental modernization, decarbonization, and the application of modern emission control technologies.

The further development of automated and digital observation systems, the integration of ground-based and satellite data, and the application of geographic information technologies and artificial intelligence create the prerequisites for establishing a fundamentally new atmospheric air quality management system. Its implementation will contribute to the timely identification of environmental risks, improve the evidence base for management decisions, reduce the adverse effects of pollution on public health, and ensure the environmentally safe and sustainable development of Ukraine.

SUMMARY

The current state and key trends in atmospheric air pollution are investigated in the context of increasing anthropogenic pressure and global climate change. The main natural and anthropogenic sources of air pollution are systematized, and the environmental hazards associated with priority pollutants, including particulate matter ($PM_{2.5}$ and PM_{10}), nitrogen and sulfur oxides, ground-level ozone, and black carbon, are characterized. The impacts of atmospheric

pollutants on public health, natural ecosystems, and climate processes are analyzed. Global and national trends in atmospheric air quality are examined, with particular attention to the transformation of anthropogenic pressures in Ukraine under wartime conditions. Contemporary approaches to atmospheric air monitoring are summarized, and the prospects for the application of automated monitoring stations, sensor networks, Earth remote sensing, and geographic information technologies are identified. Particular attention is given to the digitalization of monitoring and the application of Internet of Things (IoT), Big Data, machine learning, and artificial intelligence technologies for air quality analysis and forecasting. The need to establish an integrated atmospheric air monitoring system in Ukraine in accordance with contemporary European approaches and the objectives of environmentally sustainable post-war recovery is substantiated.

Bibliography

1. Боголюбов В. М., Клименко М. О., Мокін В. Б. та ін. Моніторинг довкілля : підручник. Херсон : Грінь Д. С., 2011. 530 с.
2. Державна служба статистики України. Викиди забруднюючих речовин і парникових газів у атмосферне повітря. URL: <https://www.ukrstat.gov.ua/>.
3. Про Основні засади (стратегію) державної екологічної політики України на період до 2030 року : Закон України від 28 лютого 2019 року № 2697-VIII URL: <https://zakon.rada.gov.ua/go/2697-19>.
- Защепкіна Н., Рудницький Р. Аналіз основних чинників, які впливають на рівень забруднення атмосферного повітря. *Вимірювальна та обчислювальна техніка в технологічних процесах*. 2026. № 1. С. 21–27. URL: <https://doi.org/10.31891/2219-9365-2026-85-3.5>. Звіт про якість повітря IQAir у світі за 2025 рік. URL: <https://www.iqair.com/newsroom/waqi-2025-pr>.
6. Екологічний моніторинг регіону: експертна оцінка стану і функціонування / за ред. д-ра. геогр. наук, проф. І. Ковальчука / [І. Ковальчук, П. Волошин, А. Михнович та ін.]. Львів : Опілля, 2009. 608 с.
7. Моніторинг забруднення повітря на території України. URL: <https://www.saveecobot.com/maps/kharkiv>.
8. Новіков М. А., Хондак І. І. Проблема забруднення атмосферного повітря в Україні. *Радіoeлектроніка та молодь у XXI столітті* : матеріали 29-го Міжнар. молодіж. форуму, 16–19 квітня 2025 р. Харків : ХНУРЕ, 2025. Т. 2. С. 132–134.
9. *Огляд стану забруднення навколишнього природного середовища на території України* за даними мережі моніторингу гідрометеорологічних організацій у 2024 році. URL: <http://cgo-sreznevskyi.kyiv.ua/uk/diialnist/>

khimichne-zabrudnennia/8-materialy-na-glavnoj/171-stan-zabrudnennya-prirodno-seredovishcha-na-teritoriji-ukrajini.

10. Про охорону навколишнього природного середовища : Закон України від 25 червня 1991 р. № 1264-XII. *Відомості Верховної Ради України*. 1991. № 41. С. 546.

11. Про охорону атмосферного повітря : Закон України від 16 жовтня 1992 р. № 2707-XII. *Відомості Верховної Ради України*. 1992. № 50. С. 678.

12. Про затвердження Порядку здійснення державного моніторингу в галузі охорони атмосферного повітря : постанова Кабінету Міністрів України від 14 серпня 2019 р. № 827.

13. *Про затвердження форми Програми державного моніторингу у галузі охорони атмосферного повітря* : наказ Міндовкілля від 25.02.2021 № 147. URL: <https://zakon.rada.gov.ua/laws/show/z0543-21>.

14. Рейтинг України у світі. URL: <https://skilky-skilky.info/novuuy-rekord-ukraini-povitra-v-ukraini-pokrashchylasia-na-11/>.

15. Скок А., Хрутьба В. Якість атмосферного повітря в Україні до і під час повномасштабного вторгнення. Міжнародний фонд відродження. Київ, 2023. С. 5–24.

16. Хондак І., Стищенко Т. Забруднення атмосферного повітря в умовах війни в Україні. Біологічні, хімічні та екологічні загрози під час війни : колективна монографія / за заг. ред. В. В. Поповича, В. О. Сергієнка, Н. О. Іванченко. Львів : ЛДУБЖД, 2025. С. 391–399.

17. Directive (EU) 2024/2881 of the European Parliament and of the Council of 23 October 2024 on ambient air quality and cleaner air for Europe (recast). *Official Journal of the European Union*. 2024.

18. World Health Organization. WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva : WHO, 2021. 290 p.

19. IPCC. *Climate Change 2023: Synthesis Report*. Geneva, 2023.

20. European Environment Agency. Air Quality Status Report 2025. Luxembourg : Publications Office of the European Union, 2025.

21. European Commission. The European Green Deal. Brussels : European Commission, 2019.

22. European Commission. Zero Pollution Action Plan: Towards Zero Pollution for Air, Water and Soil. Brussels : European Commission, 2021.

23. Seinfeld J. H., Pandis S. N. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change. 3rd ed. Hoboken: John Wiley & Sons, 2016. 1152 p.

24. Vallero D. A. Fundamentals of Air Pollution. 5th ed. Amsterdam : Elsevier, 2014. 996 p.

25. Jacob D. J. Introduction to Atmospheric Chemistry. Princeton : Princeton University Press, 1999. 266 p.
26. Paris Agreement. URL: <https://unfccc.int/process-and-meetings/the-paris-agreement>.
27. Kumar P., Morawska L., Martani C. et al. The rise of low-cost sensing for managing air pollution in cities. *Environment International*. 2015. Vol. 75. P. 199–205.
28. Snyder E. G., Watkins T. H., Solomon P. A. et al. The changing paradigm of air pollution monitoring. *Environmental Science & Technology*. 2013. Vol. 47, No. 20. P. 11369–11377.
29. Lewis A. C., Edwards P. M. Validate personal air-pollution sensors. *Nature*. 2016. Vol. 535. P. 29–31.
30. Concas F., Mineraud J., Lagerspetz E. et al. Low-Cost Outdoor Air Quality Monitoring and Sensor Calibration: A Survey and Critical Analysis. *ACM Computing Surveys*. 2021. Vol. 54, No. 3.
31. World Meteorological Organization. State of Global Air Quality and Climate. Geneva : WMO, 2024.
32. European Centre for Medium-Range Weather Forecasts. Copernicus Atmosphere Monitoring Service (CAMS): Global and Regional Air Quality Products. Reading: ECMWF, 2024.
33. Möller D. Chemistry of the Climate System. Berlin; Boston : De Gruyter, 2014. 786 p.
34. United Nations Environment Programme. Global Environment Outlook 6: Healthy Planet, Healthy People. Cambridge : Cambridge University Press, 2019. 708 p.

Information about the authors:

Pytuliak Myroslava,

Candidate of Geographic Sciences, Associate Professor,
Associate Professor at the Department of Ecology and Health Protection
West Ukrainian National University
11, Lvivska Street, Ternopil, Ukraine

Pytuliak Mykola,

Candidate of Geographic Sciences, Associate Professor,
Associate Professor at the Department of Agrobiotechnology
West Ukrainian National University
11, Lvivska Street, Ternopil, Ukraine

CONSUMERS' SELF-EXTENSION AND EXPERIENCES OF SWITCHING TO MORE SUSTAINABLE FOOD-RELATED PRODUCTS

Svietlichna Mariia, Hjelm Jonathan, Wijesinghe P. G. Sachini D.
DOI <https://doi.org/10.30525/978-9934-26-714-7-16>

INTRODUCTION

Environmental challenges increasingly shape everyday consumer behaviour, particularly in relation to food, packaging and household products. Chemical exposure, plastic pollution and climate change have moved sustainability from a specialist ethical concern into mainstream consumer culture¹. Food-related choices are therefore no longer guided solely by price or convenience; they are also connected to environmental responsibility, personal health, ethical positioning and consumers' understanding of themselves.

Sustainable consumption is especially relevant in food-related contexts because food quality, packaging materials and product transparency are directly connected to both individual well-being and environmental impact. Consumers may seek organic products, reduce plastic packaging, choose reusable containers or prefer minimally processed food not only because these practices are perceived as environmentally responsible, but also because they are associated with safety, health and a preferred way of life². Sustainable consumption can therefore be understood as a practice through which consumers express values and construct coherence between their everyday choices and their self-concept³.

Despite growing awareness of environmental and health-related issues, the transition to sustainable food consumption remains complex. Consumers often encounter tensions between sustainability values and practical constraints, including price, convenience, availability, established habits and uncertainty

¹ Bengtsson M., Alfredsson E., Cohen M., Lorek S., Schroeder P. Transforming systems of consumption and production for achieving the sustainable development goals: moving beyond efficiency. *Sustainability Science*. 2018. Vol. 13, No. 6. P. 1533–1547. DOI: 10.1007/s11625-018-0582-1.

² Ахмаді А., Светлічна М. І. Таємниці довголіття шведів: спілкування, спорт та насолода життям. *Молодий вчений*. 2024. № 4 (128). С. 14–16. DOI: 10.32839/2304-5809/2024-4-128-31.

³ Belk R. W. Possessions and the extended self in consumer behavior. *Journal of Consumer Research*. 1988. Vol. 15, No. 2. P. 139–168. URL: <https://www.jstor.org/stable/2489522>.

about product information. This tension is commonly described as the attitude-behaviour gap: consumers may express positive attitudes towards sustainability while their actual purchasing decisions remain shaped by practical and contextual factors. Sustainable consumption should therefore not be treated as a simple consequence of environmental awareness, but as a negotiated process in which health concerns, sustainability values, social influence and everyday constraints interact⁴.

Existing research has shown that sustainable consumption may carry symbolic meaning and contribute to consumers' identity construction⁵. However, much of the literature focuses on behavioural intentions, purchase outcomes or quantitative patterns of adoption. Less attention has been given to how consumers personally experience the transition towards sustainable food-related products and how they interpret these practices in relation to health, sustainability and self-extension. In particular, there remains limited qualitative research on how concerns about plastic packaging, processed food, artificial ingredients and product materials become connected to consumers' values, everyday routines and sense of self.

This study addresses this gap by examining how consumers experience the transition towards more sustainable food-related consumption. It focuses on everyday practices, including choosing organic food, reducing plastic packaging, using reusable kitchen solutions and preferring natural or minimally processed products. The study applies Extended Self Theory and Self-Congruity Theory to examine how sustainable consumption becomes connected to identity, personal values and self-perception.

The aim of this study is to explore how consumers experience the transition towards more sustainable food-related consumption and how health concerns and sustainability values contribute to self-extension and identity construction.

The study is guided by the following research questions:

(RQ1) How do consumers engage in self-extension through sustainable food consumption, including organic food choices and reduced use of plastic packaging?

(RQ2) How do health concerns and sustainability values shape this process of self-extension and the construction of the extended self?

The study also relates to Sustainable Development Goal 3, "Good Health and Well-being", and Sustainable Development Goal 12, "Responsible Consumption

⁴ Johnstone M.-L., Tan L. P. Exploring the gap between consumers' green rhetoric and purchasing behaviour. *Journal of Business Ethics*. 2020. Vol. 132, No. 2. P. 311–328. DOI: 10.1007/s10551-014-2316-3.

⁵ Chwiałkowska A. An interplay of the consumption values and green behavior. *Business Strategy and the Environment*. 2024. URL: <https://www.diva-portal.org/smash/get/diva2:1823835/FULLTEXT02.pdf>.

and Production”. These goals provide a broader context for examining how food-related consumption practices can be associated with healthier everyday choices and reduced environmental impact.

1. Theoretical Framework and Literature Review. Self-Congruity Theory

Self-Congruity Theory explains how consumers evaluate and select products in relation to their self-concept. Consumers are more likely to prefer products, brands and consumption practices that correspond with how they see themselves or how they wish to be perceived by others. Product choice is therefore not only functional; it may also communicate personal values, desired identities and social positioning.

In the context of sustainable food consumption, self-congruity helps explain why consumers may choose organic food, reusable containers, glass packaging or products with perceived natural ingredients. These choices can reflect a consumer’s desire to act consistently with values related to health, environmental responsibility and ethical consumption. A consumer who considers themselves health-conscious may prefer products perceived as less processed or less harmful, while an environmentally conscious consumer may seek to reduce plastic use or choose products with sustainable packaging.

Self-congruity also includes social dimensions. Consumers may select sustainable products because these choices correspond with the image they wish to present to family members, colleagues, friends or wider social groups. Sustainable consumption can therefore become a symbolic practice through which individuals communicate that they are responsible, health-oriented, environmentally aware or aligned with particular lifestyles. At the same time, the relationship between values and purchasing behaviour is not always consistent, because consumers also face price constraints, limited availability and convenience-based decisions.

Consumer decision-making is shaped by social norms, reference groups and cultural expectations⁶. Family members, friends, colleagues, social media content and educational experiences may influence what consumers regard as normal, desirable or responsible. Sustainable practices can receive social validation when they are visible within reference groups, while consumers may also experience discomfort when their choices diverge from perceived social norms⁷.

⁶ Confente I., Scarpi D., Russo I. Marketing a new generation of sustainable products: The role of brand symbolism and consumer values. *Journal of Business Research*. 2020. Vol. 112. P. 203–215. URL: <https://www.sciencedirect.com/science/article/pii/S0148296319306186>.

⁷ Светлічна М. І., Светлічний І. В. Enigmatic realm of global tourism production system. *Молодий вчений*. 2023. № 12 (124). С. 111–114. DOI: 10.32839/2304-5809/2023-12-124-18.

This social dimension is particularly relevant to sustainable consumption because consumers often evaluate their food-related practices not only in relation to personal preferences but also in relation to the expectations and visible behaviours of significant others.

Extended Self Theory provides a framework for understanding how possessions, objects and everyday routines become connected to identity. According to this perspective, the self extends beyond the physical individual to include objects, habits and practices that carry personal meaning. Consumption is therefore not merely a practical activity; it can become part of how individuals construct, reinforce and express who they are.

In sustainable food-related consumption, repeated practices may gradually become incorporated into the extended self. Choosing reusable kitchen products, reducing plastic packaging, purchasing local food, composting, avoiding highly processed products or using glass containers may initially be practical decisions. When these actions are repeated and associated with values such as responsibility, health, safety or environmental care, they may become part of consumers' everyday identity. Sustainable practices are thus not necessarily isolated purchasing decisions; they can form routines through which individuals express broader moral priorities and preferred ways of living⁸.

From a practice-oriented perspective, sustainable consumption is maintained not by isolated purchasing decisions but by repeated routines involving shopping, food preparation, storage and waste management. Repetition can transform initially practical choices, such as carrying reusable bags or selecting glass containers, into familiar elements of everyday life. When these routines are consistently linked to values of health, responsibility and environmental care, they may support the incorporation of sustainable practices into the extended self⁹.

The extended self is not fixed. Consumers may adopt, adjust or abandon practices as their circumstances, values and social environments change. Sustainable consumption is consequently best understood as a gradual and negotiated process rather than an immediate identity transformation. Consumers may identify with sustainability while still choosing less sustainable alternatives when price, convenience, availability or familiar routines become more influential. This tension does not invalidate sustainable values; instead, it illustrates how identity construction through consumption is shaped by competing priorities in everyday life.

⁸ Shove E., Pantzar M., Watson M. The dynamics of social practice: Everyday life and how it changes. London : SAGE Publications, 2012. DOI: 10.4135/9781446250655.

⁹ Светлічна М. І. Global tourism production systems: topical issues. *Гуманітарний і інноваційний ракурс професійної майстерності: пошуки молодих вчених*. 2023. DOI: 10.36059/978-966-397-357-9-53.

However, identity-related values do not automatically result in consistent sustainable behaviour. The relationship between self-image, environmental intentions and everyday consumption may be disrupted by price, product availability, convenience, established routines and uncertainty about product claims. This discrepancy, often described as the attitude-behaviour gap, indicates that sustainable consumption should be understood as a negotiated process rather than a direct expression of individual values.

2. Health, Social Influence and Barriers to Sustainable Consumption

Sustainable food consumption is increasingly motivated by both environmental concern and personal health. Consumers may associate plastic packaging, artificial ingredients, highly processed food and chemical exposure with perceived health risks, while organic food, natural materials and reusable products may be associated with safety, cleanliness and well-being. Health-related motivations can be particularly immediate because they concern the consumer's body, family and everyday routines.

Environmental and health concerns should not be treated as separate or competing motivations. In food-related consumption, they frequently overlap. Reducing plastic packaging may be understood as a way to limit environmental waste and reduce perceived exposure to harmful substances. Choosing fresh or minimally processed food may be associated with personal health as well as lower environmental impact. Sustainable food consumption therefore often reflects an integrated set of values in which self-care and environmental responsibility reinforce one another.

However, health-related and sustainability-related choices are shaped by uncertainty. Consumers are exposed to information from social media, advertising, family members, schools, health professionals and online platforms. These sources may provide useful knowledge but may also create confusion when claims about food, packaging and health risks are inconsistent or difficult to verify. Consumers must therefore interpret competing information while making routine decisions under practical constraints.

At the same time, sustainable consumption is constrained by structural and practical barriers. Price, product availability, convenience, limited time, habits and lack of trust in sustainability claims can prevent consumers from acting consistently with their values. These factors help explain the attitude-behaviour gap, in which positive views about sustainability do not always lead to sustainable purchasing behaviour. Sustainable food consumption should therefore be conceptualised as a complex process shaped by individual values, identity-related meanings, social influence and everyday constraints¹⁰.

¹⁰ Wijesinghe P. G. S. D., Baduge D. C. N., Світлічна М., Date M., Tusha R. Luxury wellness and medical tourism: A sustainable hospitality concept for Öland. *Grail of Science*. 2025. DOI: 10.36074/grail-of-science.23.05.2025.153.

Extended Self Theory and Self-Congruity Theory together provide an appropriate framework for examining this process. Self-Congruity Theory explains how sustainable choices may align with consumers’ self-image and desired identity, while Extended Self Theory explains how repeated consumption practices may become integrated into everyday routines and the extended self. Together, these theories support an analysis of sustainable food consumption as an identity-related process shaped by health concerns, sustainability values, social meaning and practical realities.

Methodology. This study adopts a qualitative, interpretivist approach to explore how consumers experience and interpret the transition towards more sustainable food-related consumption. The approach focuses on subjective meanings, personal experiences and socially constructed understandings of the relationships between food choices, health, environmental responsibility, identity and everyday life.

The research uses focus group discussions as the primary method of data collection. Focus groups were selected because they allow participants to discuss their experiences, compare views and respond to the perspectives of others. This interaction is particularly relevant for sustainable consumption because consumer choices are influenced not only by individual preferences but also by social norms, shared knowledge, family practices and peer expectations.

The study included three digitally conducted focus groups involving a total of nineteen participants. The first and second groups each included six participants, while the third group included seven participants. Participants were recruited through purposive sampling. The sampling strategy focused on consumers who had an interest in sustainable food-related consumption, personal health or environmentally conscious practices, including the use of organic food, reusable kitchen products or reduced plastic packaging.

Table 1

Overview of focus group discussions

Focus group	Date	Platform	Participants	Duration	Participant profile
Focus Group 1	25 April 2026	Microsoft Teams	6	80 minutes	Participants from Ukraine and Sweden; aged 18–78
Focus Group 2	26 April 2026	Microsoft Teams	6	90 minutes	Participants from Sri Lanka and Sweden; aged 18–58
Focus Group 3	28 April 2026	Google Meet	7	120 minutes	Participants from Ukraine, France, Sri Lanka and Sweden; aged 19–48
Total	–	Online	19	290 minutes	Four countries; mixed-gender sample; age range 18–78

Source: compiled by the authors based on focus-group discussion records.

In total, the study generated 290 minutes of group discussion across three online focus groups, involving nineteen participants from Ukraine, Sweden, Sri Lanka and France.

Semi-structured discussion guides were used to ensure that all groups addressed the central research themes while allowing participants to introduce additional experiences and reflections. The discussions focused on personal health, sustainability values, food and packaging choices, identity, social influence, future aspirations and barriers to sustainable consumption. The digital format enabled participation by individuals from different backgrounds and locations.

The discussions lasted 80, 90 and 120 minutes, respectively. Participants were informed about the purpose of the study, the voluntary nature of participation and their right to withdraw. Anonymity and confidentiality were maintained throughout the research process. Because some participants preferred not to be audio-recorded, the empirical material consisted of detailed discussion notes and anonymised summaries prepared by the moderators rather than verbatim transcripts. Accordingly, the findings should be interpreted as contextual thematic insights rather than verbatim representations of participants' wording.

The material was analysed through thematic analysis. This approach was used to identify, organise and interpret recurring patterns across the focus groups. The analysis focused on themes related to self-extension, health concerns, sustainability values, symbolic meanings of food and packaging, social influence, practical barriers and the gradual adoption of sustainable practices. Extended Self Theory and Self-Congruity Theory informed the interpretation of how participants connected everyday consumption practices with identity, self-perception and desired lifestyles. Table 2 summarises how participants connected particular food-related practices with symbolic meanings and identity-related processes¹¹.

Empirical Findings and Analysis. The thematic analysis identified recurring patterns concerning health-related motivations, environmental responsibility, symbolic meanings of materials and food, social influence, and practical barriers to sustainable consumption. Table 2 presents the relationship between the practices reported by participants, the meanings attributed to them, and their relevance to identity and self-extension.

These thematic patterns provide the empirical basis for the conclusions presented below.

Overall, the focus-group discussions suggest that sustainable food-related consumption was experienced as a gradual rather than a uniform transition.

¹¹ Nowell L. S, Norris J. M., White D. E., Moules N. J. Thematic analysis: Striving to meet the trustworthiness criteria. *International Journal of Qualitative Methods*. 2017. Vol. 16, No. 1.

Participants connected sustainable practices with health, environmental responsibility and preferred identities, but their ability to maintain these practices was shaped by price, availability, convenience, habits and social context. These findings are not statistically generalisable; instead, they provide context-specific insight into how participants interpreted sustainable consumption in everyday life.

Table 2

Sustainable practices, symbolic meanings and identity processes

Sustainable practice	Meaning attributed by participants	Link with identity and self-extension
Avoiding plastic packaging; choosing glass, stainless steel or ceramic	Cleanliness, safety, reduced perceived chemical exposure and environmental care	Repeated use of alternative materials may embody a health-conscious and environmentally responsible self-image
Buying organic, local or minimally processed food	Naturalness, authenticity, quality and care for personal well-being	Food choices align consumption with a preferred health-oriented identity
Reusing bags, jars and kitchen containers	Responsibility, thrift, waste reduction and routine care	Repetition transforms practical actions into normalised everyday practices
Cooking at home and reducing food waste	Control over ingredients, self-care and responsible domestic practice	Household routines become part of an individual's lifestyle narrative
Using reusable items in workplaces or public settings	Professionalism, social responsibility and role-model behaviour	Visible choices support social recognition of a sustainable identity

Source: compiled by the authors based on focus-group discussions.

CONCLUSIONS

This study examined consumers' experiences of the transition towards more sustainable food-related consumption and the role of health concerns and sustainability values in processes of self-extension and extended self-construction. The findings suggest that sustainable food consumption is better understood as an ongoing identity-related process than as a series of isolated purchase decisions. Participants described gradual changes in everyday routines, including reducing plastic packaging, choosing organic or minimally processed food, using reusable kitchen products and preferring materials perceived as safer or more natural. These practices were not described solely as functional choices; they were also connected to how participants understood themselves and the lifestyles they wished to maintain.

The findings address RQ1 by indicating that sustainable food-related practices may contribute to self-extension when they are repeated, personally meaningful and incorporated into everyday routines. Participants associated glass, wood, stainless steel, local food and minimally processed ingredients with cleanliness, safety, naturalness, authenticity and responsibility. In contrast, plastic packaging and highly processed food were often associated with perceived risks, artificiality and discomfort. These associations suggest that participants evaluated food-related products not only according to practical utility, but also according to their fit with a desired self-image. Sustainable consumption therefore emerged as a form of identity-related practice through which repeated actions could gradually become part of the extended self.

The findings also address RQ2 by showing that participants frequently described health concerns as an immediate motivation for changing food-related consumption practices. Participants referred to perceived risks connected with plastic food packaging, food storage, artificial ingredients and highly processed products. Environmental concern often coexisted with these health-related considerations. Rather than functioning as competing motivations, health and sustainability appeared to operate as complementary values: sustainable practices were understood both as forms of self-care and as ways of reducing environmental harm. This relationship was particularly visible when participants discussed reducing plastic packaging, choosing reusable containers and preferring fresh, organic or minimally processed food.

Social influence also played an important role in how sustainable consumption was interpreted and adopted. Participants referred to family members, colleagues, social media, online information and educational settings when discussing what they considered normal, responsible or desirable consumption behaviour. These influences could encourage sustainable practices by providing role models, information and social validation. At the same time, participants described uncertainty and pressure arising from conflicting information, expectations and perceived social norms. The findings therefore suggest that sustainable consumption is not solely an individual process; it is also socially negotiated through reference groups, visible practices and shared understandings of responsible behaviour.

The transition towards sustainable food-related consumption was constrained by practical barriers. Participants referred to price, limited availability, convenience, established habits and uncertainty about sustainability claims as reasons why their everyday decisions did not always correspond with their sustainability ideals. These findings are consistent with the attitude-behaviour gap, whereby positive attitudes towards sustainability do not necessarily result in consistent sustainable behaviour. Within this study, the discrepancy did not simply reflect a lack of

commitment. Instead, it reflected the need to negotiate between personal values, economic conditions, product availability and routine practices¹².

The findings are relevant to Sustainable Development Goal 3, Good Health and Well-being, because participants linked food quality and packaging choices with everyday health-oriented practices. They are also relevant to Sustainable Development Goal 12, Responsible Consumption and Production, because reusable products, reduced packaging and lower food waste were interpreted as practical expressions of responsible consumption.

Overall, the findings frame sustainable food-related consumption as a gradual and context-dependent identity process. Sustainable practices may become part of the extended self through repetition, symbolic meaning and social validation, while their adoption remains shaped by health concerns, environmental values, social influence and everyday constraints. Sustainable food consumption is therefore not only an environmental practice or a purchasing decision. It is a way through which consumers may connect personal well-being, moral responsibility and everyday consumption choices.

SUMMARY

This chapter investigates consumers' experiences of transitioning towards more sustainable food-related consumption, with a focus on identity and self-extension in everyday practices. Drawing on Extended Self Theory and Self-Congruity Theory, the study examines how practices such as using reusable kitchenware, purchasing organic food and reducing plastic packaging become connected to consumers' self-perception, values and identity. The study uses a qualitative interpretivist research design based on three focus group discussions with nineteen participants who expressed an interest in environmentally conscious consumption.

The findings suggest that participants did not perceive sustainable consumption solely as a functional activity. Instead, they often associated sustainable food-related practices with personal identity, health, responsibility and preferred lifestyles. Participants frequently described concerns about perceived health risks related to microplastics, chemicals and highly processed food as immediate motivations for changing their consumption practices. Environmental concerns commonly complemented these health-related motivations. However, price, limited product availability, convenience and established habits sometimes constrained participants' ability to act consistently with their sustainability values, reflecting an attitude-behaviour gap.

¹² Johnstone M.-L., Tan L. P. Exploring the gap between consumers' green rhetoric and purchasing behaviour. *Journal of Business Ethics*. 2020. Vol. 132, No. 2. P. 311–328. DOI: 10.1007/s10551-014-2316-3.

The study concludes that sustainable food consumption is an identity-related and socially negotiated process shaped by health concerns, environmental values, social norms and everyday constraints. The findings have implications for marketing, policy and regulatory initiatives aimed at supporting the normalisation of healthy and sustainable consumption practices.

Bibliography

1. Belk R. W. Possessions and the extended self in consumer behavior. *Journal of Consumer Research*. 1988. Vol. 15, No. 2. P. 139–168. URL: <https://www.jstor.org/stable/2489522>.
2. Braun V., Clarke V. Using thematic analysis in psychology. *Qualitative Research in Psychology*. 2006. Vol. 3, No. 2. P. 77–101. DOI: 10.1191/1478088706qp063oa.
3. Bryman A. *Social Research Methods*. 5th ed. Oxford : Oxford University Press, 2016. 747 p.
4. Morgan D. L. *Focus Groups as Qualitative Research*. 2nd ed. Thousand Oaks, CA : SAGE Publications, 1997. DOI: 10.4135/9781412984287.
5. Nowell L. S., Norris J. M., White D. E., Moules N. J. Thematic analysis: Striving to meet the trustworthiness criteria. *International Journal of Qualitative Methods*. 2017. Vol. 16, No. 1. DOI: 10.1177/1609406917733847.
6. Bengtsson M., Alfredsson E., Cohen M., Lorek S., Schroeder P. Transforming systems of consumption and production for achieving the sustainable development goals: moving beyond efficiency. *Sustainability Science*. 2018. Vol. 13, No. 6. P. 1533–1547. DOI: 10.1007/s11625-018-0582-1.
7. Çam S., Tuna M. F., Bayır T. Understanding green consumption: exploring the role of socially responsible attitudes in sustainable buying behavior. *Discover Sustainability*. 2025. Vol. 6. Art. 372. URL: <https://link.springer.com/article/10.1007/s43621-025-01223-8>.
8. Camilleri M. A., Cricelli L., Mauriello R., Strazzullo S. Consumer perceptions of sustainable products: A systematic literature review. *Sustainability*. 2023. Vol. 15, No. 11. Art. 8923. URL: <https://www.mdpi.com/2071-1050/15/11/8923>.
9. Chwialkowska A. An interplay of the consumption values and green behavior. *Business Strategy and the Environment*. 2024. URL: <https://www.diva-portal.org/smash/get/diva2:1823835/FULLTEXT02.pdf>.
10. Confente I., Scarpi D., Russo I. Marketing a new generation of sustainable products: The role of brand symbolism and consumer values. *Journal of Business Research*. 2020. Vol. 112. P. 203–215. URL: <https://www.sciencedirect.com/science/article/pii/S0148296319306186>.

11. Connolly J., Prothero A. Green consumption: Life-politics, risk and contradictions. *Journal of Consumer Culture*. 2008. Vol. 8, No. 1. P. 117–145. DOI: 10.1177/1469540507086422.
12. Gallo T., Pacchera F., Cagnetti C., Silvestri C. Do sustainable consumers have sustainable behaviors? An empirical study to understand the purchase of food products. *Sustainability*. 2023. Vol. 15, No. 5. Art. 4462. URL: <https://www.mdpi.com/2071-1050/15/5/4462>.
13. Gravelines Ž., Banytė J., Dovalienė A., Gadeikienė A. The role of green self-identity and self-congruity in sustainable food consumption behaviour. *Organizations and Markets in Emerging Economies*. 2022. URL: <https://www.econstor.eu/handle/10419/317212>.
14. Hargreaves T. Practicing behaviour change: Applying social practice theory to pro-environmental behaviour change. *Journal of Consumer Culture*. 2011. Vol. 11. P. 79–99. DOI: 10.1177/1469540510390500.
15. Johnstone M.-L., Tan L. P. Exploring the gap between consumers' green rhetoric and purchasing behaviour. *Journal of Business Ethics*. 2020. Vol. 132, No. 2. P. 311–328. DOI: 10.1007/s10551-014-2316-3.
16. Lange M., Jaeger S. R., Chervin C. et al. What influences the sustainable food consumption behaviours of university students? A systematic review. *International Journal of Public Health*. 2021. Vol. 66. P. 1–14. DOI: 10.3389/ijph.2021.1604149.
17. Li Y., Shan B. Exploring the role of health consciousness and environmental awareness in purchase intentions for green-packaged organic foods: an extended TPB model. *Frontiers in Nutrition*. 2025. Vol. 12. DOI: 10.3389/fnut.2025.1528016.
18. Pahari S., Chakraborty D., Polisetty A., Dash G., Camilleri M. A., Zhang J. Factors affecting consumer purchases of natural foods: Prioritizing health consciousness and environmental sustainability. *Business Strategy and the Environment*. 2024. Vol. 33, No. 8. DOI: 10.1002/bse.3913.
19. Pšurný M., Baláková I., Stávková J., Langr A. Perceived determinants of food purchasing behavior applicable for behavioral change toward sustainable consumption. *Frontiers in Sustainable Food Systems*. 2024. Vol. 7. DOI: 10.3389/fsufs.2023.1258085.
20. Shove E., Pantzar M., Watson M. The dynamics of social practice: Everyday life and how it changes. London : SAGE Publications, 2012. DOI: 10.4135/9781446250655.
21. United Nations Environment Programme. Plastic pollution. 2026. URL: <https://www.unep.org/plastic-pollution>.
22. United Nations. Transforming our world: The 2030 Agenda for Sustainable Development. 2015. URL: <https://sdgs.un.org/2030agenda>.

23. White K., Habib R., Hardisty D. J. How to SHIFT consumer behaviors to be more sustainable: A literature review and guiding framework. *Journal of Marketing*. 2019. Vol. 83, No. 3. P. 22–49. DOI: 10.1177/0022242919825649.
24. Warde A. The practice of eating. Cambridge : Polity Press, 2016. 220 p. URL: <https://books.google.com/books?id=0GiPCwAAQBAJ>.
25. Wijesinghe P. G. S. D., Baduge D. C. N., Светлічна М., Date M., Tusha R. Luxury wellness and medical tourism: A sustainable hospitality concept for Öland. *Grail of Science*. 2025. DOI: 10.36074/grail-of-science.23.05.2025.153.
26. Ахмаді А., Светлічна М. І. Таємниці довголіття шведів: спілкування, спорт та насолода життям. *Молодий вчений*. 2024. № 4 (128). С. 14–16. DOI: 10.32839/2304-5809/2024-4-128-31.
27. Светлічна М. І. Global tourism production systems: topical issues. *Гуманітарний і інноваційний ракурс професійної майстерності: пошуки молодих вчених*. 2023. DOI: 10.36059/978-966-397-357-9-53.
28. Светлічна М. І., Светлічний І. В. Enigmatic realm of global tourism production system. *Молодий вчений*. 2023. № 12 (124). С. 111–114. DOI: 10.32839/2304-5809/2023-12-124-18.

Information about the authors:

Svietlichna Mariia,

Bachelor

Linnaeus University

1, Universitetsplatsen, Kalmar, Sweden

Hjelm Jonathan,

Bachelor

Linnaeus University

1, Universitetsplatsen, Kalmar, Sweden

Wijesinghe P.G. Sachini D.,

Bachelor

Linnaeus University

1, Universitetsplatsen, Kalmar, Sweden

**ASSESSMENT OF THE CUMULATIVE
IMPACT OF WIND STATIONS ON SEASONAL
BIRD COMPLEXES WITHIN THE BOUNDARIES
OF MAN-MADE TERRITORIES
OF THE NORTHWESTERN AZOV REGION**

Sydorenko Andrii

DOI <https://doi.org/10.30525/978-9934-26-714-7-17>

INTRODUCTION

Bird migration corridors are among the most important ecological components to be considered during the planning and operation of wind energy facilities. Although wind power plays a key role in reducing greenhouse gas emissions and supporting the transition to renewable energy, poorly sited wind farms may adversely affect birds through collision mortality, habitat displacement, and barrier effects. Consequently, understanding the spatial and temporal characteristics of bird migration is essential for minimizing environmental impacts while ensuring sustainable wind energy development.

The Azov–Black Sea region of Ukraine represents one of the major migratory systems in Eastern Europe, forming part of the Adriatic–Black Sea flyway that connects breeding grounds across northern Eurasia with wintering areas in the Mediterranean Basin and Africa. Every year, millions of birds belonging to a wide range of ecological groups, including water birds, raptors, and passerines, pass through this region during seasonal migrations. However, migration intensity is spatially heterogeneous and largely determined by the distribution of coastal habitats, estuaries, river valleys, and suitable stopover sites¹.

The Zaporizhzhia Wind Farm is located within the Molochna–Korsak interfluvium, adjacent to the Azov–Black Sea coastal ecological corridor. Although this region lies close to one of Eastern Europe’s principal migration flyways, the actual distribution of migration routes and stopover areas at the local scale

¹ Sydorenko A. I., Gorlov P. I., Siokhin V. D. Assessment of the impact of technogenic monitoring sites on seasonal migration ornithocomplexes. *IOP Conference Series: Earth and Environmental Science*. 2022. Vol. 1049, No. 1. Art. 012060. DOI: <https://doi.org/10.1088/1755-1315/1049/1/012060>.

requires detailed assessment to evaluate potential interactions between birds and wind energy infrastructure. Such information is particularly important for estimating collision risk, barrier effects, and cumulative impacts associated with existing and planned wind farms.

This study evaluates the ornithological significance of the Zaporizhzhia Wind Farm area using long-term (2007–2022) field observations, migration surveys, and collision risk assessment. Particular attention is given to the spatial organization of seasonal migration, the identification of key stopover habitats, flight altitude distributions, and the potential cumulative effects of multiple wind farms operating within the region. The results provide a scientific basis for assessing the compatibility of wind energy development with bird conservation and contribute to evidence-based environmental management within one of the most important migratory regions of Eastern Europe.

1. Migration Corridors within the Zaporizhzhia Wind Farm Area

The Zaporizhzhia Wind Farm (WPP) is located within the Molochna–Korsak interfluvium, which forms part of one of the most important avian migration flyways in Eastern Europe—the Azov–Black Sea (Southern) Flyway. The significance of this migration route has been recognized since the late nineteenth century. It connects northern Africa and the Mediterranean Basin with southeastern Europe, western Siberia, and Kazakhstan through the seasonal movements of millions of migratory birds (Fig. 1).

More comprehensive ornithological studies conducted during the first half of the twentieth century identified two additional major migration routes across the territory of Ukraine. One of these, the Polissia Flyway, extends latitudinally across northern Ukraine and links the Pannonian Plain of Central Europe with the northern regions of Eurasia. The second route, oriented predominantly in a meridional direction, follows the floodplain of the Dnipro River, connecting the Azov–Black Sea Flyway with the Polissia Flyway. This corridor is used annually, primarily during spring migration, by several bird populations moving towards northern Eurasia².

In addition to these three principal flyways, several secondary meridional migration corridors exist, most of which follow the valleys of large and medium-sized rivers where suitable habitats provide opportunities for stopovers, allowing birds to replenish energy reserves or shelter during adverse weather conditions. The only major meridional corridor not associated with river valleys

² Черничко Й. І. Значення Азово-Чорноморського узбережжя України в підтримці внутрішньоматерикових міграційних шляхів куликів у Східній Європі. Частина 1. Особливості розміщення та міграції. *Вісник зоології. Монографічна серія*. Окремий випуск № 33. Київ, 2015. 120 с.

is the Bosphorus Flyway. This route is used predominantly by water birds, birds of prey, and several other taxa migrating from the Anatolian Peninsula across the Black Sea to Crimea, where it merges with the Azov–Black Sea Flyway³.

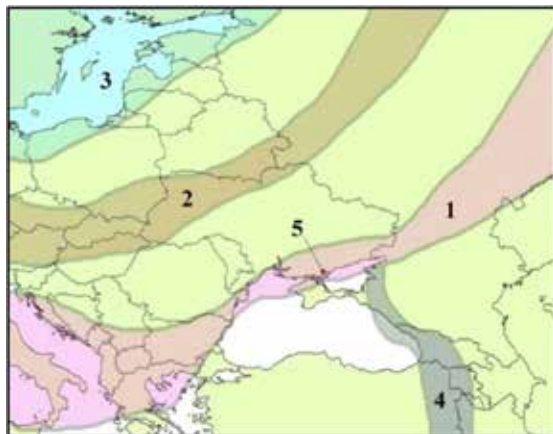


Fig. 1. Principal migration flyways of Eastern Europe:
(1) the Azov–Black Sea Flyway as part of the larger Adriatic–Black Sea Flyway; (2) the Polissia Flyway within the Central European–Siberian migration corridor; (3) the Baltic migration corridor forming part of the East Atlantic Flyway; (4) the eastern branch of the Black Sea–Mediterranean Flyway, including the Don–Caucasian migration corridor; (5) the Zaporizhzhia Wind Farm

Consequently, the spatial configuration of major flyways and subsidiary migration corridors is highly complex, as illustrated schematically in Fig. 2.

For the conservation of migratory birds, particularly water birds, an accurate understanding of the spatial organization of seasonal migration routes is essential during the planning and development of wind energy projects.

The widespread occurrence of birds across virtually all inland and especially coastal landscapes has led some researchers to question the existence of clearly defined migration corridors. When bird occurrence is considered without reference to species abundance, encounter frequency, or landscape characteristics, such an interpretation may appear plausible.

³ Черничко Й. І. Значення Азово-Чорноморського узбережжя України в підтримці внутрішньоматерикових міграційних шляхів куликів у Східній Європі. Частина 2. Біологічні та популяційні особливості міграцій окремих видів, міграційні шляхи та їх структура. *Вісник зоології. Монографічна серія*. Окремий випуск № 33. Київ, 2016. 258 с.

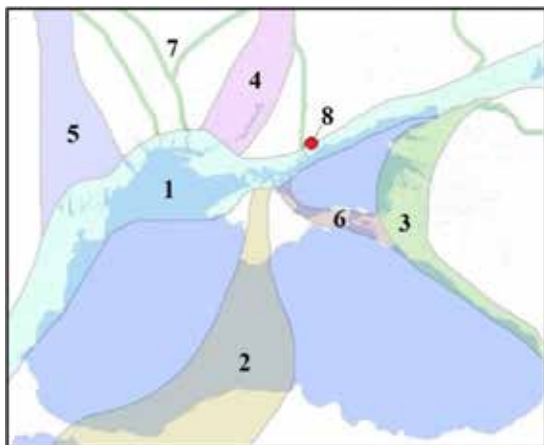


Fig. 2. Principal migration routes of southern Ukraine:
(1) Azov–Black Sea; (2) Crimean–Bosporus; (3) Don–Caucasian;
(4) Dnipro–Meshchera; (5) Prut–Dnister; (6) Taman; (7) secondary
migration corridors following river channels (shown in a common
colour); (8) the Zaporizhzhia Wind Farm

However, extensive ornithological investigations conducted during recent decades, covering large geographical regions and employing quantitative analyses of migration intensity, have demonstrated that exceptionally high concentrations of migrating birds occur only within specific geographical areas.

These findings have stimulated several hypotheses concerning the formation of migration corridors, including the guiding influence of coastlines and river basins, particularly for water birds.

More recently, the application of satellite telemetry and light-level geolocators has provided compelling evidence for well-defined spatial patterns in bird migration, confirming the existence of stable migration routes across broad geographic scales.

Although migration pathways may shift laterally from year to year in response to prevailing weather conditions, their overall spatial configuration remains remarkably stable and is characterized by a network of key stopover sites. The persistence of these stopover areas largely determines the long-term stability of migration routes, even for different populations of the same species.

This principle represents one of the fundamental concepts of practical ornithological management: effective conservation requires precise knowledge of which species or populations form the largest concentrations, where these concentrations occur, and during which migration season. Such information

The Azov–Black Sea Flyway, which constitutes the eastern continuation of the broader Adriatic–Black Sea Flyway, gradually merges into the Azov–Caspian migration system. Within Ukraine, one of the most important transcontinental ecological corridors for migratory birds is the Azov–Black Sea Coastal and Marine Ecological Corridor (Fig. 3). According to the regional ecological network of Zaporizhzhia Oblast, the Zaporizhzhia Wind Farm is situated adjacent to this corridor rather than directly within its core conservation areas⁴.

The ecological corridor extends for more than 150 km. Its maximum width, within the Molochnyi Lyman estuary and the adjacent sector of the Sea of Azov, approaches 50 km. The estimated area of the corridor within Zaporizhzhia Oblast is approximately 2.730 km². Nearly 90% of the corridor consists of open marine and estuarine waters, whereas slightly more than 10% comprises coastal spits and shoreline habitats (Fig. 3).

2. Seasonal Characteristics of Migration and Population Connectivity. Stopover Sites within the Study Region

Bird migration should be analysed within a seasonal framework because spring and autumn migrations differ fundamentally in terms of migration intensity, species composition, population structure, and geographical connectivity.

First, the number of migrating birds is influenced by population dynamics, including long-term fluctuations in breeding populations, as well as by weather conditions, which may either intensify migration or prolong its duration. The most important factor, however, is demographic structure. During spring migration, recently fledged juveniles are absent from the migratory population, whereas in autumn juvenile birds may constitute more than half of the migration flow in several species, particularly among passerines.

Second, the timing of spring migration is regulated by weather conditions and habitat availability in species such as waterfowl and herons, whereas in most bird species migration is initiated primarily by genetically controlled responses to increasing photoperiod. For species breeding at high latitudes of Eurasia, migration timing is additionally influenced by large-scale climatic processes and temperature anomalies associated with atmospheric circulation. Consequently, spring migration is generally more synchronized and occurs within a shorter time window than autumn migration.

⁴ Сіохін В. Д., Черничко Й. І., Сідоренко А. І., Горлов П. І., Алейнікова К. Г. Кумулятивна оцінка впливу вітрових станцій на природні комплекси в межах техногенних територій північно-західного Приазов'я. Повідомлення 2. Сезонні комплекси птахів. *Моніторинг та охорона біорізноманіття в Україні: прикладні аспекти моніторингу та охорони біорізноманіття. Серія «Conservation Biology in Ukraine»*. Київ ; Чернівці : Друк Арт, 2020. Вип. 16, т. 3. С. 152–168.

Third, many species and populations use different geographical routes during spring and autumn migration. Although the migration network across southern Ukraine appears highly complex, individual species often exhibit distinct patterns of geographical connectivity reflecting their evolutionary history, ecological requirements, and migration strategies.

Based on the evidence presented above, the principal potential stopover sites for migratory birds within the study area can be identified. Previous ornithological surveys conducted across selected parts of the study region have demonstrated that intrazonal landscape elements serve as important stopover habitats during seasonal migration. These areas provide opportunities for resting, intensive foraging, and the accumulation of fat reserves required for subsequent long-distance flights. The most important stopover sites are associated with the Molochnyi and Tubalskyi estuaries, river channels, and floodplain systems. Meadow habitats, pastures, and agricultural fields adjacent to river valleys also provide favourable conditions for migratory birds (Fig. 4).



Fig. 4. Distribution of the principal migratory stopover sites within the study area (1 – Zaporizhzhia Wind Farm)

Artificial forest plantations represent another important habitat type. Their greatest extent occurs along the western (right) bank of the Molochnyi Estuary, together with mature shelterbelts located mainly outside settlements. Particular attention should also be paid to the narrow coastal strip of the Sea of Azov, where dense concentrations of migratory passerines accumulate within shrub vegetation on undisturbed coastal sections during migration periods. Adjacent marine waters provide nocturnal roosting sites for geese, ducks, gulls, and

grebes. In addition to these species, gulls and other waterbirds perform regular daily movements between the coastal waters of the Sea of Azov and inland feeding areas or the shallow upper sections of estuaries, where suitable foraging conditions are available.

As shown in Figure 4, the majority of important stopover sites are associated with intrazonal estuarine and riverine landscape elements. Nevertheless, large aggregations occurring over the marine waters of the Sea of Azov also constitute a key component of the regional migration system.

Spring Migration. According to long-term ornithological observations conducted during 2009–2021, spring migration over the Molochnyi Estuary and its coastal zone begins in late February and generally ends during the final ten days of April. The principal migration directions of selected model species are presented in Figure 5.

The duration of continuous field observations in different years had relatively little influence on the cumulative number of species recorded within the study area. However, despite a cumulative total of 102 migratory species, annual species richness varied considerably, ranging from only 14 to as many as 69 species. These pronounced interannual differences reflect the combined effects of multiple environmental factors, among which weather conditions during March play the dominant role.

Species diversity along the Sea of Azov coastline is generally higher than within inland areas, although no substantial differences are observed in the total number of species recorded farther inland.

During March, the most abundant taxonomic groups comprise waterfowl (Anseriformes), passerines (Passeriformes), and shorebirds (Charadriiformes). Passerines consistently dominate either in terms of abundance or species richness because they account for more than half of the regional avifauna.

In April, the proportion of species migrating during the night increases substantially. Consequently, many shorebirds, passerines, and even geese become difficult to observe directly during migration flights. Their movements are more effectively monitored at stopover sites where birds rest or forage. During this period, the total number of recorded migratory species increases to approximately 110.

By early May, migration of most waterfowl, herons, grebes, and gulls has largely been completed, resulting in a reduction of transit migrants to approximately 37 species. Of these, 31 species are shorebirds, predominantly Arctic breeders, whose migration reaches its seasonal peak during early May. Migration intensity remains highest within the coastal zone due to extensive local bird movements. The farther observations are conducted inland, the lower the overall migration intensity becomes.

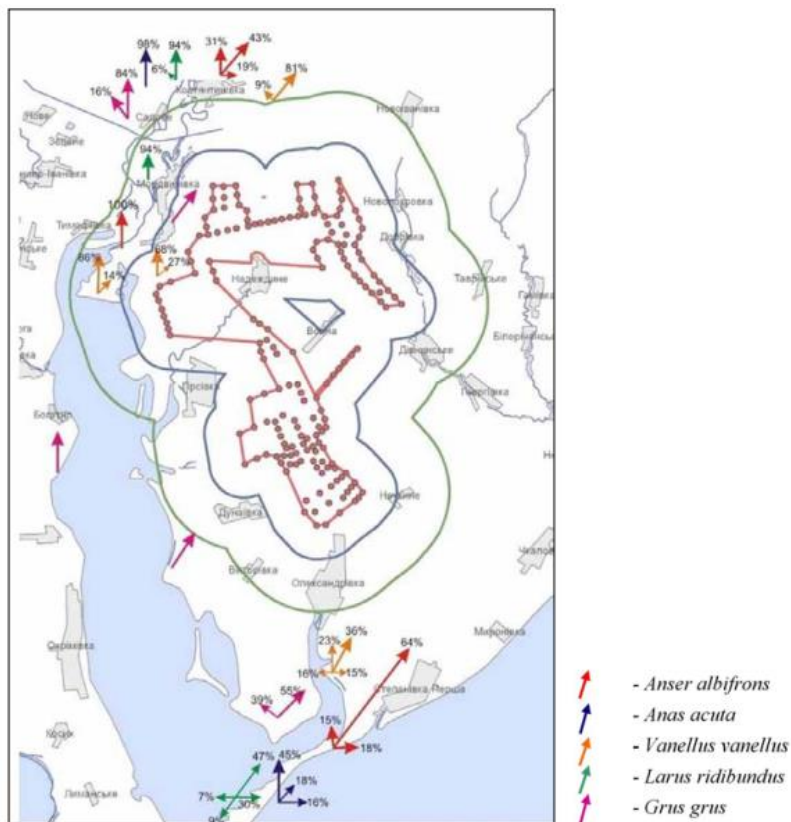


Fig. 5. Principal spring migration routes of selected waterbird species within different sectors of the Molochnyi Estuary (long-term mean values)

July–August. During July and August, the majority of transit migrants belong to shorebirds (Charadriiformes) and terns (Sternidae), although several duck species, including the Garganey (*Anas querquedula*), also initiate seasonal migration during this period. Most other abundant species form post-breeding concentrations on wetlands and perform only short-distance foraging flights. At this time, the principal migration route for most species follows the Azov Sea coastline, whereas only isolated flocks or single individuals migrate along the valleys of small rivers and ravines (Fig. 6).



Fig. 6. Main flight directions in July–August

Autumn migration along the Azov–Black Sea coast generally begins during the second half of July. Migration intensity gradually increases from mid-August, reaches its maximum during September and the first half of October, and usually ends by the middle of November. Subsequently, during the second half of November and early December, pre-winter movements occur, involving primarily geese and ducks arriving from continental regions where deteriorating weather conditions force birds to relocate towards milder coastal habitats. Similar seasonal movements are also characteristic of Common Starling (*Sturnus vulgaris*), thrushes, Rook (*Corvus frugilegus*), and several other passerine species.

The intensity of bird movements across the wind farm area begins to increase markedly in September owing to the migration of ducks, diurnal raptors, starlings, and wagtails. During late September and October, these movements are supplemented by migrating geese and cranes, while in November ducks and geese constitute the dominant component of migration across the wind farm.

A notable characteristic of autumn migration is that the majority of passerine species follow the elevated western bank of the Molochnyi Estuary. Only larks and swallows migrate across the wind farm in a broad front, but the operational

wind turbines exert virtually no measurable influence on these species. Moreover, swallows and several falcon species regularly use wind farm infrastructure, including temporarily inactive turbines, as resting perches during migration.

During the night, most transit migrants fly at considerably higher altitudes than those occupied by wind turbines. Only flocks stopping at the estuary or adjacent coastal habitats may occasionally descend into the rotor-swept zone. The probability of such events, however, is estimated to be below 0.1%.

September–October. September and the first half of October represent the peak migration period for the majority of bird species, including not only waterbirds but also numerous terrestrial ecological groups. During this period, migration intensity increases markedly over the Molochna River valley and across most sections of the wind farm. Birds migrate along a broad front extending approximately 30–50 km inland from the coastline (Fig. 7).

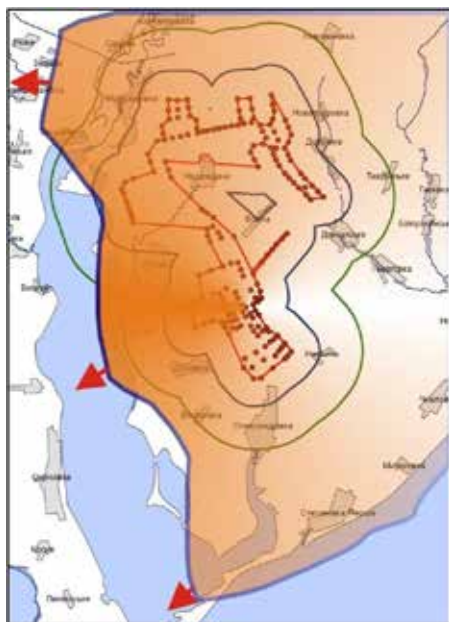


Fig. 7. Main flight directions in September–October

Late October–November. The second half of October and November marks the completion of Common Crane (*Grus grus*) migration and the period of maximum migration intensity for the Greater White-fronted Goose (*Anser albifrons*), Great Bustard (*Otis tarda*), several duck species, particularly

diving ducks, Northern Pintail (*Anas acuta*), Common Goldeneye (*Bucephala clangula*), Rook (*Corvus frugilegus*), Common Starling (*Sturnus vulgaris*), and other species. With regard to migration corridors and flight altitudes, no substantial changes occur compared with the previous period, except for a considerable increase in local bird movements within the coastal zone of the Azov Sea. In addition to the dominant latitudinal migration routes, this period is characterized by the emergence of a pronounced north–south migration corridor associated primarily with the seasonal movements of ducks and geese from continental regions of Ukraine (Fig. 8).

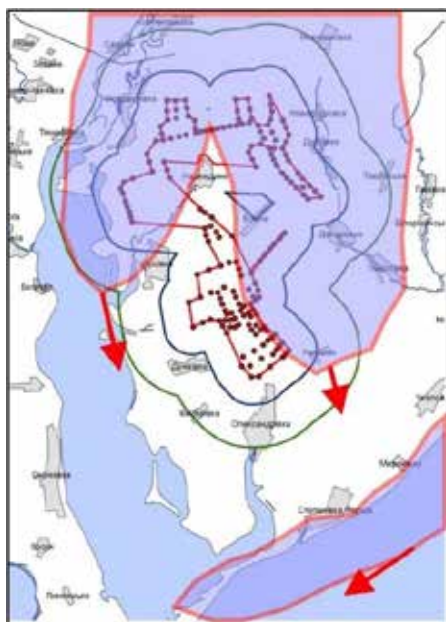


Fig. 8. Main flight directions in November

Thus, we observe the following situation: the regional migration system involves 265 bird species, including 37–110 species recorded during spring migration and 65–184 species during autumn migration. The taxonomic composition remains relatively stable and includes representatives of 13 avian orders, with waterfowl (Anseriformes), shorebirds (Charadriiformes), and passerines (Passeriformes) consistently representing the most abundant groups.

Daily migration intensity is strongly influenced by the geographical location of the survey area. The highest migration activity is consistently observed in

coastal habitats. During spring, daily numbers range from approximately 0.7 to 2.1 thousand individuals, while during autumn migration the abundance increases substantially to 1.2–20.8 thousand individuals per day. Daily migration patterns also differ seasonally. In spring, the highest migration intensity occurs during the morning hours throughout the migration period. In contrast, during July and September, evening migration exceeds both morning and daytime activity. Nevertheless, the first hour after sunrise consistently represents the period of highest migration intensity throughout the year.

Flight altitude depends not only on the geographical position of the study site but also on the species composition of migrating birds. The proportion of birds crossing the monitored areas within the rotor-swept zone potentially posing a collision risk to wind turbines averages 13.6% during spring migration and 6.3% during autumn migration.

The closer a study site is located to the seacoast, the greater the diversity of migration directions observed. This pattern reflects the high proportion of local daily movements associated with feeding, roosting, and stopover behaviour in coastal habitats.

The assessment of migration intensity within the study area should consider not only the absolute abundance of birds recorded in different functional landscape zones but also the spatial distribution of migratory movements. The principal conclusion consistently supported by long-term observations is that the highest migration intensity occurs within the coastal strip due to extensive local bird movements associated with marine and estuarine habitats. Migration intensity progressively decreases with increasing distance inland from the Azov Sea coast.

3. Cumulative Impacts within the Wind Farm Area

Wind turbines located along migration flyways, long-distance flight routes, or regular local movements between feeding, roosting, and breeding sites may potentially interfere with bird movements and create a barrier effect⁵.

To evaluate the actual significance of this effect, the results of long-term studies conducted at the Nysted offshore wind farm in Denmark provide an illustrative example. Since 2005, migratory flight routes in the vicinity of the wind farm have been monitored using radar techniques. Each year, approximately 200,000–300,000 geese migrate through the area during spring and autumn while travelling to agricultural feeding grounds⁶. Monitoring began prior to wind farm construction and has continued throughout its operational

⁵ European Commission. Guidance document on wind energy developments and EU nature legislation. Brussels : Publications Office of the European Union, 2020. 184 p. DOI: <https://doi.org/10.2779/095188>.

⁶ Gémard C., Duriez O., Chappe O., Duclos G., Besnard A. Towards a better understanding of avian collision in wind energy facilities using automatic detection systems. *Journal of Applied Ecology*. 2025. Vol. 62, No. 6. P. 1437–1448. DOI: <https://doi.org/10.1111/1365-2664.70055>.

period. The results demonstrate that birds consistently avoid the wind farm by deviating approximately 500 m from their original flight paths. Considering that the overall migration distance exceeds 1.400 km, this detour results in only a minor increase in energy expenditure regardless of species endurance. Even the need to circumvent approximately 160 similar wind farms along an entire migration route would result in an estimated body mass loss of only about 1%⁷.

As illustrated in Figures 9 and 10, migrating birds are able to detect and avoid wind farms during flight.

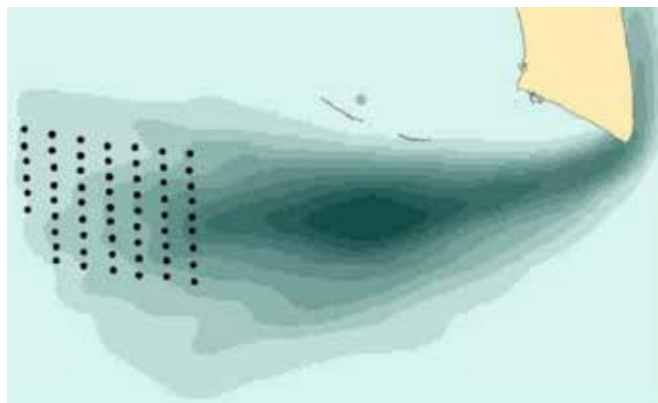


Fig. 9. Flight trajectories of migratory birds at the Nysted offshore wind farm (Denmark) before construction. Black dots indicate the planned turbine locations, while grey dots represent radar detections of birds⁸

These findings indicate that the barrier effect should be regarded as a significant impact only under specific circumstances, particularly where intensive local bird movements occur or where multiple large wind farms are concentrated over extensive areas along major seasonal migration routes.

Numerous studies have documented avoidance behaviour in many bird species, especially waterbirds and passerines. However, this response is strongly species-specific. During daylight hours, birds generally maintain distances of approximately 100–3.000 m from wind farms, whereas at night these avoidance

⁷ Keane A., Nisbet I., Calvo G., Pickering G., Tulloch J., More G., Koronka N. Wind farm cumulative induction zone effect and the impact on energy yield estimation. *Renewable Energy*. 2022. Vol. 181. P. 1209–1222. DOI: <https://doi.org/10.1016/j.renene.2021.09.056>.

⁸ Petersen I. K., Christensen T. K., Kahlert J., Desholm M., Fox A. D. Final Results of Bird Studies at the Offshore Wind Farms at Nysted and Homs Rev, Denmark. Rønde : National Environmental Research Institute, 2006. 161 p. URL: https://tethys.pnnl.gov/sites/default/files/publications/NERI_Bird_Studies.pdf (дата звернення: 08.07.2026).

distances may be considerably reduced⁹. Although avoidance behaviour clearly reduces the immediate risk of collision, altered flight routes inevitably require additional time and energy expenditure. In theory, repeated energetic costs over long periods could influence individual condition and consequently affect demographic parameters such as survival and reproductive success.

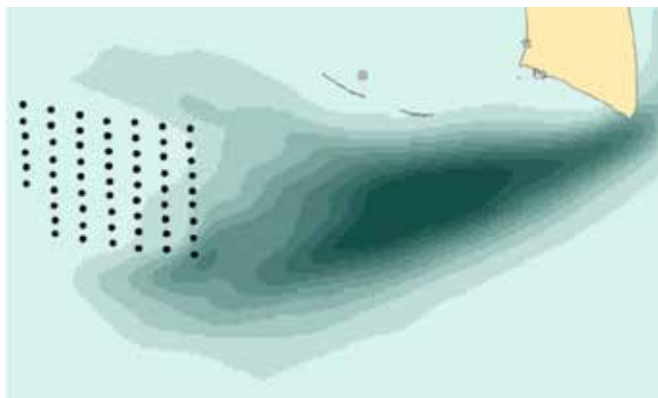


Fig. 10. Flight trajectories of migratory birds at the Nysted offshore wind farm (Denmark) during the operational phase. Black dots indicate turbine locations, while grey dots represent radar detections of birds¹⁰

Available scientific evidence nevertheless indicates that the barrier effect alone is unlikely to have measurable consequences at the population level¹¹. However, potential cumulative impacts resulting from multiple wind farms located along a migration corridor should not be disregarded¹². The magnitude of the barrier effect depends largely on wind farm design, including turbine dimensions, spatial configuration, and distances between turbines. Consequently, optimization of wind farm layout is widely recognized as one of the most effective measures for minimizing interference with bird migration.

⁹ Christiansen N., Daewel U., Schrum C. Cumulative hydrodynamic impacts of offshore wind farms on North Sea currents and surface temperatures. *Communications Earth & Environment*. 2026. Vol. 7. Art. 164. DOI: <https://doi.org/10.1038/s43247-026-03186-8>.

¹⁰ Petersen I. K., Christensen T. K., Kahlert J., Desholm M., Fox A. D. Final Results of Bird Studies at the Offshore Wind Farms at Nysted and Horns Rev, Denmark. Ronde : National Environmental Research Institute, 2006. 161 p. URL: https://tethys.pnnl.gov/sites/default/files/publications/NERI_Bird_Studies.pdf (дата звернення: 08.07.2026).

¹¹ Drewitt A. L., Langston R. H. W. Collision effects of wind-power generators and other obstacles on birds. *Annals of the New York Academy of Sciences*. 2008. Vol. 1134. P. 233–266.

¹² Yordanov N., Zehindjiev P., Whitfield D. P. Long-term decline in bird collisions at operational wind farms: evidence from systematic monitoring to support sustainable wind energy development (2010–2024). *Sustainability*. 2026. Vol. 18, No. 2. Art. 992. DOI: <https://doi.org/10.3390/su18020992>.

Birds flying within the rotor-swept zone frequently avoid potential collisions by altering either their flight direction or flight altitude. The energetic cost of bypassing a single wind turbine is generally negligible because the resulting detour typically increases the flight path by only 5–10% relative to the original trajectory. Considering that most migration routes extend over more than 1,000 km, such additional energy expenditure is minimal and comparable to that required to compensate for lateral wind drift^{13, 14}.

Early assessments estimated that approximately 99.5% of birds successfully avoid collisions with wind turbines, implying that only about 0.5% enter the rotor-swept zone. More conservative assessments subsequently reduced the avoidance rate to 98.0%, providing a precautionary estimate for collision risk modelling. For the White-tailed Eagle (*Haliaeetus albicilla*), the avoidance rate has been estimated at 92.5%¹⁵.

4. Barrier Effects and Cumulative Impacts of Wind Farms on Birds

During ornithological surveys conducted within the Zaporizhzhia Wind Farm project area, particular attention was given to assessing the barrier effect and potential flight obstruction. Bird species using the wind farm site as a feeding area generally fly at altitudes below 50 m above ground level. Consequently, the operational impact of wind turbines on these local movements is considered low and, for most species, negligible. In contrast, the technical characteristics of wind turbines, particularly the rotor-swept area, create a potential collision hazard for migratory birds flying within the altitude range of approximately 50–170 m (or, depending on turbine specifications, 40–180 m).

According to ornithological surveys conducted between September 2017 and May 2018 using fixed observation points and standardized transects, a total of 10,258 migrating birds were recorded within the project area. Most individuals (8,065; 78.62%) flew below the rotor-swept zone. A further 1,750 individuals (17.06%) migrated above the operational height of the turbines, typically at altitudes of 300–400 m. Only 443 individuals (4.32%) were observed within the rotor-swept zone, where collision risk exists.

Surveys carried out in 2019 revealed a similar pattern. A total of 21,250 birds were recorded during migration, of which 16,755 individuals (78.85%) flew

¹³ Masden E. A., Cook A. S. C. P., McCluskie A., Bouten W., Burton N. H. K., Thaxter C. B. When speed matters: The importance of flight speed in an avian collision risk model. *Environmental Impact Assessment Review*. 2021. Vol. 90. Art. 106622. DOI: <https://doi.org/10.1016/j.eiar.2021.106622>.

¹⁴ Desholm M., Fox A. D., Beasley P. D. L., Kahlert J. Remote techniques for counting and estimating the number of bird–wind turbine collisions at sea: a review. *Ibis*. 2006. Vol. 148, Suppl. 1. P. 76–89.

¹⁵ May R., Hoel P. L., Langston R., Dahl E. L., Bevanger K., Reitan O., Nygård T., Pedersen H. C., Røskoft E., Stokke B. G. Collision Risk in White-tailed Eagles: Modelling Collision Risk Using Vantage Point Observations in Smøla Wind-power Plant. NINA Report No. 639. Trondheim : Norwegian Institute for Nature Research, 2010.

below rotor height, while 4.400 individuals (20.71%) were recorded above the turbines. Only 95 birds (0.44%) were observed within the collision-risk zone.

These results accurately reflect the general ornithological situation in the region and are consistent with observations from other wind farm sites. Long-term monitoring conducted at the operational Botiieve Wind Farm between 2013 and 2019 demonstrated that the overwhelming majority of birds moved outside the collision-risk zone, either below 50 m above ground level or above 150 m, and most frequently at altitudes exceeding 200 m. Only 252 individuals (0.86%) were recorded within the potentially hazardous altitude interval of 51–150 m, indicating that bird movements through the rotor-swept zone were infrequent and represented a typical migration pattern for the region^{16, 17}.

Bird movements within potentially hazardous flight altitudes were not recorded every year. No birds were observed within the 51–150 m altitude interval during 2013, 2015, 2016, 2018 or 2021. In the remaining years, the proportion of birds flying within the collision-risk zone never exceeded 6%, accounting for 3.64% in 2014, 5.90% in 2017, 4.35% in 2019 and 4.44% in 2020. Similar patterns have also been documented during three-year monitoring programmes at the Prymorska, Orlivska, and Prymorska-2 wind farms.

Cumulative impacts may arise when several wind farms and their associated infrastructure are located within areas supporting consistently high bird abundance, along major migration corridors, or where wind energy developments interact with other forms of human activity. Importantly, cumulative impact should not be interpreted simply as the arithmetic sum of impacts from individual wind farms. Depending on ecological conditions and species-specific responses, cumulative effects may be either greater or smaller than the sum of individual project impacts.

For example, a single wind farm may cause a relatively low level of bird mortality that remains well within the natural recovery capacity of the affected population and therefore has little influence on its long-term viability. However, when mortality associated with multiple wind farms is combined, total losses may exceed the population's capacity for compensation, ultimately resulting in population decline. Consequently, although the impacts of individual projects may each be considered insignificant, their combined effect may become

¹⁶ Горлов П. І., Сіохін В. Д., Долинний В. І., Сидоренко А. І. Сезонні орнітологічні особливості території Ботієвського вітропарку (Запорізька область) за результатами спостережень у весняні періоди 2013–2014 років. *Бранта* : збірник наукових праць Азово-Чорноморської орнітологічної станції. 2014. № 17. С. 19–38. URL: <https://branta.org.ua/ua/branta-issues/branta-17/17-02.html> (дата звернення: 08.07.2026).

¹⁷ Горлов П. І., Сидоренко А. І., Сіохін В. Д. Багаторічний орнітологічний моніторинг на Ботієвській вітровій станції як об'єктивна складова оцінки впливу вітропарку на птахів. *Вісник Біосферного заповідника «Асканія-Нова»*. 2016. Т. 18. С. 116–130.

ecologically significant and influence planning decisions for both existing and proposed developments.

The principal objective of cumulative impact assessment is therefore to determine the threshold at which the combined effects of habitat loss (including permanent habitat removal), habitat fragmentation, barrier effects associated with increased energetic costs of flight, and collision mortality become ecologically significant.

Cumulative assessment must also consider the interaction of the proposed development with other existing or planned projects within the surrounding landscape, including developments unrelated to wind energy. While the environmental effect of an individual project may be negligible, its combination with other plans or activities may produce significant cumulative consequences. Habitat fragmentation should likewise be incorporated into cumulative impact assessments because it may adversely affect population structure, connectivity, and long-term demographic dynamics across a broad range of bird species.

During the planning phase of the Zaporizhzhia Wind Farm and the associated 330 kV transmission line, comprehensive ornithological field surveys were conducted in 2014–2016 and 2017–2018 to identify local bird populations and develop appropriate mitigation measures. Based on the survey results, the avifauna of the study area was found to be typical of the region. Most of the project area consists of agricultural landscapes, including arable fields and shelterbelts. Consequently, the areas designated for the wind farm and its associated infrastructure – including underground cable lines and the 330 kV overhead transmission line connecting the proposed Nadezhdyne substation to the existing Melitopol 330 kV substation – were not considered to be of high conservation value or of particular importance for biodiversity protection.

Monitoring conducted at the Zaporizhzhia Wind Farm during 2017–2018 recorded a relatively large number of birds (13,113 individuals). However, this result primarily reflects the high intensity of ornithological observations rather than an exceptional attractiveness of the project area for birds. This conclusion is supported by the very low proportion of rare species recorded during the survey period. Between September 2017 and May 2018, species of conservation concern accounted for only 0.37% of all recorded individuals. Subsequent monitoring in 2019–2021 likewise revealed no important local migration routes crossing either the wind farm site or the associated 330 kV transmission line. Accordingly, both facilities were assessed as unlikely to produce significant adverse impacts on birds and were considered environmentally acceptable within the proposed project boundaries.

The abundance of threatened bird species within the project area was consistently low. To evaluate conservation significance, three principal conservation reference

lists were analysed: the Red Data Book of Ukraine, the European Red List, and the IUCN Red List of Threatened Species. Particular attention was given to species classified as Critically Endangered (CR) or Endangered (EN) by the IUCN, as well as species listed as Endangered in the Red Data Book of Ukraine. The spatial distribution of these threatened species recorded within the project area between 2007 and 2021 is presented in Figure 11. In total, 111 individuals representing seven threatened species were recorded. The Eurasian Curlew (*Numenius arquata*) was the dominant species, accounting for 58 individuals (52.3%), followed by the Pallas's Gull (*Larus ichthyaetus*) with 22 individuals (19.8%) and the European Roller (*Coracias garrulus*) with 15 individuals (13.5%).

As illustrated in Figure 11, most records of bird species classified as Endangered or Critically Endangered were concentrated in adjacent wetlands, particularly the Tubalskyi Estuary and the upper reaches of the Molochnyi Estuary. Only four individuals (3.6%) representing two threatened species were recorded within the actual project area.

Cumulative impacts resulting from several neighbouring wind energy projects may become significant where turbine layouts overlap with key bird habitats, including important feeding areas, migration corridors, regular commuting routes between feeding and breeding sites, or major stopover locations. However, the available monitoring data indicate that the wind farm complex investigated in this study is not situated along any major migration corridor and is therefore unlikely to interfere with regular bird movements. Furthermore, because the individual wind farms are located within extensive agricultural landscapes and are spatially separated, their cumulative effects on bird populations are expected to remain relatively limited¹⁸.

Collision Risk Modelling (CRM)¹⁹ predicted that the Zaporizhzhia Wind Farm would result in approximately 31 bird fatalities per year, assuming the operation of 167 wind turbines. Importantly, monitoring conducted during the construction phase demonstrated that predicted collisions primarily involved common and abundant species typical of open agricultural landscapes, including the Eurasian Skylark (*Alauda arvensis*), Common Buzzard (*Buteo buteo*), Common Swift (*Apus apus*), and Northern Raven (*Corvus corax*). In addition, several water-associated species recorded within the project area, including the Yellow-legged Gull (*Larus cachinnans*) and Grey Heron (*Ardea cinerea*), were also identified as potentially susceptible to collisions.

¹⁸ Josimović B., Bezbradica L., Manić B., Sрниć D., Srebrić N. Cumulative impact of wind farm noise. *Applied Sciences*. 2023. Vol. 13, No. 15. Art. 8792. DOI: <https://doi.org/10.3390/app13158792>.

¹⁹ Scottish Natural Heritage. Use of Avoidance Rates in the SNH Wind Farm Collision Risk Model. SNH Avoidance Rate Information and Guidance Note. Inverness : SNH, 2024. URL: <https://www.nature.scot/doc/guidance-using-updated-collision-risk-model-assess-bird-collision-risk-onshore-wind-farms> (дата звернення: 08.07.2026).

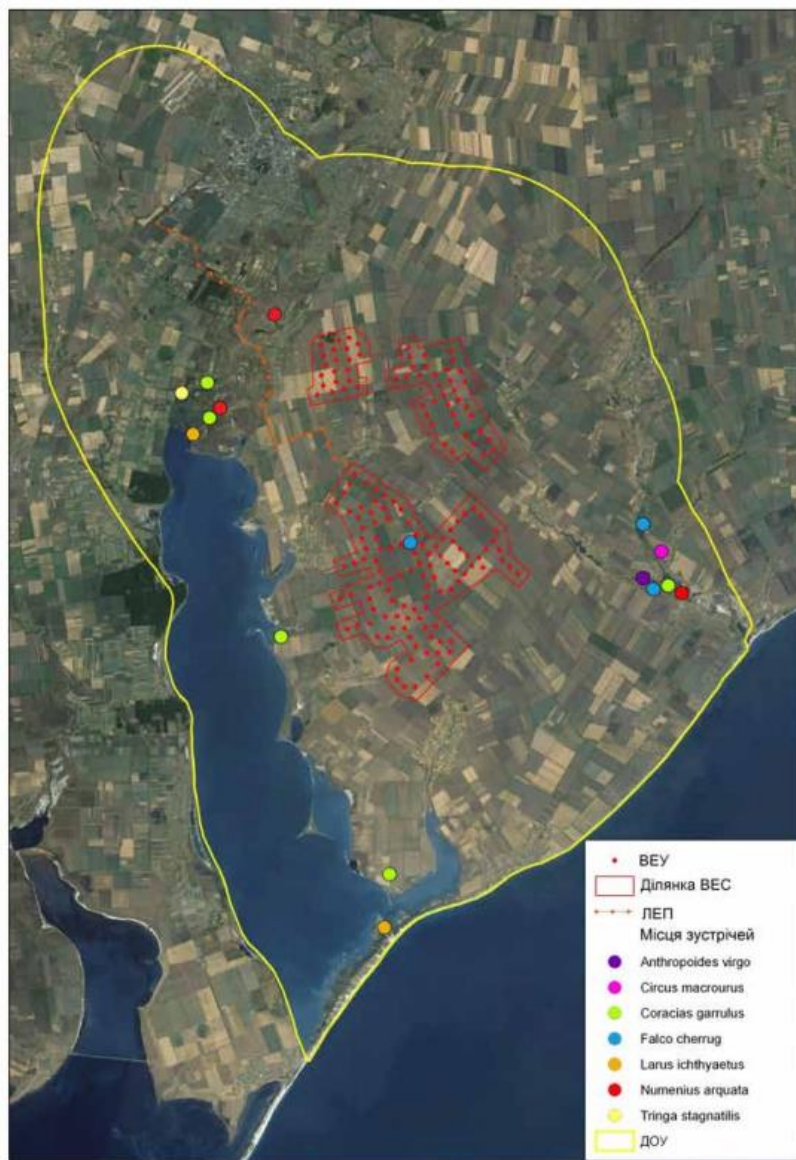


Fig. 11. Distribution of bird species classified as Endangered or Critically Endangered within the environmental assessment area during 2007–2021

Unfortunately, no ornithological monitoring data are available for the Azov Wind Farm (“Zofia”) for 2019, including information on bird abundance, species composition, or migration intensity within the project area. Consequently, it is not possible to estimate collision mortality directly for this facility. Nevertheless, according to published estimates²⁰, wind farms cause an average mortality of approximately 0.1 bird per MW of installed capacity per year. Assuming an installed capacity of 750 MW and approximately 167 operating wind turbines, the Zofia Wind Farm could theoretically result in about 75 bird fatalities annually. Owing to the similar landscape characteristics of both wind farms, the assemblage of species potentially affected at the Zofia Wind Farm is expected to be broadly comparable to that recorded at the Zaporizhzhia Wind Farm.

Potential Biological Removal (PBR)²¹ analysis further demonstrated that the predicted collision mortality at the Zaporizhzhia Wind Farm remains below the maximum biologically sustainable level for all assessed species. Consequently, the expected losses are not anticipated to have measurable adverse effects on the long-term viability of local or regional bird populations.

If the predicted annual mortality is expressed as a percentage of the species-specific Potential Biological Removal (PBR) threshold, a similar pattern emerges. For most species, this proportion is very low, ranging from 0.04% to 9.24%. Consequently, the combined mortality predicted for the Zaporizhzhia and Azov wind farms is also unlikely to exceed the allowable PBR threshold, since most species occurring in the area do not use fixed migration corridors and are widely distributed throughout the region.

Theoretically, the spatial arrangement of turbines belonging to several neighbouring wind farms could alter the foraging behaviour and breeding territory configuration of certain species, particularly birds of prey, through displacement effects. However, the abundance of these species in the vicinity of the wind farms considered in this study is relatively low; therefore, this potential impact is expected to be negligible.

Large numbers of wind turbines may also potentially create a barrier to migrating birds. Nevertheless, the study area is situated outside the principal migration routes. Although the Zaporizhzhia Wind Farm and the Zophia Wind Farm are located relatively close to one another (the shortest distance between

²⁰ Ogłęcki P., Dąbrowski P., Gnatowski T., Małuszyńska I., Małuszyński M. J., Przybyłowski J., Pawluśkiewicz B. Bird collision risk at the interface of solar and wind farms – a cumulative effect? *Journal of Water and Land Development*. 2025. No. 66. P. 152–161. DOI: <https://doi.org/10.24425/jwld.2025.155311>.

²¹ Punt A. E., Siple M., Francis T. B., Hammond P. S., Heinemann D., Long K. J., Moore J. E., Sepúlveda M., Reeves R. R., Sigurðsson G. M., Vikingsson G., Wade P. R., Williams R., Zerbini A. N. Robustness of potential biological removal to monitoring, environmental, and management uncertainties. *ICES Journal of Marine Science*. 2020. Vol. 77, Issue 7–8. P. 2491–2507. DOI: <https://doi.org/10.1093/icesjms/fsaa096>.

them is approximately 12 km), the turbine layout provides sufficient open space for bird movements. The main migration corridor between the two facilities, extending over the waters of the Molochnyi Estuary, remains unaffected by either project. Furthermore, numerous studies have demonstrated that 92.5–98% of birds are able to avoid wind turbines located in open agricultural landscapes, even during regular migratory flights. With respect to the Botiieve Wind Farm, the distances separating it from the two aforementioned projects (15.5 km and 37 km, respectively) are sufficiently large to preclude any meaningful barrier effect in the conventional sense. Long-term monitoring conducted by the authors has shown that bird concentrations within the Botiieve Wind Farm and adjacent areas reach their highest levels along the coastal zone and over the Sea of Azov, exhibiting behavioural patterns very similar to those documented at the Nysted Offshore Wind Farm in Denmark (Figs. 9–10)²².

Moreover, the wind turbines are located within extensive agricultural landscapes corresponding to areas of relatively low bird density. Consequently, the partial loss of these habitats is unlikely to be ecologically significant, even when potential displacement effects resulting in reduced breeding bird densities near turbines are taken into account. Habitat transformation associated with wind farm construction, including the development of access roads and crane pads, may locally influence the abundance of bird species characteristic of open, dry agricultural habitats associated with human activities, such as the Corn Bunting (*Emberiza calandra*), European Stonechat (*Saxicola rubicola*), and Crested Lark (*Galerida cristata*).

Overall, the wind farms considered in this assessment are not expected to cause significant increases in mortality of species of conservation concern, substantial habitat loss, changes in land-use patterns, or cumulative barrier effects²³.

Despite the implementation of mitigation measures, a residual risk of collisions between birds or bats and wind turbines cannot be completely eliminated. Therefore, post-construction monitoring has been planned for the above-mentioned projects. The results of this monitoring will provide an empirical basis for evaluating the operational impacts of the wind farms on bird and bat populations.

²² Williams K. A., Gulka J., Cook A. S. C. P., Diehl R. H., Farnsworth A., Goyert H., Hein C., Loring P., Mizrahi D., Petersen I. K., Peterson T., Press K. M., Stenhouse I. J. A framework for studying the effects of offshore wind energy development on birds and bats in the Eastern United States. *Frontiers in Marine Science*. 2024. Vol. 11. Article 1274052. DOI: <https://doi.org/10.3389/fmars.2024.1274052>.

²³ Горлов П. І., Сіохін В. Д. Аналіз міжнародного досвіду вивчення впливу вітрових електростанцій на птахів. *Біологічний вісник Мелітопольського державного педагогічного університету імені Богдана Хмельницького*. 2012. № 1. С. 37–47.

CONCLUSIONS

This study demonstrates that the Zaporizhzhia Wind Farm is located adjacent to the Azov–Black Sea migration system, one of the principal flyways in Eastern Europe, while the wind farm itself is situated outside the areas of highest migration intensity. Seasonal bird movements are concentrated primarily along the coastal zone of the Sea of Azov, the Molochnyi Estuary, and associated river valleys, whereas the project area is characterized by comparatively low migration density and the absence of stable, high-intensity migration corridors.

Long-term ornithological monitoring confirmed that the overwhelming majority of birds crossed the study area either below or above the rotor-swept zone, with only a small proportion of individuals recorded within collision-risk heights. The observed flight altitude distribution was consistent across monitoring years and comparable to results obtained at other wind farms in the Azov–Black Sea region, indicating that the project area presents a relatively low level of collision risk.

The analysis of barrier effects indicates that birds generally avoid wind turbines by making minor adjustments to their flight direction or altitude, resulting in negligible additional energetic costs. Consequently, under the existing turbine layout, the Zaporizhzhia Wind Farm is unlikely to create a significant obstacle to seasonal migration or to disrupt ecological connectivity between key migration stopover sites.

Assessment of cumulative impacts further suggests that the combined operation of the Zaporizhzhia Wind Farm with neighbouring wind energy projects is not expected to result in biologically significant adverse effects. The spacing between wind farms, the preservation of major migration corridors, the predominantly agricultural character of the surrounding landscape, and the low density of sensitive species collectively reduce the likelihood of cumulative barrier effects or population-level consequences.

Collision Risk Modelling (CRM) and Potential Biological Removal (PBR) analyses indicate that the predicted annual mortality remains well below biologically sustainable thresholds for all assessed species. The projected collision rates represent only a small fraction of the allowable population losses defined by PBR, suggesting that wind farm operation is unlikely to compromise the long-term viability of local or migratory bird populations.

Rare and threatened bird species were recorded only sporadically within the project area, with the majority of observations occurring in adjacent wetlands rather than within the wind farm itself. This spatial distribution further supports the conclusion that the project does not overlap with habitats of critical conservation importance.

Overall, the available evidence indicates that the Zaporizhzhia Wind Farm has a low ornithological impact with respect to collision risk, habitat loss, barrier effects, and cumulative impacts. Nevertheless, continued post-construction monitoring remains essential to verify predicted impact levels, detect any long-term changes in bird behaviour or migration patterns, and support adaptive management. Such an approach will ensure that renewable energy development is implemented in accordance with biodiversity conservation objectives and international best practices for environmentally sustainable wind energy planning.

SUMMARY

The rapid expansion of wind energy requires reliable assessments of its potential impacts on migratory bird populations, particularly within internationally important flyways. This study evaluates the ornithological significance of the Zaporizhzhia Wind Farm, located adjacent to the Azov–Black Sea migratory corridor in southeastern Ukraine. Long-term field observations, migration surveys, flight altitude analyses, and collision risk assessments were used to characterize seasonal bird movements and estimate potential impacts of wind turbines. The results demonstrate that the highest migration intensity is concentrated along the Sea of Azov coastline, estuaries, and river valleys, whereas the wind farm is situated outside the principal migration corridor. Most birds were recorded either below or above the rotor-swept zone, while only a small proportion of individuals crossed the area at collision-risk heights. The predicted collision mortality remained within biologically acceptable limits according to Collision Risk Modelling (CRM) and Potential Biological Removal (PBR) assessments. Rare and threatened bird species occurred only sporadically within the project area and were primarily associated with adjacent wetland habitats. Evaluation of cumulative impacts indicated that the existing configuration of neighbouring wind farms is unlikely to create significant barrier effects or adversely affect bird populations at the regional scale. The findings suggest that the Zaporizhzhia Wind Farm has a low overall impact on migratory birds when appropriate siting principles and environmental planning measures are applied. Continued post-construction monitoring is recommended to verify long-term predictions and support adaptive biodiversity management.

Bibliography

1. Sydorenko A. I., Gorlov P. I., Siokhin V. D. Assessment of the impact of technogenic monitoring sites on seasonal migration ornithocomplexes. *IOP Conference Series: Earth and Environmental Science*. 2022. Vol. 1049, No. 1. Art. 012060. DOI: <https://doi.org/10.1088/1755-1315/1049/1/012060>.

2. Черничко Й. І. Значення Азово-Чорноморського узбережжя України в підтримці внутрішньоматерикових міграційних шляхів куликів у Східній Європі. Частина 1. Особливості розміщення та міграції. *Вісник зоології. Монографічна серія*. Окремий випуск № 33. Київ, 2015. 120 с.

3. Черничко Й. І. Значення Азово-Чорноморського узбережжя України в підтримці внутрішньоматерикових міграційних шляхів куликів у Східній Європі. Частина 2. Біологічні та популяційні особливості міграцій окремих видів, міграційні шляхи та їх структура. *Вісник зоології. Монографічна серія*. Окремий випуск № 33. Київ, 2016. 258 с.

4. Сіохін В. Д., Черничко Й. І., Сідоренко А. І., Горлов П. І., Алейнікова К. Г. Кумулятивна оцінка впливу вітрових станцій на природні комплекси в межах техногенних територій північно-західного Приазов'я. Повідомлення 2. Сезонні комплекси птахів. *Моніторинг та охорона біорізноманіття в Україні: прикладні аспекти моніторингу та охорони біорізноманіття. Серія «Conservation Biology in Ukraine»*. Київ ; Чернівці : Друк Арт, 2020. Вип. 16, т. 3. С. 152–168.

5. European Commission. *Guidance document on wind energy developments and EU nature legislation*. Brussels : Publications Office of the European Union, 2020. 184 p. DOI: <https://doi.org/10.2779/095188>.

6. Gémard C., Duriez O., Chappe O., Duclos G., Besnard A. Towards a better understanding of avian collision in wind energy facilities using automatic detection systems. *Journal of Applied Ecology*. 2025. Vol. 62, No. 6. P. 1437–1448. DOI: <https://doi.org/10.1111/1365-2664.70055>.

7. Keane A., Nisbet I., Calvo G., Pickering G., Tulloch J., More G., Koronka N. Wind farm cumulative induction zone effect and the impact on energy yield estimation. *Renewable Energy*. 2022. Vol. 181. P. 1209–1222. DOI: <https://doi.org/10.1016/j.renene.2021.09.056>.

8. Petersen I. K., Christensen T. K., Kahlert J., Desholm M., Fox A. D. *Final Results of Bird Studies at the Offshore Wind Farms at Nysted and Horns Rev, Denmark*. Rønde : National Environmental Research Institute, 2006. 161 p. URL: https://tethys.pnnl.gov/sites/default/files/publications/NERI_Bird_Studies.pdf (дата звернення: 08.07.2026).

9. Christiansen N., Daewel U., Schrum C. Cumulative hydrodynamic impacts of offshore wind farms on North Sea currents and surface temperatures. *Communications Earth & Environment*. 2026. Vol. 7. Art. 164. DOI: <https://doi.org/10.1038/s43247-026-03186-8>.

10. Drewitt A. L., Langston R. H. W. Collision effects of wind-power generators and other obstacles on birds. *Annals of the New York Academy of Sciences*. 2008. Vol. 1134. P. 233–266.

11. Yordanov N., Zehtindjiev P., Whitfield D. P. Long-term decline in bird collisions at operational wind farms: evidence from systematic monitoring to support sustainable wind energy development (2010–2024). *Sustainability*. 2026. Vol. 18, No. 2. Art. 992. DOI: <https://doi.org/10.3390/su18020992>.
12. Masden E. A., Cook A. S. C. P., McCluskie A., Bouten W., Burton N. H. K., Thaxter C. B. When speed matters: The importance of flight speed in an avian collision risk model. *Environmental Impact Assessment Review*. 2021. Vol. 90. Art. 106622. DOI: <https://doi.org/10.1016/j.eiar.2021.106622>.
13. Desholm M., Fox A. D., Beasley P. D. L., Kahlert J. Remote techniques for counting and estimating the number of bird–wind turbine collisions at sea: a review. *Ibis*. 2006. Vol. 148, Suppl. 1. P. 76–89.
14. May R., Hoel P. L., Langston R., Dahl E. L., Bevanger K., Reitan O., Nygård T., Pedersen H. C., Røskoft E., Stokke B. G. Collision Risk in White-tailed Eagles: Modelling Collision Risk Using Vantage Point Observations in Smøla Wind-power Plant. *NINA Report No. 639*. Trondheim : Norwegian Institute for Nature Research, 2010.
15. Горлов П. І., Сіохін В. Д., Долинний В. І., Сидоренко А. І. Сезонні орнітологічні особливості території Ботієвського вітропарку (Запорізька область) за результатами спостережень у весняні періоди 2013–2014 років. *Бранта* : збірник наукових праць Азово-Чорноморської орнітологічної станції. 2014. № 17. С. 19–38. URL: <https://branta.org.ua/ua/branta-issues/branta-17/17-02.html> (дата звернення: 08.07.2026).
16. Горлов П. І., Сидоренко А. І., Сіохін В. Д. Багаторічний орнітологічний моніторинг на Ботієвській вітровій станції як об’єктивна складова оцінки впливу вітропарку на птахів. *Вісті Біосферного заповідника «Асканія-Нова»*. 2016. Т. 18. С. 116–130.
17. Josimović B., Bezbradica L., Manić B., Srnić D., Srebrić N. Cumulative impact of wind farm noise. *Applied Sciences*. 2023. Vol. 13, No. 15. Art. 8792. DOI: <https://doi.org/10.3390/app13158792>.
18. Scottish Natural Heritage. Use of Avoidance Rates in the SNH Wind Farm Collision Risk Model. SNH Avoidance Rate Information and Guidance Note. Inverness : SNH, 2024. URL: <https://www.nature.scot/doc/guidance-using-updated-collision-risk-model-assess-bird-collision-risk-onshore-wind-farms> (дата звернення: 08.07.2026).
19. Ogłęcki P., Dąbrowski P., Gnatowski T., Małuszyńska I., Małuszyński M. J., Przybyłowski J., Pawluśkiewicz B. Bird collision risk at the interface of solar and wind farms – a cumulative effect? *Journal of Water and Land Development*. 2025. No. 66. P. 152–161. DOI: <https://doi.org/10.24425/jwld.2025.155311>.

20. Punt A. E., Siple M., Francis T. B., Hammond P. S., Heinemann D., Long K. J., Moore J. E., Sepúlveda M., Reeves R. R., Sigurðsson G. M., Vikingsson G., Wade P. R., Williams R., Zerbin A. N. Robustness of potential biological removal to monitoring, environmental, and management uncertainties. *ICES Journal of Marine Science*. 2020. Vol. 77, Issue 7–8. P. 2491–2507. DOI: <https://doi.org/10.1093/icesjms/fsaa096>.

21. Williams K. A., Gulka J., Cook A. S. C. P., Diehl R. H., Farnsworth A., Goyert H., Hein C., Loring P., Mizrahi D., Petersen I. K., Peterson T., Press K. M., Stenhouse I. J. A framework for studying the effects of offshore wind energy development on birds and bats in the Eastern United States. *Frontiers in Marine Science*. 2024. Vol. 11. Article 1274052. DOI: <https://doi.org/10.3389/fmars.2024.1274052>.

22. Горлов П. І., Сіохін В. Д. Аналіз міжнародного досвіду вивчення впливу вітрових електростанцій на птахів. *Біологічний вісник Мелітопольського державного педагогічного університету імені Богдана Хмельницького*. 2012. № 1. С. 37–47.

Information about the author:

Sydorenko Andrii,

Senior Lecturer at the Department of Biology and Physical Rehabilitation
Bogdan Khmelnytsky Melitopol State Pedagogical University
59, Naukovoho mistechka Street, Zaporizhzhia, Ukraine

**THE IMPACT OF MILITARY ACTIONS
ON THE ECOLOGICAL STATE OF UKRAINE'S
TERRITORIAL COMMUNITIES AND DIRECTIONS
FOR THE RESTORATION OF NATURAL ECOSYSTEMS**

Sovhira Svitlana

DOI <https://doi.org/10.30525/978-9934-26-714-7-18>

INTRODUCTION

The full-scale armed aggression of the Russian Federation against Ukraine, ongoing since 24 February 2022, has caused not only a humanitarian catastrophe but an environmental disaster unprecedented in scale, the consequences of which are felt at every level of the state's territorial organization – from the national level down to that of an individual territorial community. Hostilities, missile and artillery strikes, the mining of vast areas, the destruction of industrial and municipal facilities, as well as deliberate acts of environmental terrorism (above all, the destruction of the Kakhovka hydroelectric dam) have led to a cumulative environmental crisis affecting atmospheric air, surface and ground water, soils, forests, protected-area-fund sites, and biodiversity.

The relevance of this study stems from the fact that territorial communities, as the basic units of local self-government under decentralization, bear the main burden of the initial documentation of environmental damage, of ensuring the environmental safety of the population, and of planning environmental-recovery measures, while frequently lacking sufficient methodological, human-resource and financial capacity to carry out these tasks. International organizations – the United Nations Environment Programme (UNEP), the Organisation for Economic Co-operation and Development (OECD), and the Joint Research Centre of the European Commission – are conducting a systematic assessment of the environmental consequences of the war in Ukraine, providing an important empirical basis for the scholarly understanding of the problem at the regional and local levels.

The purpose of this chapter is to analyse the scale, nature and categories of the environmental damage inflicted on Ukraine's territorial communities as a result of hostilities, and to systematize the directions and practical

instruments for restoring natural ecosystems at the local level in the context of the country's post-war reconstruction.

Structurally, the chapter consists of two subsections. The first subsection analyses the scale, categories and regional differentiation of the environmental consequences of hostilities for territorial communities, in particular through the example of the Kakhovka dam breach. The second subsection systematizes the directions, instruments and institutional mechanisms for restoring natural ecosystems and the environmental rehabilitation of communities in the context of the state's post-war reconstruction.

1. The ecological state of territorial communities under armed aggression: scale, categories and consequences of destructive processes

Methodological foundations for classifying environmental damage.

The first systematic attempt to classify the environmental consequences of the war in Ukraine was made by UNEP in its 2022 preliminary review, which identifies five main categories of damage: impacts on the chemical industry and the handling of hazardous chemical substances; damage to fuel and associated infrastructure; disruption of waste-management systems; destruction of urban and critical infrastructure, including water supply; and damage to agriculture and natural ecosystems¹. This classification is methodologically universal and applicable to assessing the environmental state of individual territorial communities regardless of their location relative to the front line (Table 1).

The practical value of this classification for analysis at the level of a territorial community lies in the fact that it allows each category of damage to be linked to specific objects of local infrastructure and the economic complex that fall within the remit of local self-government bodies. At the same time, an independent perspective review of the war's environmental consequences confirms that the key drivers of environmental degradation are not only direct combat impacts but also associated processes – mass displacement of the population, disruption of logistical chains for handling hazardous waste, and the temporary suspension of environmental regulations under martial law.

Air pollution and health risks for the population of communities.

Shelling of industrial facilities, fires at fuel-and-lubricant and agricultural-produce storage sites, as well as large-scale fires in forest and steppe ecosystems, cause periodic but intense emissions of pollutants into the atmospheric air of territorial communities. Unlike the chronic industrial pollution of peacetime, wartime conditions are characterized by the unpredictability of sources and the

¹ United Nations Environment Programme. The Environmental Impact of the Conflict in Ukraine: A Preliminary Review. Nairobi : UNEP, 2022. 56 p.

episodic nature of large-volume emissions, which complicates both forecasting and the organization of systems for alerting the population to deteriorating air quality within a given community.

Table 1

Categories and nature of the environmental damage inflicted on Ukraine’s territorial communities as a result of hostilities

№.	Category of impact	Nature of the environmental damage	Examples of affected objects in territorial communities
1	Chemical industry and substances associated with the armed conflict	Emissions and spills of hazardous chemicals resulting from shelling of industrial facilities	Chemical, oil-refining and metallurgical enterprises in combat zones
2	Fuel and associated infrastructure	Contamination of soils and water with petroleum products, fires at fuel and energy facilities	Oil depots, filling stations, elements of communities’ energy infrastructure
3	Waste and waste-management infrastructure	Destruction of landfills and sorting stations, accumulation of construction and military debris	Municipal solid-waste landfills, destroyed community buildings
4	Urban and critical infrastructure	Damage to water supply, sewerage and wastewater treatment systems	Water utility facilities, treatment plants of territorial communities
5	Damage to agriculture and natural ecosystems	Soil degradation, biodiversity loss, damage to protected areas	Arable land, forests, protected-area-fund objects of communities

Source: compiled by the author

A perspective analytical review emphasizes that armed conflict creates a specific class of environmental risks associated with breaches to the integrity of hazardous facilities located within or near populated areas, including the risk of secondary technogenic accidents at chemically hazardous sites. For territorial communities on whose territory such facilities are located, this necessitates the advance mapping of potential sources of air pollution and the integration of this information into local civil-protection plans.

Degradation and contamination of the soil cover. A special place among studies of the war’s environmental consequences is occupied by research on qualitative changes in the state of the soil cover, based on a combination of field measurements and remote-sensing methods, which record soil degradation and contamination in zones of active hostilities². The combined approach – field

² Solokha M., Pereira P., Symochko L., Vynokurova N., Demyanyuk O., Sementsova K., Inacio M., Barceló D. Russian-Ukrainian war impacts on the environment: Evidence from the field on soil properties and remote sensing. *Science of the Total Environment*. 2023. Vol. 902. Art. 166122. DOI: 10.1016/j.scitotenv.2023.166122.

sampling together with satellite data – makes it possible not only to confirm the fact of contamination but also to trace its spatial dynamics over large territories that are inaccessible for comprehensive field surveys under ongoing hostilities.

Researchers distinguish several main types of impact of hostilities on the soil cover: mechanical disturbance of soil structure caused by the digging of fortifications, the movement of heavy machinery and explosions; chemical contamination from munition-detonation products, heavy metals and residual rocket fuel; and indirect degradation caused by the cessation of agrotechnical measures (tillage, fertilization, erosion-control measures) on land temporarily withdrawn from economic use due to the danger of mines.

A generalizing study of the scale of land degradation and contamination resulting from the armed conflict at the national level confirms the systemic nature of these processes and the need to develop a unified methodology for assessing soil quality on territories affected by hostilities³. For territorial communities located in the front-line zone and on de-occupied territories, these processes are decisive for assessing the suitability of agricultural land for further economic use, as well as for planning the priority of humanitarian demining and reclamation works. An additional risk factor is the presence of explosive objects in the upper soil horizons, which makes it impossible to conduct a conventional soil survey without a prior pyrotechnic inspection of the territory.

Damage to critical infrastructure and waste-management systems.

A separate group of consequences, distinguished in the UNEP classification, consists of damage to urban and critical infrastructure, above all water supply, sewerage and waste-management systems. For territorial communities, the destruction of water-utility facilities means not only a temporary loss of access to drinking water but also the risk of untreated wastewater uncontrollably entering surface water bodies, which increases the burden on already vulnerable aquatic ecosystems, discussed below.

At the same time, the war has created an unprecedented burden on territorial communities' systems for handling household and construction waste: the destruction of residential buildings generates significant volumes of construction debris, often mixed with hazardous components (asbestos-containing materials, residual fuel and lubricant substances), while some existing solid-waste landfills have themselves been damaged or have temporarily lost access to environmental-safety monitoring systems. The lack of sorting and proper handling of this mixed waste stream increases, in the

³ Solokha M., Demyanyuk O., Symochko L., Mazur S., Vynokurova N., Sementsova K., Mariychuk R. Soil degradation and contamination due to armed conflict in Ukraine. *Land*. 2024. Vol. 13, No. 10. Art. 1614. DOI: 10.3390/land13101614.

medium term, the risk of secondary contamination of soils and groundwater in areas adjacent to landfills.

A specific challenge for territorial communities is also the accumulation of waste of military origin – remnants of weapons, munitions and military equipment – the handling of which requires specialized pyrotechnic and environmental procedures that go beyond the ordinary competence of municipal services. This necessitates the creation, at the regional level, of specialized facilities for the safe disposal of this type of waste, accessible to several territorial communities simultaneously.

Impact on agriculture and food security of communities. The agricultural sector of territorial communities is affected by the war along several interrelated dimensions: physical destruction of agricultural infrastructure (grain elevators, livestock complexes, land-reclamation systems), temporary or permanent loss of access to land due to the danger of mines, and deterioration of the qualitative characteristics of soils on land that remains in economic use⁴. These processes directly affect the food security and economic resilience of territorial communities for which agriculture has traditionally been the basis of the local economy.

Studies of changes in the physico-chemical properties of soils, based on field measurements and remote sensing, record, in particular, increased soil density resulting from the movement of heavy machinery, disruption of the natural structure of the arable layer, and localized contamination from detonation products, which together reduce the potential yield of affected land even in the absence of direct chemical contamination capable of rendering economic use impossible. For territorial communities, this implies the need for a differentiated approach to planning the restoration of agricultural production – prioritizing the return to use of land with a minimal degree of damage, coupled with the phased reclamation of more severely affected plots.

Water ecosystems of territorial communities. No less vulnerable are the surface water bodies of territorial communities: a telling case is that of the Irpin River basin, for which changes in the structure of microalgae and macrophyte communities have been documented as a result of war-related anthropogenic activity⁵, in particular due to changes in the hydrological regime caused by the destruction of hydraulic structures in 2022, as well as the influx of pollutants

⁴ Rawtani D., Gupta G., Khatri N., Rao P. K., Hussain C. M. Environmental damages due to war in Ukraine: A perspective. *Science of the Total Environment*. 2022. Vol. 850. Art. 157932. DOI: 10.1016/j.scitotenv.2022.157932.

⁵ Nezbrytska I., Bilous O., Sereda T., Ivanova N., Pohorielova M., Shevchenko T., Dubniak S., Lietytska O., Zhezherya V., Polishchuk O. et al. Effects of war-related human activities on microalgae and macrophytes in freshwater ecosystems: A case study of the Irpin River Basin, Ukraine. *Water*. 2024. Vol. 16, No. 24. Art. 3604. 17 p. DOI: 10.3390/w16243604.

from destroyed wastewater-management infrastructure. The recorded changes in the structure of aquatic communities are regarded by researchers as an indicator of broader, often visually imperceptible, disruptions to the functioning of freshwater ecosystems.

The case of the Irpin River basin has important methodological significance for territorial communities located in the basins of other affected rivers, as it demonstrates the value of incorporating hydrobiological monitoring – not merely chemical analysis of water-quality indicators – into standard environmental-assessment procedures. Structural changes in microalgae and macrophyte communities can signal disruption of an aquatic ecosystem long before corresponding changes become apparent through conventional chemical water-quality indicators, making biological monitoring an important complement to the existing control systems used by territorial communities.

Protected-area fund and biodiversity. Particular attention should be paid to the impact of hostilities on protected-area-fund sites: studies confirm that Ukraine's protected areas have suffered substantial direct and indirect impacts from hostilities, including physical destruction, fires and breaches of protection regimes⁶. Direct impact manifests as the physical destruction of vegetation cover due to explosions and fires caused by hostilities, while indirect impact is associated with the impossibility of carrying out planned conservation measures (population regulation, control of invasive plants, fire-prevention work) on territories to which access is restricted for security reasons.

Since a significant share of protected-area-fund sites are located within or near the territorial communities of front-line and de-occupied regions, this issue must be considered in inseparable connection with local environmental-recovery programmes. Researchers emphasize the need to restore the protected status and functions of the most valuable territories as soon as possible after the end of active hostilities, since a prolonged absence of a protection regime increases the risk of irreversible biodiversity loss, in particular of rare and endangered species.

The case of the Kakhovka dam breach. The single most destructive act from an environmental standpoint was the breach of the Kakhovka hydroelectric dam in June 2023. A rapid environmental assessment conducted by UNEP at the request of the Government of Ukraine established that a significant share of the damage caused by the breach is irreversible, and that the hydrological, geomorphic, chemical and ecological consequences extend to territories both

⁶ Leal Filho W., Fedoruk M., Eustachio J. H. P. P., Splodytel A., Smaliychuk A., Szykowska-Jóźwik M. I. The environment as the first victim: The impacts of the war on the preservation areas in Ukraine. *Journal of Environmental Management*. 2024. Vol. 364. Art. 121399. DOI: 10.1016/j.jenvman.2024.121399.

upstream and downstream of the dam, including protected-area-fund sites⁷ (Table 2, Fig. 1). The assessment drew on official data, satellite imagery and remote-sensing methods, since the security situation in the combat zone limited the possibilities for conducting full-scale field studies.

The consequences of the breach differ depending on a territory’s position relative to the dam. Upstream of the dam, the rapid drawdown of the Kakhovka reservoir caused a change in the hydrological regime of adjacent territories, disrupted the functioning of water-supply systems that depended on the reservoir, and altered the conditions for aquatic and riparian ecosystems that had developed over decades of the reservoir’s existence. Downstream of the dam, large-scale flooding led to the inundation of settlements, agricultural land and natural territories, including areas within the Black Sea Biosphere Reserve.

For the territorial communities of Kherson and adjacent oblasts, this event caused a comprehensive transformation of the hydrological regime, the loss of agricultural land through flooding and subsequent desiccation, and long-term risks to drinking-water supply systems, a significant share of which had depended on a stable water level in the reservoir. Of particular note is that UNEP’s assessment classified a significant share of the damage as irreversible – this concerns, in particular, the loss of riparian and wetland ecosystems formed over decades, the restoration of which in their original form is practically impossible even with the complete reconstruction of hydraulic infrastructure.

Table 2

Consequences of the Kakhovka dam breach for natural ecosystems

Indicator of the dam-breach consequences	Value / characteristic
Area of affected natural and semi-natural habitats	over 500 thousand ha upstream and downstream of the dam
Number of potential pollution sources flooded as a result of the breach	over 1000 sites (treatment plants, filling stations, landfills, industrial enterprises)
Protected areas affected	the Black Sea Biosphere Reserve and other areas of national and international importance
Type of damage caused	hydrological and geomorphic changes, chemical contamination, waste accumulation, ecosystem degradation
Reversibility of the consequences	a significant share of the damage is classified as irreversible; consequences are projected to last decades

Source: compiled by the author

⁷ United Nations Environment Programme. *Rapid Environmental Assessment of Kakhovka Dam Breach, Ukraine, 2023*. Nairobi : UNEP, 2023. XIII, 115 p. DOI: 10.59117/20.500.11822/43696.

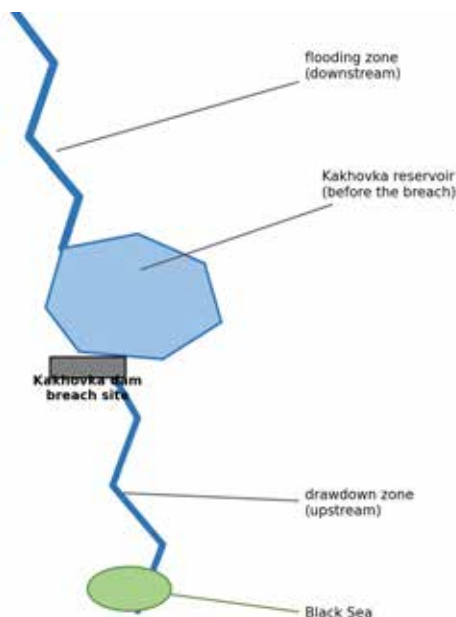


Fig. 1. Schematic map of the spread of environmental consequences of the Kakhovka dam breach along the Dnipro river basin (simplified, not a cartometric base; prepared by the author)

Special attention should be paid to the indirect consequences of the breach for irrigation and industrial water-supply systems: the Kakhovka reservoir historically supplied water to a significant share of the irrigation systems of the southern oblasts of Ukraine and also served as a source of technical water for a number of industrial facilities in the region, including the cooling systems of large energy facilities located in the lower Dnipro basin. The loss of a stable water source for these systems poses a long-term risk both to agricultural production in the territorial communities of the southern region and to broader industrial and energy security, extending well beyond purely environmental concerns.

In comparative terms, the Kakhovka dam breach is regarded by researchers as one of the largest technogenic incidents caused by the armed conflict, in terms of the area affected, the number of ecosystem functions disrupted, and the projected duration of recovery. Unlike point sources of pollution (individual industrial facilities or fuel depots), the consequences of the breach display

a pronounced cascading character: the change in hydrological regime triggers further changes in soil conditions and the structure of plant communities, and ultimately in the living conditions of the population of nearby territorial communities, which complicates both the assessment of the overall scale of damage and the planning of comprehensive recovery measures.

Regional differentiation of environmental consequences. A synthesis of the material analysed makes it possible to state that the intensity and nature of environmental consequences differ substantially depending on a territorial community's position relative to the front line. For front-line and temporarily occupied territorial communities (in particular, significant parts of Kharkiv, Luhansk, Donetsk, Zaporizhzhia and Kherson oblasts), the maximum intensity of destructive processes is characteristic – direct destruction of infrastructure, fires, the mining of large areas, and the practical impossibility of conducting environmental or recovery measures before the end of hostilities.

For communities in de-occupied territories (individual districts of Chernihiv, Sumy, Kyiv, Mykolaiv and Kherson oblasts), the defining tasks are related to humanitarian demining, the reclamation of disturbed land, and the restoration of damaged infrastructure, whereas the direct threat of hostilities is already absent. Rear-area communities – mostly in the central and western oblasts – mostly experience secondary consequences of the war: an increased burden on local waste-management systems due to the placement of internally displaced persons, changes in the water regime of transit water bodies, and logistical and economic effects associated with the redirection of production and transport flows. A schematic regional differentiation is presented in Fig. 2.

Managerial and socio-economic consequences for territorial communities. In addition to purely environmental consequences, hostilities generate a number of managerial challenges for the local self-government bodies of territorial communities. These include, in particular, the need for the initial documentation of environmental damage for the purposes of subsequent compensation, the limited human-resource capacity of local environmental services, and the lack of unified methodological guidance on a community's course of action in the event of detecting pollution or damage to natural objects under martial law.

An additional challenge is the financial capacity of communities: a significant share of the budgets of territorial communities affected by hostilities is directed toward urgent humanitarian and reconstruction needs, while environmental measures – monitoring, reclamation, restoration of natural objects – require separate, often long-term financing, sources of which are largely absent at the local level. This underscores the critical importance of external – state and international – support, a matter examined in more detail in the second subsection.

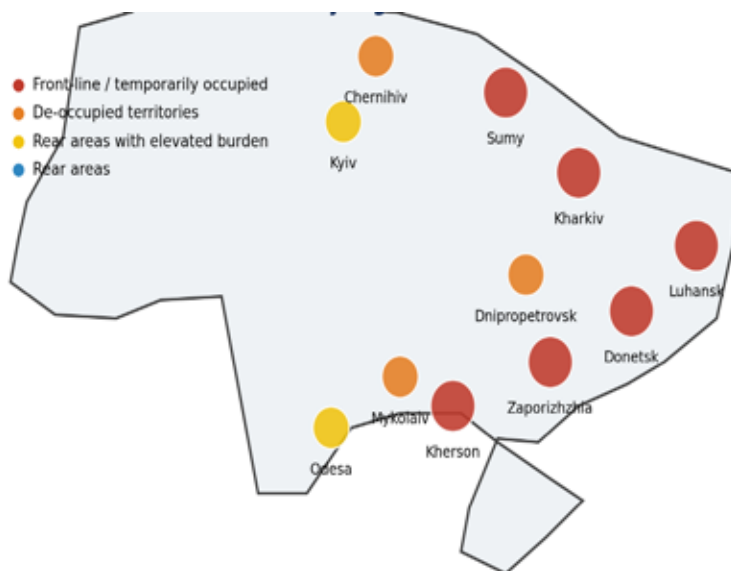


Fig. 2. Schematic map of the regional differentiation of environmental consequences of hostilities for territorial communities of Ukraine (simplified, not a cartometric base; prepared by the author)

No less important is the issue of documenting the environmental damage inflicted, for the purposes of subsequent compensation – both within national compensation mechanisms and in the context of possible international-legal procedures to hold the aggressor state accountable for damage to the environment. Rigorous, methodologically sound documentation of pollution incidents at the level of a territorial community – linked to specific objects, dates and, where possible, laboratory-analysis results – forms an evidentiary base whose significance extends far beyond purely environmental tasks and acquires weight in the broader context of post-war justice.

These managerial challenges indicate that the environmental restoration of territorial communities is not merely an environmental issue but a complex socio-economic and institutional problem, the resolution of which requires coordinated action at all levels of public administration – from an individual village, settlement or city council to the central executive authorities and Ukraine’s international partners.

A qualitative comparison of the relative prominence of the categories of environmental damage discussed above in the sources reviewed is presented in a supplementary Excel file accompanying this chapter.

Summarizing the material presented in this subsection, it can be stated that the environmental state of Ukraine's territorial communities under armed aggression is characterized by a combination of direct (physical destruction, contamination) and indirect (managerial, socio-economic) consequences, the intensity of which is determined both by the nature of hostilities and by the natural-economic specifics of a given territory. This forms the basis for developing differentiated, rather than uniform, strategies for restoring natural ecosystems, which are the subject of the second subsection.

2. Directions for the restoration of natural ecosystems and strategies for the ecological rehabilitation of territorial communities

Monitoring and assessment of the environmental state. Formulating an effective strategy for the post-war environmental recovery of territorial communities requires a consistent combination of several interrelated directions. The methodological basis for planning recovery measures is systematic monitoring and assessment of the environmental state using remote sensing of the Earth combined with field studies, which makes it possible to record the dynamics of degradation processes promptly and objectively, even in territories with limited physical access. The quality of the source monitoring data directly determines the effectiveness of subsequent planning of recovery measures at the national and regional levels.

In the practice of environmental monitoring of territories affected by the war in Ukraine, a combination of several types of instruments is applied – from international satellite emergency-response services to local observation systems operating directly within a territorial community (Table 3). For local self-government bodies, what matters is not so much direct access to complex geospatial platforms as the ability to interpret and use the results of such monitoring when planning priority recovery measures, as well as to establish cooperation with the relevant state and international bodies that possess the corresponding technical capabilities.

An important complement to remote methods is the development of local civic (citizen) environmental-monitoring systems, which allow residents of territorial communities to independently record cases of air, water or soil pollution and pass the relevant information on to the responsible authorities. Despite limited metrological accuracy compared with official instrumental observations, this form of monitoring plays an important role in the prompt detection of local environmental incidents, especially in territories with a limited presence of state regulatory bodies during periods of active hostilities.

Table 3

**Examples of environmental-monitoring tools and platforms
applicable to territorial communities**

Tool / platform	Purpose	Level of application
Copernicus Emergency Management Service (EU)	Satellite monitoring of emergencies, mapping of affected areas	International / national
PREEO (UNEP rapid environmental assessment methodology)	Structured field and remote assessment of the environmental consequences of conflict	International
Geospatial services of Ukrainian remote-sensing providers	Analysis of damage to land, crops and infrastructure based on satellite imagery	National / regional
Interactive maps of environmental incidents (state and civic initiatives)	Recording reports of pollution, fires and discharges accessible to communities	Regional / local (community)
Local environmental monitoring systems of territorial communities	Regular monitoring of air, water and soil conditions within a specific community	Local (community)

Source: compiled by the author

Building the human-resource and methodological capacity of communities. The effective use of monitoring results at the level of a territorial community is impossible without appropriate human-resource and methodological training of local self-government officials. Practice indicates an uneven institutional capacity among Ukraine's territorial communities to work with environmental data, which justifies the development of centralized training programmes and methodological guidance adapted to the needs of communities with varying levels of resource availability.

In this context, an important role can be played by higher-education institutions specializing in environmental, geographical and natural sciences, which can act as methodological partners of territorial communities – both in training specialists for local self-government bodies and in directly supporting individual environmental-assessment and recovery projects on a contractual or grant basis. Such a format of cooperation simultaneously addresses the task of providing natural-science students with practical training on real-world cases and strengthens the methodological capacity of communities.

Soil remediation and humanitarian demining. The second key direction is the remediation of contaminated and disturbed land in territorial communities, encompassing humanitarian demining, soil reclamation, and the elimination of chemical and oil-contamination hotspots. In its analysis of the prospects for a green reconstruction, the OECD emphasizes the merit of integrating environmental priorities directly into the process of post-war infrastructure

reconstruction, rather than treating them as a separate, secondary task⁸, which is of fundamental importance precisely at the level of the territorial community, where the reconstruction of housing stock, roads and municipal infrastructure inevitably involves earthworks.

Humanitarian demining is a prerequisite for any further recovery measures across much of the de-occupied and formerly front-line territories, since without it neither a full-scale field soil survey nor practical land reclamation is possible. Studies of land degradation and contamination resulting from the armed conflict point to the merit of combining demining with the parallel collection of soil samples for subsequent chemical analysis, which shortens the overall time required to return land to safe economic use.

Reclamation measures for territorial communities should be differentiated according to the type and intensity of contamination: for territories with mechanical disturbance of the soil cover (craters, trenches, tracks left by heavy machinery), measures to restore the terrain and fertile layer are the priority; for territories with chemical contamination, containment and, where necessary, removal of contaminated soil followed by disposal or neutralization; for agricultural land withdrawn from cultivation for a prolonged period, the phased restoration of agrotechnical practices with due regard to the changes in soil physico-chemical properties recorded by field studies.

Given the limited resources available to most territorial communities, a phased approach to land remediation is practically advisable: at the first stage – demining and surveying territories of the highest priority for use (land near settlements, plots with the best agro-productive characteristics); at the second stage – reclamation of land with a moderate degree of damage; at the third, long-term stage – restoration of the most complex, heavily contaminated or structurally disturbed plots, which may require specialized remediation technologies unavailable at the level of an individual community without external technical support. The OECD emphasizes that precisely such consistent, phased planning, aligned with a realistic assessment of available resources, is the key to avoiding a situation in which begun but unfinished reclamation works create additional, secondary environmental risks.

Restoration of water bodies. The third direction concerns the restoration of aquatic ecosystems – the reconstruction of hydraulic structures, the cleanup of water bodies from pollutants, and the restoration of the natural hydrological regime of rivers and reservoirs affected both by direct destruction (above all in the Dnipro basin following the Kakhovka dam breach) and by the indirect

⁸ OECD. Environmental impacts of the war in Ukraine and prospects for a green reconstruction. OECD Policy Responses on the Impacts of the War in Ukraine. Paris : OECD Publishing, 2022. 11 p. DOI: 10.1787/9e86d691-en.

anthropogenic burden of wartime, documented in particular for the Irpin River basin.

For territories affected by the Kakhovka dam breach, recovery measures must take into account the dual nature of the damage caused: upstream of the dam – measures aimed at stabilizing the hydrological regime and restoring water-supply systems that depended on the reservoir; downstream of the dam – measures to restore the natural state of floodplain and riparian ecosystems, as well as to eliminate the consequences of the flooding of settlements and agricultural land. Given the significant irreversibility of part of the damage, as established by international experts, the recovery strategy should be oriented not toward the literal reproduction of the previous state but toward the formation of a new, environmentally resilient state of the affected territories.

For territorial communities located in the basins of smaller rivers, affected by the indirect anthropogenic burden of wartime, priority measures include the restoration of wastewater-treatment systems, the elimination of unauthorized discharges, and the restoration of natural vegetation along riparian buffer zones, which performs an important buffering function against pollution entering from adjacent territories.

A separate task, closely linked to the restoration of aquatic ecosystems, is the reconstruction of drinking-water supply and sewerage systems, damage to which was recorded among the principal categories of environmental damage as early as UNEP's preliminary review. For territorial communities, the restoration of such systems should be regarded not merely as an engineering task but also as a component of a broader strategy for restoring aquatic ecosystems, since the quality of wastewater treatment directly determines the burden placed on receiving water bodies. This warrants coordination between water-utility enterprises and the relevant environmental services of a community when planning the sequence of reconstruction works.

Restoration of forest and protected ecosystems. The fourth direction is the restoration of forest and protected ecosystems. Research on pathways for Ukraine's post-war nature recovery, focused on forest socio-ecological systems, emphasizes the importance of combining active reforestation with support for natural succession processes, as well as the need to reconcile economic and conservation interests in communities where forests simultaneously perform resource, protective and recreational functions⁹. This approach allows territorial communities to view forest restoration not only as an environmental resource but also as a socio-economic resource for local development.

⁹ Melnykovych M., Nijnik M., Soshenskyi O. et al. Pathways for Ukraine's post-war nature recovery: Focus on forest socio-ecological systems. *Ambio*. 2026. Vol. 55. P. 817–843. DOI: 10.1007/s13280-025-02263-0.

Researchers note that the choice between active reforestation (artificial planting) and support for natural succession should be made with regard to the specific conditions of a given territory – the degree of soil-cover damage, the presence of mine danger, the availability of locally sourced planting material, and the community's long-term goals for the use of forest resources. In cases of significant contamination or mechanical soil disturbance, natural succession may prove a slower but ecologically more resilient alternative to artificial reforestation.

For protected-area-fund sites that have suffered direct or indirect impacts from hostilities, the priority task is the swiftest possible restoration of the protection regime and the corresponding management functions, since a prolonged absence of oversight increases the risks of illegal resource use, spontaneous fires and poaching on these territories. Territorial communities within whose boundaries such sites are located can play an active role in restoring the protection regime by organizing civic oversight and involving the local population in conservation measures.

The concept of forest socio-ecological systems, which underlies research on pathways for Ukraine's post-war nature recovery, entails viewing a forest not merely as an aggregate of woody vegetation but as a complex system of interaction among natural processes, economic use, and local communities dependent on forest resources. A practical implication of this approach for territorial communities is the need to align state (sectoral) reforestation programmes with local interests – the recreational use of forests, traditional forms of use of non-timber forest resources, and the role of forest tracts in protecting agricultural land from erosion and adverse climatic phenomena, the significance of which is growing under climate change.

Researchers also highlight the risk of a conflict of interest between rapid commercial restoration of tree stands (dominated by monoculture plantations of fast-growing species) and the long-term goal of restoring biologically diverse, resilient forest ecosystems. For territorial communities primarily interested in the fastest possible restoration of the protective and recreational functions of forests, this creates a need for a conscious choice between faster but potentially less resilient solutions and longer, yet more ecologically sound, reforestation strategies that combine artificial planting with support for natural succession on the plots best suited to it.

Institutional and legal support and financing for recovery. The fifth, institutional-legal direction involves integrating the principles of sustainable and green recovery into the local development strategies of territorial communities, aligning them with national reconstruction programmes, and attracting international technical and financial support. The success of green recovery depends to a large extent on how consistently environmental criteria are

integrated into the decision-making system at every level of governance – from the national to the local – including the procedures for planning and financing reconstruction projects at the territorial-community level.

Analytical materials of the Joint Research Centre of the European Commission on the state of the environment and climate in Ukraine emphasize that integrating the environmental dimension into the post-war reconstruction process is not only an internal need of the state but also an important precondition for further European integration, since a significant share of the EU's environmental legislation (the *acquis communautaire* in the field of the environment) will need to be progressively implemented at the national and regional levels¹⁰. For territorial communities, this means the need to progressively adapt local regulatory practices to European standards of environmental governance, in particular in the areas of waste management, water quality, and environmental impact assessment.

The practical dimension of such adaptation includes, in particular, the gradual alignment of local waste-handling practices with the waste hierarchy (prevention, reuse, recycling, disposal) enshrined in EU environmental legislation, the introduction of a river-basin approach to water-resource management, and the phased extension of environmental-impact-assessment procedures to a wider range of recovery projects implemented within territorial communities. Although full implementation of these approaches is a task for the national level, territorial communities that begin adapting their own practices to the relevant standards at an early stage gain an additional advantage when preparing applications for European financing.

Climate resilience of restored ecosystems. The planning of natural-ecosystem restoration in territorial communities should be carried out with due regard not only for the damage caused by the war but also for long-term climatic trends, since ecosystems restored without accounting for this factor risk proving vulnerable to droughts, rising temperatures, or changes in precipitation patterns characteristic of most regions of Ukraine. It is advisable to combine the tasks of post-war environmental recovery with the tasks of climate-change adaptation, in particular through the selection of drought-resistant species during reforestation and the prioritization of measures that simultaneously increase ecosystem resilience and reduce the climate-related vulnerability of territorial communities.

The formation of mixed-age and mixed-species stands, rather than monoculture plantations, is likewise advisable for reasons of long-term resilience

¹⁰ Belis C. A., Petrosian A., Turos O. et al. Status of Environment and Climate in Ukraine: Assessing the Impact of War and Its Implications for Reconstruction. Luxembourg : Publications Office of the European Union, 2025. JRC141480. DOI: 10.2760/6292177.

to climatic and other stress factors, including the risk of repeated fires and pests. For territorial communities, this means that decisions which at first glance appear purely environmental in nature have, in the long term, a direct bearing on the climatic and economic resilience of the local community as a whole.

Financing environmental-recovery measures at the level of territorial communities requires a combination of several sources: the state budget within national reconstruction programmes, international technical and financial assistance, and the attraction of private investment through public-private partnership mechanisms (Table 4). The practice of preparing applications for international financing of environmental projects requires territorial communities to build a solid evidentiary base regarding the damage inflicted, which further underscores the importance of the systematic monitoring discussed above.

Table 4

**Typology of financing sources for environmental-recovery measures
in territorial communities**

Source of financing	Characteristics	Suitability for the community level
State budget (national recovery programmes)	Centralized financing of priority recovery projects	Through regional coordination mechanisms
International technical and financial assistance	Grants and programmes of international organizations and donors for environmental projects	Direct community participation in competitive procedures
EU pre-accession funds and programmes	Support for adaptation to EU environmental legislation	Through national/regional programme operators
Public-private partnership	Attraction of private investment into recovery infrastructure	Where investment-attractive community projects exist

Source: compiled by the author

Practice shows that territorial communities with more developed institutional capacity – the presence of qualified specialists, experience in preparing project applications, and established cooperation with regional and central authorities – have substantially higher chances of attracting external financing compared with communities that rely solely on their own resources. This creates a risk of deepening the unevenness of recovery among territorial communities, which justifies targeted methodological support for communities with limited capacity from regional administrations, relevant ministries and partner organizations.

The role of territorial communities and public participation in recovery. The practical implementation of the directions presented depends to a large extent on the capacity of territorial communities themselves to act

not as passive recipients of assistance but as active subjects of the planning and implementation of recovery measures. This entails establishing, within the structure of local self-government bodies, units or officials responsible for coordinating environmental issues, developing local environmental-rehabilitation programmes, and maintaining ongoing communication with the population regarding the state of the environment within the community.

Involving the residents of territorial communities in recovery measures – through civic monitoring, participation in reforestation campaigns, and oversight of compliance with the protection regime of local natural areas – is not merely an additional resource for local self-government bodies with limited human-resource capacity but also an important factor in building public support for long-term, often costly, environmental-recovery programmes. The experience of citizen science in the field of environmental monitoring, which has become more widespread in Ukraine in recent years, demonstrates the potential of such involvement given appropriate methodological support from relevant specialists and educational institutions, including higher-education institutions that train specialists in the environmental and natural sciences.

Practical forms for institutionalizing public participation in territorial communities can include standing or ad hoc advisory bodies on environmental recovery (community environmental councils attached to local self-government bodies), the inclusion of environmental projects in participatory (community) budgeting procedures, and cooperation agreements with relevant civic organizations and higher-education institutions for the joint implementation of specific components of a local environmental-rehabilitation programme. Engaging a broad range of stakeholders, including local communities, improves both the quality of recovery-measure planning and the level of public trust in their implementation, which is especially important given the constrained resources of the post-war period.

At the same time, researchers caution against formal, purely declarative public involvement that is not accompanied by real influence on decision-making: effective community participation requires transparent access to environmental-monitoring results, an explanation of the data obtained that is comprehensible to a non-specialist audience, and a clear indication of how residents' proposals and comments are taken into account when adjusting a local environmental-rehabilitation programme.

The monitoring-recovery cycle: the importance of feedback. An important methodological principle uniting all the directions discussed is the understanding of natural-ecosystem restoration not as a one-off set of measures but as a cyclical process that requires constant feedback between monitoring and practical action. The results of an initial assessment of

a territory’s environmental state determine the priority and content of recovery measures, while subsequent, repeat monitoring makes it possible to evaluate their effectiveness and, if necessary, to adjust the strategy adopted.

For territorial communities, the practical implementation of such a cycle means the need to build repeat (control) surveys into the plan of any recovery project – whether the reclamation of a contaminated plot, reforestation works, or measures to clean up a water body. The absence of such control creates a risk of inefficient expenditure of a community’s limited resources on measures whose real effectiveness remains unconfirmed, and also complicates the justification of further applications for external financing, which typically require demonstration of the results of previous stages of work.

A generalized model for a territorial community’s environmental-rehabilitation programme. A synthesis of the directions discussed makes it possible to propose a generalized sequence of actions for a territorial community when developing its own environmental-rehabilitation programme: inventory and categorization of the environmental damage sustained, in accordance with the classification presented in Table 1; determination of the priority of recovery directions taking into account available resources and the degree of risk to the population; alignment of the local programme with regional and national reconstruction strategies; and the formulation of a plan for attracting external financing and technical support (Table 5, Fig. 3).

Table 5

**Directions and instruments for the restoration of natural ecosystems
in territorial communities**

Restoration direction	Main measures at the territorial-community level	Expected outcome
Monitoring and assessment	Remote sensing, field surveys, local environmental monitoring systems	Up-to-date picture of the community’s environmental state, basis for planning
Soil and land remediation	Humanitarian demining, reclamation of disturbed land, elimination of pollution hotspots	Return of land to safe economic use
Restoration of water bodies	Restoration of hydrological regime, cleanup of water bodies, reconstruction of water infrastructure	Improved water quality, restored aquatic ecosystems
Restoration of forest and protected ecosystems	Reforestation, support for natural succession, expansion of the protected-area fund	Conservation of biodiversity, increased ecosystem resilience
Institutional and legal support	Integration of green-recovery principles into local development strategies, attraction of international support	Alignment of community actions with national recovery policy

Source: compiled by the author

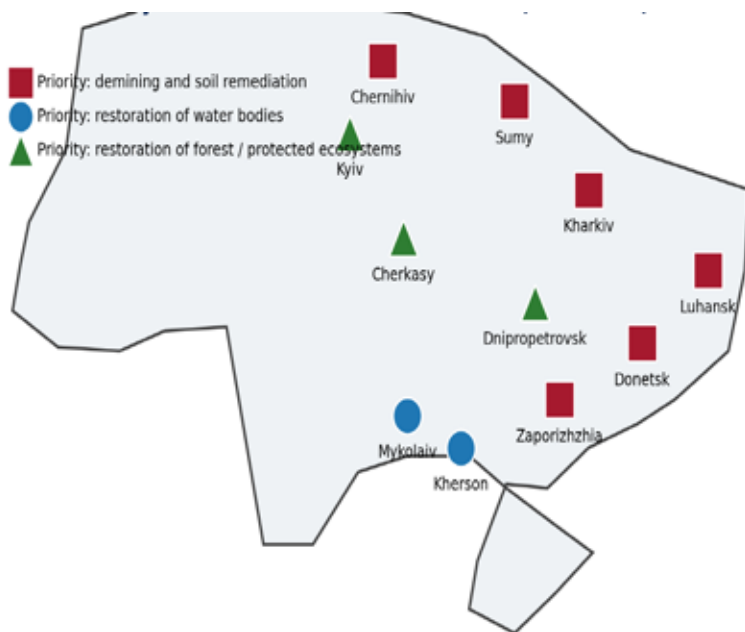


Fig. 3. Schematic map of priority directions for the restoration of natural ecosystems of Ukraine's territorial communities (simplified, not a cartometric base; prepared by the author)

The generalized model presented is not a rigid algorithm but rather a methodological reference point, which each territorial community must adapt to its own specific circumstances – the nature and scale of the damage sustained, available natural resources, and the human-resource and financial capacity of local self-government bodies. At the same time, the availability of such a reference point ensures a minimum level of methodological consistency among the environmental-rehabilitation programmes of different territorial communities, which is an important precondition for their further coordination at the regional and national levels.

In summary, effective restoration of the natural ecosystems of territorial communities is possible only through a comprehensive combination of technical (monitoring, remediation, reconstruction), environmental (forest and biodiversity restoration), and institutional-managerial (legal support, financing, community participation) components, discussed in this subsection.

CONCLUSIONS

The analysis conducted demonstrates that hostilities have caused a multidimensional environmental crisis which, at the level of territorial communities, manifests itself in the pollution of atmospheric air, soils and water bodies, the destruction of critical infrastructure, land degradation, and biodiversity loss, with the intensity of these processes varying substantially depending on a community's position relative to the front line. The most destructive factor is the combination of direct combat impacts with technogenic disasters triggered by the war, the most illustrative example being the Kakhovka dam breach, the consequences of which have been recognized as largely irreversible.

Restoring the natural ecosystems of territorial communities requires a comprehensive approach combining systematic monitoring, the remediation of contaminated territories, the restoration of aquatic and forest ecosystems, and institutional-legal support for integrating environmental priorities into local development strategies. The practical capacity of territorial communities to implement such programmes depends to a large extent on access to external financing, methodological support, and the active participation of the local population.

Prospects for further research are associated with developing a methodology for the quantitative assessment of environmental damage at the level of an individual territorial community, forming standard models of local environmental-rehabilitation programmes for communities in different categories of affected territories, and studying the long-term dynamics of ecosystem recovery following the Kakhovka dam breach as the largest technogenic incident of the war from an environmental standpoint.

SUMMARY

This chapter examines the impact of hostilities caused by the full-scale armed aggression of the Russian Federation against Ukraine on the ecological state of territorial communities and substantiates directions for the restoration of natural ecosystems. The international classification of categories of war-inflicted environmental damage is systematized and adapted for analysis at the level of a territorial community. The scale of soil-cover degradation, water-body contamination, and damage to protected areas resulting from hostilities is examined, along with the managerial and socio-economic consequences for local self-government bodies. Particular attention is devoted to the breach of the Kakhovka hydroelectric dam in June 2023 as the most destructive single act of environmental impact of the war, the consequences of which are recognized as largely irreversible. It is shown that the intensity of environmental consequences is differentiated according to a community's position relative to the front

line – front-line, de-occupied and rear-area territories require different response strategies. Five directions for restoring the natural ecosystems of territorial communities are systematized: monitoring and assessment, land remediation, restoration of water bodies, restoration of forest and protected ecosystems, and institutional-legal support. The need to integrate environmental priorities into the local post-war development strategies of territorial communities and to secure active public participation in recovery measures is substantiated. The material of this chapter may be used by local self-government bodies in developing environmental-rehabilitation programmes, as well as in the educational process of higher-education institutions in the environmental and geographical fields.

Bibliography

1. United Nations Environment Programme. The Environmental Impact of the Conflict in Ukraine: A Preliminary Review. Nairobi : UNEP, 2022. 56 p. URL: офіційна сторінка UNEP (дата звернення: 23.07.2026).
2. Solokha M., Pereira P., Symochko L., Vynokurova N., Demyanyuk O., Sementsova K., Inacio M., Barceló D. Russian-Ukrainian war impacts on the environment: Evidence from the field on soil properties and remote sensing. *Science of the Total Environment*. 2023. Vol. 902. Art. 166122. DOI: 10.1016/j.scitotenv.2023.166122.
3. Solokha M., Demyanyuk O., Symochko L., Mazur S., Vynokurova N., Sementsova K., Mariychuk R. Soil degradation and contamination due to armed conflict in Ukraine. *Land*. 2024. Vol. 13, No. 10. Art. 1614. DOI: 10.3390/land13101614.
4. Rawtani D., Gupta G., Khatri N., Rao P. K., Hussain C. M. Environmental damages due to war in Ukraine: A perspective. *Science of the Total Environment*. 2022. Vol. 850. Art. 157932. DOI: 10.1016/j.scitotenv.2022.157932.
5. Nezbrytska I., Bilous O., Sereda T., Ivanova N., Pohorielova M., Shevchenko T., Dubniak S., Lietytska O., Zhezherya V., Polishchuk O. et al. Effects of war-related human activities on microalgae and macrophytes in freshwater ecosystems: A case study of the Irpin River Basin, Ukraine. *Water*. 2024. Vol. 16, No. 24. Art. 3604. 17 p. DOI: 10.3390/w16243604.
6. Leal Filho W., Fedoruk M., Eustachio J. H. P. P., Splodytel A., Smaliychuk A., Szykowska-Jóźwik M. I. The environment as the first victim: The impacts of the war on the preservation areas in Ukraine. *Journal of Environmental Management*. 2024. Vol. 364. Art. 121399. DOI: 10.1016/j.jenvman.2024.121399.
7. United Nations Environment Programme. Rapid Environmental Assessment of Kakhovka Dam Breach, Ukraine, 2023. Nairobi : UNEP, 2023. XIII, 115 p. DOI: 10.59117/20.500.11822/43696.

8. OECD. Environmental impacts of the war in Ukraine and prospects for a green reconstruction. *OECD Policy Responses on the Impacts of the War in Ukraine*. Paris : OECD Publishing, 2022. 11 p. DOI: 10.1787/9e86d691-en.

9. Melnykovych M., Nijnik M., Soshenskyi O. et al. Pathways for Ukraine's post-war nature recovery: Focus on forest socio-ecological systems. *Ambio*. 2026. Vol. 55. P. 817–843. DOI: 10.1007/s13280-025-02263-0.

10. Belis C. A., Petrosian A., Turos O. et al. Status of Environment and Climate in Ukraine: Assessing the Impact of War and Its Implications for Reconstruction. Luxembourg : Publications Office of the European Union, 2025. JRC141480. DOI: 10.2760/6292177.

Information about the author:

Sovhira Svitlana,

Doctor of Pedagogical Sciences, Professor,
Professor at the Department of Biology and Chemistry
Uman National University
1, Instytutaska Street, Uman, Ukraine

ПОТЕНЦІАЛ ЕКОЛОГІЧНОЇ РЕЗИЛЬЄНТНОСТІ МІСЬКИХ ҐРУНТОВО-ЛАНДШАФТНИХ СИСТЕМ ДО КОМБІНОВАНИХ ШОКІВ: МЕТОДИЧНІ ЗАСАДИ ОБҐРУНТУВАННЯ РОБАСТНИХ УПРАВЛІНСЬКИХ РІШЕНЬ

Тітенко Ганна

DOI <https://doi.org/10.30525/978-9934-26-714-7-19>

ВСТУП

Міські території функціонують як складні, відкриті, просторово неоднорідні, адаптивні соціо-еколого-технологічні системи, в яких екологічні проблеми зазвичай зумовлені кількома чинниками, ідентифікація, аналіз та врахування яких є обов'язковою умовою наближення до ефективного управління міськими територіями^{1, 2}. Серед таких чинників – поєднання кліматичних екстремумів, хронічних урбанізаційних тисків, техногенних навантажень, змін землекористування, деградації природних систем та багато іншого. Для міст, що зазнають воєнного впливу, до них додаються різні типи порушення ґрунтового покриву, пожежі, накопичення будівельних уламків, локальне забруднення та інші.

Попри визнання множинності впливів, у сучасній науковій літературі відсутня чітка методологія розрізнення хронічних тисків, стресорів і гострих шоків, а також оцінки їх взаємодії в межах міських ґрунтово-ландшафтних систем (ІЛС). Транспортне забруднення, запечаткування поверхні та поступове ущільнення ґрунту формують тривалі тиски, тоді як екстремальна злива, пожежа або аварійне надходження забруднювача мають ознаки раптового шоку. Важливим є не об'єднання всіх

¹ McPhearson T., Cook E. M., Berbés-Blázquez M. et al. A social-ecological-technological systems framework for urban ecosystem services. *One Earth*. 2022. Vol. 5, No. 5. P. 505–518. DOI: <https://doi.org/10.1016/j.oneear.2022.04.007>.

² Pickett S. T. A., Cadenasso M. L., Grove J. M., Nilon C. H., Pouyat R. V., Zipperer W. C., Costanza R. Urban ecological systems: Linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. *Annual Review of Ecology and Systematics*. 2001. Vol. 32. P. 127–157. DOI: <https://doi.org/10.1146/annurev.ecolsys.32.081501.114012>.

несприятливих чинників, а встановлення того, як гостра подія взаємодіє з уже сформованою вразливістю території³.

У цьому дослідженні поняття «комбіновані шоки» застосовується до сценаріїв, за яких кілька небезпечних подій, стресорів або фонових тисків одночасно, послідовно чи просторово пов'язано формують спільний функціональний наслідок. Такий підхід узгоджується із сучасною типологією compound events, у межах якої розрізняють попередньо зумовлені, багатофакторні, послідовні та просторово поєднані події.

Поняття «резильєнтність» у сучасних наукових підходах пов'язують зі здатністю системи підтримувати або досить швидко відновлювати бажані функції, адаптуватися до змін, а за необхідності трансформувати структури, що обмежують майбутню адаптивну спроможність. Для міських ГЛС це означає, що потенціал резильєнтності не є універсальною властивістю території, він залежить від виду шоку, критичної функції, вихідного стану ґрунту, просторової структури ландшафту та можливостей управлінського реагування.

Водночас значна частина традиційних процедур екологічного контролю та територіального планування орієнтована на порівняння окремих показників із нормативними значеннями. Такий підхід є необхідним для виконання регламентів безпеки, але має обмежену здатність характеризувати поведінку системи за нестандартних або змінних умов.

Альтернативою прогнозно-оптимізаційній логіці є обґрунтування робастних управлінських рішень як таких, що забезпечують прийнятний рівень збереження або відновлення критичних функцій за широкого спектра умов. Методологія Robust Decision Making (RDM) передбачає формування множини сценаріїв, тестування управлінських альтернатив та пошук рішень, найменш чутливих до невизначеності вихідних припущень.

Метою дослідження є розроблення методичних засад оцінювання потенціалу екологічної резильєнтності міських ГЛС до комбінованих шоків та обґрунтування робастних управлінських рішень за умов глибокої невизначеності.

Для досягнення поставленої мети передбачено уточнити понятійний апарат дослідження, визначити критичні функції міських ГЛС, розробити типологію комбінованих шоків і механізмів їх взаємодії, сформувати систему показників опірності, відновлюваності та адаптивності, обґрунтувати принципи визначення референтних і порогових станів, запропонувати процедуру побудови функціонально-сценарного профілю

³ Zscheischler J., Westra S., van den Hurk B. J. J. M. et al. Future climate risk from compound events. *Nature Climate Change*. 2018. Vol. 8. P. 469–477. DOI: <https://doi.org/10.1038/s41558-018-0156-3>.

резильєнтності, визначити критерії сценарного тестування та робастності управлінських альтернатив.

Об'єктом дослідження є міські ГЛС, що функціонують під впливом природних та антропогенних чинників.

Предметом дослідження є структурні, функціональні, просторові та управлінські передумови потенціалу резильєнтності міських ГЛС, а також методи обґрунтування управлінських рішень, здатних забезпечувати прийнятний результат за певних сценаріїв комбінованих шоків.

В основу дослідження покладено припущення, що потенціал резильєнтності міської ГЛС визначається не лише її поточним екологічним станом, а поєднанням функціональних резервів, просторової організації, характеру можливих шоків, природної відновлюваності та адаптивної спроможності управління.

1. Концептуальні засади екологічної резильєнтності міських ґрунтово-ландшафтних систем (ГЛС)

Міська ГЛС як об'єкт екологічного менеджменту. Міські території являють собою складні просторово неоднорідні системи, у межах яких природні процеси взаємодіють із забудовою, транспортною та інженерною інфраструктурою, різними формами землекористування й управлінськими практиками. Ґрунтовий покрив у такій системі не існує ізольовано, його властивості визначаються походженням і складом ґрунтоутворних порід (субстрату), рельєфом, водним режимом, рослинністю, ступенем запечатування поверхні, характером техногенного навантаження та історією використання території. Водночас стан ґрунту впливає на інфільтрацію атмосферних опадів, формування поверхневого стоку, життєздатність рослинності, міський мікроклімат, геохімічну міграцію речовин і безпеку використання незапечатаних територій. З огляду на це необхідна системна одиниця аналізу, яка поєднує ґрунтові властивості з просторовою організацією ландшафту, потоками речовини та енергії, зовнішніми впливами й механізмами управління. У вітчизняних дослідженнях методичні передумови формалізації урболандшафту пов'язуються з визначенням системи критеріїв і параметрів, необхідних для побудови його моделі. Це створює підґрунтя для переходу від загального опису міської території до виокремлення її структурних компонентів, просторових зв'язків і функціонально значущих характеристик⁴.

⁴ Тітенко Г. В., Баскакова Л. В. Критерії та параметри для розробки моделі урболандшафту. *Вісник Харківського національного університету імені В. Н. Каразіна. Серія «Екологія»*. 2013. № 1070, вип. 9. С. 91–95. URL: http://nbuv.gov.ua/UJRN/VKhNU_2013_1070_9_15 (дата звернення: 30.07.2026).

У межах дослідження під міською ГЛС розуміємо просторово обмежену та ієрархічно організовану соціоекологічну підсистему міста, утворену природними, антропогенно трансформованими і техногенними ґрунтами та ґрунтоподібними тілами, рельєфом, рослинністю, поверхневими й ґрунтовими водами, запечатаними поверхнями та інженерною інфраструктурою, функціонування яких визначається характером землекористування, зовнішніми навантаженнями, внутрішніми взаємозв'язками та управлінськими практиками⁵.

Просторові межі міської ГЛС не збігаються з адміністративними межами ділянки, кварталу або району. Вони мають визначатися відповідно до процесу, функції або ризику, що досліджується. Для оцінювання регулювання поверхневого стоку функціональною одиницею може бути локальний водозбір, який включає як незапечатані ґрунтові ділянки, так і дороги, покритві, дренажні споруди та пониження рельєфу. Для аналізу життєздатності міських дерев межі системи мають охоплювати доступний кореневий простір, зону надходження води, прилегли запечатані поверхні та підземні комунікації. Для оцінювання забруднення територіальна одиниця має враховувати джерело надходження речовин, напрям поверхневого та внутрішньогрунтового перенесення речовин та зони потенційної акумуляції речовин.

Отже, залежно від мети оцінювання вбачаємо можливість використання кількох вкладених типів меж, як-то морфологічні (за рельєфом і ґрунтовим покривом); гідрологічні (за напрямками стоку й межами водозбору); функціональні (за реалізацією певної екосистемної функції); експозиційні (за поширенням певного впливу) та управлінські (за юрисдикцією та відповідальністю суб'єктів управління).

Вибір меж є не технічним, а методологічним рішенням. Надто вузькі межі можуть приховувати каскадні процеси, які виходять за межі пробної ділянки. Надто широкі можуть ускладнювати встановлення причинних зв'язків і створювати хибне усереднення просторово неоднорідних умов⁶⁷.

В аспекті ієрархічної організації міська ГЛС є багаторівневою. З урахуванням ієрархічно-патчового підходу⁸ до моделювання міських

⁵ Meerow S., Newell J. P., Stults M. Defining urban resilience: A review. *Landscape and Urban Planning*. 2016. Vol. 147. P. 38–49. DOI: <https://doi.org/10.1016/j.landurbplan.2015.11.011>.

⁶ Lempert R. J. Robust Decision Making (RDM). *Decision Making under Deep Uncertainty: From Theory to Practice* / eds. V. A. W. J. Marchau, W. E. Walker, P. J. T. M. Bloemen, S. W. Popper. Cham : Springer, 2019. P. 23–51. DOI: https://doi.org/10.1007/978-3-030-05252-2_2.

⁷ Hall J. W., Lempert R. J., Keller K. et al. Robust climate policies under uncertainty: A comparison of Robust Decision Making and Info-Gap methods. *Risk Analysis*. 2012. Vol. 32, No. 10. P. 1657–1672. DOI: <https://doi.org/10.1111/j.1539-6924.2012.01802.x>.

⁸ Zhang C., Wu J., Grimm N. B., McHale M., Buyantuyev A. A hierarchical patch mosaic ecosystem model for urban landscapes: Model development and evaluation. *Ecological Modelling*. 2013. Vol. 250. P. 81–100. DOI: <https://doi.org/10.1016/j.ecolmodel.2012.09.020>.

екосистем, відповідно до якого локальні екосистеми, типи земного покриву, землекористування, ландшафти та урбанізовані регіони утворюють вкладену просторово-функціональну структуру, у дослідженні запропоновано чотирирівневу класифікацію міських ґрунтово-ландшафтних систем: мікроділянка, функціональна ділянка, локальний ландшафтний комплекс і міська мережа. Авторська класифікація, розроблена на основі Hierarchical Patch Mosaic-Urban Ecosystem Model (HPM-UEM) та концепції динамічної неоднорідності⁹.

1. Мікроділянка як окремий ґрунтовий профіль, пристовбурна зона, газон, спеціально створений біоретенційний елемент або локальний осередок порушення ґрунтового покриву.

2. Функціональна ділянка, а саме парк, двір, сквер, транспортна смуга, промисловий майданчик, територія житлової забудови.

3. Локальний ландшафтний комплекс, а саме водозбір, зелений коридор, заплавна або схилова система.

4. Міська мережа як сукупність пов'язаних ґрунтових (коричневих), зелених, блакитних та інженерних елементів міста.

Показник, отриманий на мікроділянці, не може автоматично переноситися на вищий рівень. Наприклад, висока інфільтрація в окремій точці не означає ефективного регулювання стоку на рівні кварталу, якщо більша частина поверхні запечатана, а потоки води обминають відкриту ґрунтову ділянку. Аналогічно задовільний стан ґрунту в межах окремого парку не характеризує зв'язність усієї зеленої мережі міста.

Щодо компонентної структури та внутрішніх зв'язків міська ГЛС охоплює не лише ґрунтовий покрив, а щонайменше п'ять взаємозалежних компонентів.

Ґрунтовий компонент представлений природними ґрунтами, урбоземами, техноземами, насипними субстратами, похованими горизонтами, змішаними ґрунтово-будівельними матеріалами та іншими ґрунтоподібними тілами.

Геоморфологічний компонент включає природний і штучно змінений рельєф, схили, пониження, насипи, виїмки, вирви та мікроформи, які визначають перерозподіл води, осадів і забруднювальних речовин.

Біотичний компонент охоплює деревну, чагарникову й трав'янисту рослинність, кореневі системи, ґрунтову мікробіоту, безхребетних та інші організми, які беруть участь у формуванні структури ґрунту, кругообігу речовин і відновленні системи. Так, дослідження міських зелених просторів

⁹ Pickett S. T. A., Cadenasso M. L., Rosi-Marshall E. J. et al. Dynamic heterogeneity: A framework to promote ecological integration and hypothesis generation in urban systems. *Urban Ecosystems*. 2017. Vol. 20. P. 1–14. DOI: <https://doi.org/10.1007/s11252-016-0574-9>.

у 56 муніципалітетах на шести континентах¹⁰ показало зв'язок ґрунтового й рослинного біорізноманіття з широким набором екосистемних функцій, що підтверджує недоцільність розгляду біоти лише як другорядного індикатора озеленення.

Гідрологічний компонент включає надходження атмосферних опадів, інфільтрацію, перерозподіл вологи в профілі, поверхневий стік, підтоплення, дренаж і зв'язок із водними об'єктами.

Антропогенно-інженерний компонент представлений будівлями, дорогами, покриттями, підземними комунікаціями, дренажними системами, об'єктами благоустрою та джерелами навантаження.

Між цими компонентами наявні горизонтальні та вертикальні зв'язки. Горизонтальні зв'язки забезпечують переміщення води, осадів, забруднювачів, органічної речовини та біоти між ділянками. Вертикальні охоплюють взаємодію поверхні, ґрунтового профілю, кореневої зони, насипних матеріалів, підґрунтя та підземних вод.

Особливе значення мають зворотні зв'язки. Так, наприклад, ущільнення ґрунту → зменшення інфільтрації → посилення поверхневого стоку → ерозія і втрата органічної речовини → подальше погіршення структури ґрунту. Або інший приклад, дефіцит ґрунтової вологи → пригнічення рослинності → зменшення затінення та транспірації → підвищення температури поверхні → зростання випаровування → подальше посилення дефіциту вологи. Такі зворотні зв'язки можуть переводити тимчасове порушення у стійкий деградований стан.

Також вважаємо за потрібне розділяти зовнішню дію та властивості системи, які визначають її реакцію (внутрішню вразливість). Екстремальна злива є зовнішнім впливом, але масштаб підтоплення залежить від частки запечатування, ущільнення, особливостей рельєфу, стану дренажної системи й попередньої вологості ґрунту. Хвиля спеки є кліматичним шоком, однак функціональні втрати рослинності визначаються доступним об'ємом ґрунту, запасом вологи, глибиною кореневмісного шару та видовим складом насаджень.

Отже, реакцію міської ГЛС не можна пояснювати лише силою шоку. Вона є результатом взаємодії характеру небезпеки + експозиції + вихідної вразливості + функціональних резервів + управлінської спроможності.

Таке розмежування відповідає загальній логіці оцінювання кліматичних ризиків, у якій наслідки формуються не самим існуванням небезпеки, а її взаємодією з експозицією та вразливістю системи.

¹⁰ Fan K., Chu H., Eldridge D. J. et al. Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nature Ecology & Evolution*. 2023. Vol. 7. P. 113–126. DOI: <https://doi.org/10.1038/s41559-022-01935-4>.

Міська ГЛС стає об'єктом екологічного менеджменту, коли для неї визначені критичні функції та цільовий стан, суб'єкти управління, правові й організаційні повноваження, система моніторингу, можливі управлінські заходи, ресурси для їх реалізації, показники результативності, а також пороги й тригери перегляду рішень.

Управлінський контур не є зовнішнім доповненням до природної системи. Режим прибирання листя, частота косіння, організація руху відвідувачів, використання протиожеледних матеріалів, розміщення нової забудови, спосіб поводження зі знятим ґрунтом і характер відновлення пошкоджених територій безпосередньо змінюють структуру та функціональність системи.

Тому екологічний менеджмент має охоплювати повний адаптивний цикл. Від діагностики та встановлення цільових функцій через аналіз шоків і сценаріїв, вибір заходів, їх реалізацію, моніторинг та оцінювання результатів до коригування рішення.

Підхід до міських екологічних інфраструктур також підкреслює необхідність інтегрованого управління природними компонентами міста¹¹ та подолання інституційної фрагментації, за якої ґрунти, рослинність, вода й інженерні системи плануються окремо¹².

Ґрунтова інфраструктура (brown infrastructure) у структурі міста. Концепція brown infrastructure використовується в окремих напрямках урбоєкології для позначення ґрунтової основи міських екологічних систем. У концепції brown urban ecological infrastructure виділяється поряд із зеленими, блакитними та іншими екологічними компонентами й охоплює неозеленені наземні елементи та міські ґрунти, які забезпечують екосистемні функції й послуги. У літературі з міського ґрунтознавства ґрунти також описуються як brown infrastructure^{13, 14} за аналогією із зеленою інфраструктурою.

Водночас термін ще не має настільки уніфікованого й інституційно закріпленого змісту, як green infrastructure. Тому в роботі до поняття «ґрунтова інфраструктура (brown infrastructure)» пропонується таке робоче

¹¹ Fan K., Chu H., Eldridge D. J. et al. Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nature Ecology & Evolution*. 2023. Vol. 7. P. 113–126. DOI: <https://doi.org/10.1038/s41559-022-01935-4>.

¹² Childers D. L., Bois P., Hartnett H. E. et al. Urban Ecological Infrastructure: An inclusive concept for the non-built urban environment. *Elementa: Science of the Anthropocene*. 2019. Vol. 7. Article 46. DOI: <https://doi.org/10.1525/elementa.385>.

¹³ Pouyat R. V., Day S. D., Brown S. et al. Urban soils. Forest and Rangeland Soils of the United States Under Changing Conditions / eds. R. V. Pouyat, D. S. Page-Dumroese, T. Patel-Weynand, L. H. Geiser. Cham : Springer, 2020. P. 127–144. DOI: https://doi.org/10.1007/978-3-030-45216-2_7.

¹⁴ Shankar M., Ng M., Rogers M. et al. Unearthing the role of soils in urban climate resilience planning. *Nature Sustainability*. 2024. Vol. 7. P. 1374–1376. DOI: <https://doi.org/10.1038/s41893-024-01436-1>.

визначення. Під ґрунтовою інфраструктурою міста розуміємо просторово-функціональну сукупність відкритих і частково трансформованих міських ґрунтів, техноземів, ґрунтових конструкцій та доступних ґрунтових об'ємів, стан, розміщення і зв'язність яких забезпечують реалізацію водорегулювальних, біопродукційних, біогеохімічних, кліматорегулювальних і захисних функцій міського середовища.

Сучасна дискусія про міську кліматичну резильєнтність дедалі чіткіше підкреслює роль ґрунтів як essential infrastructure, необхідної для підтримання водорегулювання, рослинності та інших функцій міста.

Ґрунтова, зелена й блакитна інфраструктура не мають розглядатися як три незалежні категорії. Ґрунтова інфраструктура забезпечує субстрат, кореневий простір, інфільтрацію, утримання води, сорбцію речовин і середовище існування ґрунтової біоти. Втрата ґрунтової ланки порушує роботу всієї системи. Систематичні огляди підтверджують широкий спектр екосистемних послуг міських ґрунтів, але водночас показують, що їх мультифункціональність і зв'язки з іншими міськими компонентами часто оцінюються фрагментарно. Відповідно, ґрунтову інфраструктуру ми розглядаємо як функціональну рамку оцінювання міського ґрунтового ресурсу.

Потенціал екологічної резильєнтності. Екологічну резильєнтність міської ГІС доцільно розглядати не як її статичну характеристику або синонім задовільного екологічного стану, а як динамічну здатність системи реагувати на порушення, зберігаючи або відновлюючи критично важливі функції. У цьому контексті поточний стан ґрунту характеризує властивості системи на певний момент часу, тоді як резильєнтність описує характер її реакції на конкретний шок або сукупність взаємодіючих впливів.

Методологічно важливо розрізняти потенційну і фактично проявлену резильєнтність. Потенціал резильєнтності є сукупністю структурних, функціональних, просторових та управлінських передумов, які можуть забезпечити опірність системи, її відновлення або адаптацію до змін. Фактично проявлена резильєнтність може бути встановлена лише після виникнення конкретного порушення на основі спостереження за величиною функціональних втрат, тривалістю відновлення та станом системи після завершення відновного періоду. Отже, за відсутності часових рядів, що охоплюють стан системи до, під час і після шоку, науково коректніше оцінювати саме потенціал резильєнтності.

Для міських ГІС потенціал резильєнтності пропонується розглядати через три взаємопов'язані здатності: опірність, відновлюваність та адаптивність. Така модель дає змогу перейти від загального твердження про «стійкість території» до аналізу конкретних механізмів збереження і відтворення її функціональності.

Опірність міської ГЛС характеризує здатність системи обмежувати масштаб функціональних втрат під час дії шоку. Вона показує, наскільки істотно змінюється функціонування системи безпосередньо під впливом посухи, хвилі спеки, інтенсивних опадів, механічного порушення, забруднення, пожежі або іншої несприятливої події.

Опірність не означає повної відсутності змін. Навіть резильєнтна система може зазнавати тимчасового погіршення стану, проте величина цього погіршення не повинна перевищувати межу, після якої відбувається втрата критичної функції або перехід системи до іншого, небажаного функціонального стану.

Для міських ГЛС критичними можуть бути такі функції¹⁵, як:

- інфільтрація та утримання атмосферних опадів;
- регулювання поверхневого стоку;
- підтримання життєздатності міських насаджень;
- буферизація і сорбція забруднювачів;
- підтримання ґрунтового біорізноманіття;
- акумулювання органічної речовини;
- забезпечення безпечного використання території;
- участь у регулюванні локального мікроклімату.

Опірність залежить не від одного показника, а від поєднання властивостей системи. Наприклад, здатність ґрунту протистояти наслідкам посухи визначається гранулометричним складом, пористістю, вмістом органічної речовини, глибиною функціонального ґрунтового шару, характером кореневої системи, рівнем затінення та можливістю поповнення запасів вологи. Високий вміст гумусу може підвищувати водоутримувальну здатність, але не гарантує опірності, якщо ґрунт сильно ущільнений або ізольований від надходження води запечатаними поверхнями.

У разі оцінювання опірності важливо визначати:

- 1) вихідний рівень функціональності до впливу;
- 2) мінімальний рівень функціональності під час або одразу після шоку;
- 3) величину функціональної втрати;
- 4) наявність критичного порога;
- 5) просторовий масштаб порушення;
- 6) можливість поширення наслідків на суміжні компоненти міського ландшафту.

¹⁵ O’Riordan R., Davies J., Stevens C., Quinton J. N., Boyko C. The ecosystem services of urban soils: A review. *Geoderma*. 2021. Vol. 395. Article 115076. DOI: <https://doi.org/10.1016/j.geoderma.2021.115076>.

Висока опірність однієї функції не означає високої опірності всієї системи. Наприклад, ґрунт може зберігати здатність підтримувати трав'яний покрив, але втрачати інфільтраційну функцію через ущільнення¹⁶. Так само буферна здатність ґрунту щодо окремих забруднювачів може залишатися високою, тоді як біологічна активність і коренепроникність уже суттєво знижені.

Відновлюваність міської ГЛС характеризує здатність системи повертати втрачені функції після припинення або послаблення дії шоку. Її не можна оцінювати лише за фактом появи рослинності або часткового покращення окремого показника. Необхідно враховувати швидкість, повноту, стабільність і просторову рівномірність відновлення.

До основних характеристик відновлюваності належать:

- час початку відновної реакції;
- швидкість відновлення;
- частка відновленої функціональності;
- тривалість періоду до досягнення прийнятного стану;
- стабільність відновленого стану;
- залежність від зовнішнього втручання;
- імовірність повторної деградації;
- можливість відновлення кількох функцій одночасно.

Відновлення не обов'язково означає повернення до точного попереднього стану. Міські ґрунти переважно є антропогенно трансформованими, мозаїчними та просторово неоднорідними системами. Тому повернення до історичного або умовно природного стану може бути неможливим або недоцільним. Практичним орієнтиром має бути відновлення визначеного набору критичних функцій до рівня, достатнього для вибраного функціонального використання території.

Наприклад, для міського парку пріоритетними можуть бути підтримання деревної рослинності, інфільтрація опадів, терморегуляція та безпечне рекреаційне використання. Для біоретенційної ділянки основним критерієм буде здатність приймати, утримувати та очищувати поверхневий стік. Для території житлової забудови першочергового значення можуть набувати безпечність контакту населення з ґрунтом і відсутність вторинного пилового забруднення.

Відновлюваність визначається збереженням функціональних резервів системи. До них можуть належати:

- збережена структура ґрунту;
- достатня потужність незруйнованого ґрунтового шару;

¹⁶ Frene J. P., Pandey B. K., Castrillo G. Under pressure: Elucidating soil compaction and its effect on soil functions. *Plant and Soil*. 2024. Vol. 502. P. 267–278. DOI: <https://doi.org/10.1007/s11104-024-06573-2>.

- наявність органічної речовини;
- життєздатна ґрунтова біота;
- насіннєвий банк;
- збережені кореневі системи;
- гідрологічний зв'язок із незапечатаними територіями;
- відсутність незворотного токсичного забруднення;
- можливість проведення відновних заходів.

Після сильного механічного порушення, змішування горизонтів або значного забруднення природне відновлення може бути повільним або недостатнім. У такому разі слід розрізняти внутрішню відновлюваність системи та кероване відновлення, яке досягається за допомогою розуцільнення, внесення органічних матеріалів, відновлення рослинного покриву, регулювання стоку, локалізації забруднення або інших управлінських заходів.

Швидке відновлення окремого видимого компонента не завжди свідчить про відновлення системи. Наприклад, поява трав'янистої рослинності на порушеній ділянці не доводить відновлення ґрунтової структури, водного режиму, біологічного різноманіття чи буферної здатності. Відновлення необхідно оцінювати за функціями, а не лише за зовнішніми ознаками.

Адаптивність міської ГЛС характеризує здатність системи та пов'язаної з нею системи управління змінювати структуру, просторову організацію, режими функціонування або практики управління у відповідь на нові умови, не допускаючи неприйнятної втрати критичних функцій¹⁷.

На відміну від опірності, орієнтованої на обмеження безпосередніх втрат, і відновлюваності, спрямованої на повернення функціональності, адаптивність передбачає можливість функціональної перебудови. У міському середовищі вона часто залежить не лише від природних властивостей ґрунту, а й від інституційної спроможності, доступності даних, гнучкості планування та можливості змінювати управлінські рішення.

Адаптивність може передбачати:

- зміну складу рослинності відповідно до нового водного або температурного режиму;
- перерозподіл поверхневого стоку;
- створення додаткових інфільтраційних або біоретенційних ділянок;
- зменшення частки запечатаних поверхонь;
- зміну режиму рекреаційного використання;

¹⁷ Ribeiro P. J. G., Pena Jardim Gonçalves L. A. Urban resilience: A conceptual framework. *Sustainable Cities and Society*. 2019. Vol. 50. Article 101625. DOI: <https://doi.org/10.1016/j.scs.2019.101625>.

- просторове перенесення найбільш чутливих функцій;
- зміну функціонального призначення сильно порушеної території;
- поетапну ремедіацію;
- запровадження адаптивного моніторингу;
- перегляд управлінських рішень після досягнення визначених порогів.

Адаптивність не зводиться до необмеженого пристосування системи до будь-яких змін. Деякі зміни можуть призводити до збереження певних процесів, але супроводжуватися втратою бажаних екосистемних послуг. Наприклад, заміщення різноманітного рослинного угруповання кількома стійкими рудеральними або інвазійними видами може забезпечувати постійний рослинний покрив, але необов'язково підтримувати біорізноманіття, рекреаційну цінність або регулювання мікроклімату на необхідному рівні.

У певних концепціях міської резильєнтності поряд із адаптацією виділяють трансформацію¹⁸. Трансформація потрібна тоді, коли збереження попередньої структури або функціонального використання стає неможливим, економічно невиправданим чи екологічно небезпечним. У межах запропонованої трикомпонентної моделі трансформаційну здатність ми розглядаємо як вищий рівень адаптивності.

Прикладом трансформаційного рішення може бути перетворення території, непридатної для житлового або рекреаційного використання через складне забруднення чи повторюване підтоплення, на контрольовану буферну зону, водорегулювальний простір або ділянку з обмеженим доступом. У такому разі система не повертається до попереднього стану, але набуває нової конфігурації, здатної безпечніше виконувати визначені функції.

Опірність, відновлюваність і адаптивність не є взаємозамінними характеристиками. Система може мати високу опірність, але низьку відновлюваність, якщо після перевищення критичного порога її функції відновлюються дуже повільно. Інша система може сильно реагувати на короткочасний шок, але швидко відновлюватися після його завершення. Висока адаптивність може частково компенсувати недостатню опірність, однак лише за наявності своєчасного моніторингу, ресурсів і можливості змінювати управління.

Для характеристики потенціалу резильєнтності доцільно використовувати не один інтегральний бал, а функціональний профіль, який окремо відображає опірність кожної критичної функції, очікувану

¹⁸ Cariolet J.-M., Vuillet M., Diab Y. Mapping urban resilience to disasters – A review. *Sustainable Cities and Society*. 2019. Vol. 51. Article 101746. DOI: <https://doi.org/10.1016/j.scs.2019.101746>.

швидкість і повноту її відновлення, доступні механізми адаптації, критичні пороги, ключові невизначеності та залежність від управлінського втручання.

Такий профіль дає змогу виявити асиметрію резильєнтності. Наприклад, територія може мати достатню здатність підтримувати рослинність, але низьку здатність регулювати зливовий стік; або прийнятний водний режим, але неприйнятний рівень хімічного ризику для населення. Усереднення таких характеристик в одному індексі може приховати критичну проблему.

Екологічний стан є важливою передумовою резильєнтності, але не її достатнім доказом. Поточний стан описує систему в конкретний момент, тоді як резильєнтність стосується її поведінки під впливом порушення і після нього. Ґрунт може бути відносно хімічно незабрудненим, але переущільненим, малопотужним, біологічно збідненим або ізольованим запечатаними поверхнями. Після посухи, пожежі чи механічного порушення відновлення його функцій може бути тривалим і потребувати значного зовнішнього втручання.

Так само наявність зеленої зони не гарантує функціональної стійкості. Площа, позначена на карті як парк або сквер, може мати деградований ґрунтовий покрив, низьку інфільтраційну здатність, дефіцит доступної для рослин вологи, фрагментовану кореневу зону та недостатню різноманітність насаджень. Формальна наявність рослинного покриву в такому разі приховує високу вразливість до посухи, спеки, рекреаційного навантаження або інтенсивних опадів.

Показовим є приклад міського парку з прийнятним хімічним станом ґрунту. Відсутність значного забруднення може свідчити про безпечність території за хімічними критеріями, але не характеризує її здатність протистояти кліматичним шокам. За високої щільності складення, слабкої інфільтрації, низької водоутримуючої здатності та обмеженого об'єму ґрунту для розвитку коренів парк може бути вразливим до тривалої посухи. У період спеки дерева відчуватимуть дефіцит вологи, їхня транспірація і охолоджувальна функція зменшуватимуться, а відмирання частини насаджень посилюватиме нагрівання поверхні. У такій ситуації прийнятний хімічний стан поєднується з низькою функціональною опірністю і недостатньою відновлюваністю.

Отже, оцінювання потенціалу резильєнтності має виходити за межі нормативного порівняння концентрацій забруднювачів. Воно повинно охоплювати фізичні, хімічні, біологічні, гідрологічні, просторові та управлінські характеристики, безпосередньо пов'язані зі збереженням і відновленням критичних функцій.

Під потенціалом екологічної резильєнтності міської ГЛС пропонується розуміти сукупність її структурних, функціональних, просторових та управлінських властивостей, які визначають здатність обмежувати втрату критичних функцій під час комбінованих шоків, відновлювати ці функції після порушення та адаптувати структуру і режими управління до зміни умов без переходу до екологічно або соціально неприйнятного стану.

Таке визначення векторизує дослідження не на пошук абстрактної «стійкості», а на встановлення конкретних функцій, порогів, часових горизонтів, механізмів відновлення та можливостей адаптивного управління.

2. Методичний підхід до оцінювання потенціалу резильєнтності в умовах комбінованих шоків

Оцінювання потенціалу екологічної резильєнтності міських ГЛС потребує переходу від традиційного опису екологічного стану території до функціонально та сценарно-орієнтованого аналізу. Визначення концентрацій забруднювачів, фізичних властивостей ґрунту або частки зелених насаджень саме по собі не дає відповіді на питання, як система поводитиметься під час екстремального впливу, які функції вона зможе зберегти, наскільки швидко відновиться та чи буде здатною адаптуватися до нових умов.

У межах запропонованого підходу потенціал резильєнтності розглядається як сценарно залежна характеристика. Одна й та сама територія може демонструвати відносно високий потенціал резильєнтності до короточасної посухи, але бути вразливою до екстремальної зливи, хімічного забруднення або поєднання пожежі з наступним ерозійним зливом. Тому оцінка не повинна формулюватися абстрактно як «територія має високий рівень резильєнтності». Коректним є твердження про потенціал збереження або відновлення конкретних функцій за визначеного сценарію впливу, просторового масштабу та часового виміру.

Методичну основу дослідження становить причинно-функціональний ланцюг: вихідний стан системи → хронічні тиски і стреси → шок або комбінація шоків → первинна реакція компонентів → втрата критичних функцій → каскадні наслідки → відновлення або перехід до іншого стану → управлінська відповідь. Такий підхід дає змогу відокремити властивості самої ГЛС від характеристик зовнішнього впливу, а природну відновлюваність від результатів цілеспрямованого управлінського втручання.

Віднесення певного чинника до тиску, стресу або шоку не може ґрунтуватися лише на його назві. Воно залежить від швидкості виникнення, тривалості, інтенсивності, повторюваності, просторового охоплення,

попереднього стану системи, характерного часу її реакції та наявності порогу функціонального порушення.

Наприклад, посуха може бути тривалим стресором, якщо дефіцит вологи нарастає поступово, але може функціонувати як шок, якщо швидка та інтенсивна атмосферна посуха виникає в критичний період розвитку рослинності. Забруднення може бути хронічним тиском за поступового накопичення або шоком за аварійного викиду. Ущільнення може формуватися поступово внаслідок рекреаційного навантаження або виникати раптово після проходження важкої техніки.

Відповідно, доцільно класифікувати вплив за набором часово-просторових параметрів, а не за формальною назвою (табл. 1).

«Комбінований шок» пропонується визначати як ситуацію, у якій два або більше драйверів, стресорів чи небезпечних подій діють одночасно, послідовно або в просторово пов'язаних частинах системи та спільно визначають функціональний наслідок. При цьому комбінований шок необов'язково передбачає доведений синергетичний ефект.

Таблиця 1

Критерії ідентифікації впливів у разі оцінювання потенціалу екологічної резильєнтності міських ГЛС

№	Критерій	Хронічний тиск	Тривалий стресор	Шок
1.	Початок	Поступовий або постійний	Поступовий чи сезонний	Відносно раптовий
2.	Тривалість	Тривала, часто невизначена	Від днів до років	Переважно коротша за час відновлення
3.	Повторюваність	Систематична	Періодична або тривала	Разова або серійна
4.	Основний ефект	Накопичення змін	Виснаження резервів	Різка функціональне відхилення
5.	Роль у резильєнтності	Формує фонову вразливість	Зменшує функціональний резерв	Перевіряє опірність і запускає відновлення
-	Типовий приклад	Транспортне забруднення	Тривала посуха	Екстремальна злива

Наприклад, комбінацію «спека + посуха + ущільнення» точніше описувати як кліматичний комбінований вплив, посилений попередньо сформованою фізичною вразливістю ґрунту. Саме ущільнення в цьому випадку є необов'язково шоком, а умовою, яка зменшує доступний функціональний резерв.

Комбінація «вибухове порушення + пожежа + забруднений поверхневий стік» є складним послідовно-каскадним шоком. Механічне та термічне

порушення змінюють властивості поверхні, а подальші опади забезпечують мобілізацію та просторове перенесення речовин.

Типологію комбінованих шоків для міських ГЛС доцільно формувати за двома критеріями – за походженням чинників та за механізмом їх поєднання (табл. 2).

Класифікація лише за походженням є недостатньою, оскільки одна подія може одночасно належати до кількох категорій. Наприклад, руйнування дренажу внаслідок воєнного впливу¹⁹, подальша злива та перенесення забруднювачів формують воєнний, гідрологічний і каскадний компоненти одного сценарію.

Наслідки кліматичного шоку значною мірою залежать від попереднього стану ґрунту. Ущільнений або малопотужний ґрунт із низьким вмістом органічної речовини може мати значно нижчу опірність до посухи, ніж ґрунт із розвиненою структурою та доступним для коренів профілем. Водночас після тривалої посухи інтенсивна злива може викликати не відновлення запасів вологи, а поверхневий стік, ерозію та локальне підтоплення.

Воєнний вплив не слід автоматично ототожнювати з токсичним забрудненням. Наявність вирви, уламків або слідів пожежі є підставою для скринінгу, але не доказом перевищення концентрацій конкретних речовин. Хімічний ризик має встановлюватися аналітично з урахуванням типу події, джерела забруднення, властивостей ґрунту та шляхів перенесення²⁰.

Каскадний шок характеризується передаванням наслідків між компонентами системи. Каскадний аналіз потребує виходу за межі окремої пробної ділянки. Необхідно враховувати рельєф, водозбір, напрями стоку, просторову зв'язність зелених зон, розташування інженерних мереж і можливість перенесення речовин.

Більшість урбанізаційно-техногенних впливів є хронічними тисками або накопичувальними стресорами, а не шоками у вузькому значенні. Проте вони визначають попередню вразливість і можуть суттєво посилювати наслідки кліматичних або аварійних подій. Прикладом є поєднання запечатування, ущільнення залишкових незабудованих ділянок та інтенсивних опадів. У цьому сценарії запечатування скорочує площу інфільтрації, ущільнення зменшує водопроникність відкритого ґрунту, а злива стає тригером швидкого формування поверхневого стоку.

¹⁹ Solokha M., Demyanyuk O., Symochko L. et al. Soil degradation and contamination due to armed conflict in Ukraine. *Land*. 2024. Vol. 13, No. 10. Article 1614. DOI: <https://doi.org/10.3390/land13101614>.

²⁰ Bonchkovskiy O., Ostapenko P., Bonchkovskiy A., Shvaiko V. War-induced soil disturbances in north-eastern Ukraine (Kharkiv region): Physical disturbances, soil contamination and land use change. *Science of the Total Environment*. 2025. Vol. 964. Article 178594. DOI: <https://doi.org/10.1016/j.scitotenv.2025.178594>.

Таблиця 2

Групи шоків та ризики, які вони створюють (приклади)

Група шоків та впливів	Явища	Функції під ризиком
Кліматичні та гідрометеорологічні шоки	<ul style="list-style-type: none"> – Хвилі спеки; – атмосферні та ґрунтові посухи; – інтенсивні опади; – швидке танення снігу; – різкі переходи температури через 0°C; – сильні вітри; – поєднання спеки з посухою; – посуха, за якою настає екстремальна злива. 	<ul style="list-style-type: none"> – Утримання вологості; – інфільтрація; – регулювання поверхневого стоку; – підтримання рослинності; – біологічна активність; – регулювання температури міського середовища; – протигерозійна функція.
Воєнно зумовлені шоки	<ul style="list-style-type: none"> – Вибухове механічне порушення; – утворення вивр і штучного мікрорельєфу; – перемішування ґрунтових горизонтів; – пожежі; – руйнування будівель; – осідання пилу та продуктів горіння; – надходження уламків і будівельних матеріалів; – ущільнення важкою технікою; – руйнування дренажних систем; – зміну напрямів поверхневого стоку; – потенційне локальне хімічне забруднення. 	<ul style="list-style-type: none"> – Фізична цілісність ґрунтового профілю; – інфільтрація; – підтримання рослинності; – буферизація забруднювачів; – безпечність контакту з ґрунтом; – можливість подальшого землекористування; – природна відновлюваність
Каскадні та системні шоки	<p><i>1 варіант.</i></p> <p>Руйнування дренажу → → локальне підтоплення → → зміна окисно-відновних умов → → мобілізація забруднювачів → → перенесення до понижень рельєфу → → ризик для водних об'єктів або населення.</p> <p><i>2 варіант.</i></p> <p>пожежа → → втрата рослинного й органічного покриву → зниження структурної стабільності → → екстремальна злива → → ерозійний змив → → замулення дренажної системи → → повторне підтоплення.</p>	<ul style="list-style-type: none"> – Регулювання поверхневого стоку та інфільтрація опадів; – акумулювання та підтримання доступних запасів ґрунтової вологості; – підтримання життєздатності міської рослинності; – терморегуляція міського середовища; – буферизація, сорбція і трансформація забруднювальних речовин; – збереження фізичної стабільності ґрунтового покриву; – підтримання біогеохімічних циклів і ґрунтового біорізноманіття; – просторової та функціональної зв'язності міської природної інфраструктури.
Урбанізаційно-техногенні впливи	<ul style="list-style-type: none"> – Ущільнення, – запечаткування поверхні; – зняття або перемішування ґрунтових горизонтів; – забруднення потенційно небезпечними елементами; – надходження нафтопродуктів та органічних забруднювачів; – засолення, – зміна рельєфу; – фрагментація зелених ділянок; – порушення природних шляхів стоку; – теплове навантаження від штучних поверхонь. 	<ul style="list-style-type: none"> – Інфільтраційно-водорегулювальна; – структурно-фізична; біопродукційна; – буферно-фільтраційна; – біогеохімічна; – вуглецерегулювальна; – біотопічна; – кліматорегулювальна функції.

Управлінська неспроможність працює як модифікатор вразливості. Відсутність моніторингу, затримка реагування, невизначеність відповідальності або дефіцит фінансування не є фізичними чи екологічними шоками. Їх доцільно трактувати як інституційні модифікатори вразливості. Вони можуть збільшувати тривалість негативного впливу; зменшувати ймовірність раннього виявлення; перешкоджати локалізації забруднення; збільшувати час відновлення; сприяти повторенню порушення; переводити оборотну деградацію у стійкий небажаний стан.

Отже, потенціал резильєнтності міської території визначається не лише природними властивостями ґрунту, а й здатністю системи управління розпізнавати шоки, своєчасно реагувати й коригувати рішення.

Щодо формування системи показників потенціалу резильєнтності вважаємо, що вибір показників має здійснюватися за принципом «функція – механізм – показник».

Алгоритм пропонується такий:

- 1) визначення критичної функції;
- 2) встановлення механізму її порушення;
- 3) вибір властивостей, які контролюють цей механізм;
- 4) визначення вимірюваних індикаторів;
- 5) встановлення референтного або порогового значення;
- 6) оцінювання невизначеності інтерпретації.

Наприклад, для функції регулювання зливого стоку важливими є не всі хімічні показники ґрунту, а передусім водопроникність, щільність, структура, потужність ґрунтового профілю, положення в рельєфі, частка запечатування та зв'язок із дренажною мережею.

Блок А. Параметри шоку й експозиції. Запропоновані чотири блоки показників (табл. 2) необхідно доповнити окремим блоком, який характеризує зовнішній вплив. Без цього неможливо відокремити високу резильєнтність від слабкої інтенсивності шоку.

До блоку належать тип події; інтенсивність; тривалість; швидкість виникнення; частота повторення; сезон; просторове охоплення; послідовність подій; час між повторними подіями; частка території, яка зазнала впливу; наявність зовнішнього надходження забруднювачів; потенційні шляхи поширення наслідків. Наприклад, невелика функціональна втрата після слабкої зливи не є доказом високої опірності до екстремальних опадів.

Блок Б. Стан і функціональність ґрунту. Фізичні показники: гранулометричний склад; щільність складення; загальна та функціональна пористість; опір penetрації; водостійкість агрегатів; інфільтраційна здатність; водоутримувальна здатність; потужність

функціонального ґрунтового шару; наявність ущільнених або техногенних горизонтів.

Хімічні показники: рН; електропровідність; вміст органічного вуглецю або гумусу; ємність катіонного обміну; доступні елементи живлення; валові та рухомі форми потенційно небезпечних елементів; нафтопродукти, поліциклічні ароматичні вуглеводні або інші органічні забруднювачі (лише за наявності обґрунтованого джерела ризику).

Біологічні показники: ґрунтове дихання; мікробна біомаса; активність ферментів; чисельність або різноманітність ґрунтових безхребетних; інтенсивність розкладання органічної речовини; стан кореневої системи; ознаки біологічного відновлення після порушення. Включення біологічних показників є принциповим, оскільки фізико-хімічно прийнятний ґрунт може мати низьку біологічну функціональність. Однак конкретний набір має визначатися можливостями лабораторії та цінністю показника для обраної функції.

Блок В. Просторово-ландшафтні характеристики. Показники для визначення: частка запечатаних поверхонь; площа й конфігурація незапечатаних ділянок; структура рослинного покриву; частка деревної, чагарникової та трав'янистої рослинності; зв'язність зелених ділянок; положення в рельєфі; крутизна схилу; належність до водозбору; напрями поверхневого стоку; відстань до джерел забруднення; близькість до транспортної мережі; фрагментація кореневого простору; просторове сусідство з водними об'єктами; частка території з порушеним ґрунтовым профілем.

Ці показники можуть визначатися за допомогою польового картування, GIS, цифрових моделей рельєфу, дистанційного зондування та аналізу землекористування.

Блок Г. Ознаки вразливості. Вразливість характеризує схильність системи до втрати конкретної функції за визначеного сценарію. Ознаками вразливості можуть бути: ущільнення; низька структурна стабільність; дефіцит органічної речовини; мала потужність кореневмісного шару; ерозійна нестійкість; токсичне забруднення; засолення; порушення дренажу; велика частка запечатування; просторове роз'єднання зелених ділянок; низьке видове та функціональне різноманіття рослинності; відсутність альтернативних шляхів відведення стоку; залежність від постійного зовнішнього догляду; попередня історія повторних порушень.

Один і той самий показник може характеризувати стан, вразливість або функціональність залежно від мети оцінювання. Наприклад, щільність складення є фізичною властивістю, але її високе значення стає ознакою вразливості щодо інфільтрації й розвитку коренів.

Блок Д. Управлінська та адаптивна спроможність. До цього блоку належать: наявність систематичного моніторингу; доступність актуальних просторових і лабораторних даних; визначеність відповідального суб'єкта; наявність плану реагування; швидкість мобілізації ресурсів; доступність території для втручання; технічна здійсненність заходів; фінансова забезпеченість; можливість поетапної реалізації; наявність резервних сценаріїв; можливість зміни функціонального використання; нормативні та безпекові обмеження; участь місцевих зацікавлених сторін; система індикаторів і тригерів перегляду рішення.

Цей блок не слід механічно поєднувати з ґрунтовими показниками. Він характеризує соціоекологічний контур управління та здатність компенсувати природні обмеження системи.

Щодо класифікації показників за динамічністю. Для оцінювання резильентності корисно розрізняти відносно сталі властивості (гранулометричний склад, природне положення в рельєфі), повільно змінні властивості (потужність профілю, запас органічного вуглецю), динамічні властивості (вологість, температура, електропровідність, біологічна активність), швидкі індикатори реакції (інфільтрація після опадів, стан рослинності, поверхневий стік), управлінські індикатори (час реагування, наявність даних, реалізація заходів).

Поєднання показників із різним часом реакції дає змогу відрізнити структурні передумови резильентності від короткострокової реакції на подію.

Пропонуємо таку процедуру оцінювання потенціалу резильентності. По-перше, вважаємо за доцільне не створювати один універсальний індекс резильентності. Агрегований бал може приховати критичну вразливість, бо високі значення другорядних показників здатні математично компенсувати втрату функції, яка фактично не може бути компенсована. По-друге, вважаємо за максимально надійний підхід формування функціонально-сценарного профілю резильентності.

Етап 1. Визначення меж системи. Необхідно встановити просторові межі; вертикальну потужність ґрунтового профілю; тип землекористування; пов'язані зелені, водні та інженерні елементи; суб'єктів управління; часовий горизонт оцінювання.

Межі мають визначатися відповідно до процесів. Для аналізу стоку доцільною одиницею може бути локальний водозбір²¹, для оцінки деревостану – кореневий простір, для забруднення – зона потенційного перенесення.

²¹ Casal-Campos A., Sadr S. M. K., Fu G., Butler D. Reliable, resilient and sustainable urban drainage systems: An analysis of robustness under deep uncertainty. *Environmental Science & Technology*. 2018. Vol. 52, No. 16. P. 9008–9021. DOI: <https://doi.org/10.1021/acs.est.8b01193>.

Етап 2. Визначення критичних функцій. Для конкретної території вибираються функції, втрата яких є екологічно або соціально неприйнятною, а саме інфільтрація; регулювання стоку; підтримання рослинності; буферизація забруднювачів; біологічна активність; терморегуляція; безпечність використання; стабілізація поверхні. Не всі функції повинні мати однакову пріоритетність. Вона залежить від призначення території та потреб зацікавлених сторін.

Етап 3. Оцінювання поточної забезпеченості функцій. Для кожної функції визначається набір прямих і непрямих показників; референтна основа; поточний рівень; просторове варіювання; достовірність даних; основні обмежувальні фактори. Результатом є не загальна «якість ґрунту», а профіль забезпеченості функцій.

Етап 4. Формування сценаріїв комбінованих шоків. Сценарій має містити склад чинників; послідовність; інтенсивність; тривалість; частоту повторення; просторове охоплення; сезон; фонові тиски; управлінські умови.

Доцільно формувати не один прогноз, а набір правдоподібних сценаріїв: помірний, несприятливий, екстремальний та сценарій із невизначеними параметрами.

Етап 5. Визначення чутливості функцій. Для кожної комбінації «функція – сценарій» встановлюється механізм впливу; напрям зміни; очікувана величина втрати; імовірність перевищення порога; можливість каскадного поширення; рівень доказовості причинного зв'язку.

Чутливість бажано подавати окремо від експозиції. Територія може бути дуже чутливою до підтоплення, але не експонованою до нього через положення на вододілі²².

Етап 6. Оцінювання опірності. Опірність характеризується очікуваною або фактичною величиною втрати критичної функції під час шоку. Оцінювання опірності передбачає визначення максимальної відносної втрати критичної функції в межах заданого сценарію та часового вікна. Вихідний і мінімальний рівні функціональності мають бути визначені за зіставних умов, а показники приведені до єдиного напрямку інтерпретації. За наявності часових спостережень опірність розраховують за фактичною траєкторією функцій; за їх відсутності формують сценарну оцінку на основі механістичного моделювання, просторових або історичних аналогів та структурованого експертного судження. Результат слід подавати разом із діапазоном невизначеності, характеристиками шоку та інформацією про можливе перетинання критичних функціональних порогів.

²² Urich C., Rauch W. Exploring critical pathways for urban water management to identify robust strategies under deep uncertainties. *Water Research*. 2014. Vol. 66. P. 374–389. DOI: <https://doi.org/10.1016/j.watres.2014.08.020>.

Етап 7. Оцінювання відновлюваності. Оцінюються очікуваний час початку відновлення; тривалість повернення до прийнятного рівня; повнота відновлення; стабільність відновленого стану; імовірність повторного порушення; залежність від зовнішнього втручання; наявність природних резервів відновлення.

Є сенс окремо розрізнати природне відновлення; кероване відновлення; відновлення до попереднього стану; відновлення до іншого, але функціонально прийнятного стану.

Етап 8. Оцінювання адаптивності. Адаптивність визначається через наявність альтернативних способів виконання функції; можливість зміни просторової організації; наявність функціональної надлишковості; можливість зміни режиму використання; гнучкість управління; доступність ресурсів; якість моніторингу; можливість поетапного втручання; наявність тригерів перегляду рішення.

Етап 9. Формування профілю резильєнтності. Для кожної критичної функції формується набір характеристик, у т.ч. поточне забезпечення, опірність, відновлюваність, адаптивність, критичний поріг, невизначеність.

Профіль може відображатися у вигляді матриці, теплової карти або радарної діаграми. Однак графічне узагальнення не повинно замінювати аналіз причин функціональних обмежень.

Етап 10. Виявлення критичних вразливостей. Доцільно застосовувати принцип функціонального «вузького місця», якщо одна критична функція переходить у неприйнятний стан, високі значення інших функцій не завжди можуть її компенсувати. Наприклад, висока водопроникність не компенсує небезпечне забруднення або добре озеленення не компенсує ризик контакту дітей із токсичним ґрунтом²³, або високий вміст органічної речовини не компенсує повне запечаткування, або висока природна відновлюваність не усуває необхідності термінової локалізації забруднення. Тому функції є сенс поділити на взаємозамінні; частково компенсовані; незамінні або обмежувальні.

Етап 11. Оцінювання невизначеності. Профіль резильєнтності має супроводжуватися оцінкою невизначеності. Основні її типи: вимірювальна (похибка аналізу та просторового відбору), структурна (неповне розуміння причинних зв'язків), модельна (залежність результату від вибраної моделі), сценарна (невідомі характеристики майбутніх шоків), нормативна (невизначеність щодо прийнятного стану), управлінська (невідомі

²³ Yin D., Zhang X., Cheng Y., Jia H., Jia Q., Yang Y. Can flood resilience of green-grey-blue system cope with future uncertainty? *Water Research*. 2023. Vol. 242. Article 120315. DOI: <https://doi.org/10.1016/j.watres.2023.120315>.

ресурси, строки та спроможність реалізації), епістемічна (нестача знань, яку потенційно можна зменшити), варіабельність (природна або соціальна мінливість, яку не можна усунути додатковими дослідженнями).

Результатом оцінювання має бути:

- 1) визначення критичних функцій конкретної території;
- 2) ідентифікація шоків і фонових стресорів;
- 3) встановлення причинних механізмів функціональних втрат;
- 4) оцінка опірності, відновлюваності та адаптивності;
- 5) виявлення функціональних «вузьких місць»;
- 6) визначення рівня невизначеності;
- 7) формування інформаційної основи для сценарного тестування робастних управлінських рішень.

Таким чином, оцінювання потенціалу резильєнтності є не завершальним рейтингуванням, а проміжною аналітичною процедурою, яка пов'язує дані про міські ґрунти, ландшафтну структуру та управлінську спроможність із вибором заходів, здатних забезпечувати прийнятний результат за різних сценаріїв комбінованих шоків.

3. Обґрунтування робастних управлінських рішень для міських територій

Управлінські рішення щодо міських ІЛС ухвалюються за умов неповноти даних, просторової неоднорідності території, мінливості кліматичних впливів, обмеженого фінансування та невизначеної спроможності відповідальних суб'єктів реалізовувати й підтримувати заплановані заходи. Для міст, що функціонують в умовах воєнного ризику, до цих чинників додаються можливість повторних пошкоджень, порушення енергопостачання, обмеження доступу до територій, нестабільність бюджетів і зміна пріоритетів відновлення. За таких умов орієнтація на одне «найкраще» рішення, розраховане для найбільш імовірного сценарію, є недостатньою.

У межах цього дослідження робастним управлінським рішенням пропонується вважати рішення або портфель взаємопов'язаних заходів, який:

- 1) забезпечує збереження або відновлення критичних функцій за широкого діапазону правдоподібних сценаріїв;
- 2) не призводить до неприйнятних наслідків за несприятливих умов;
- 3) залишається технічно, фінансово й інституційно здійсненним;
- 4) допускає коригування, розширення або заміну окремих компонентів у міру надходження нової інформації;
- 5) не блокує реалізацію перспективних альтернатив у майбутньому.

Робастність не означає, що рішення є найефективнішим у кожному окремому сценарії. Воно може поступатися вузько оптимізованому рішенням за сприятливих умов, але має менший ризик функціонального провалу, неприйнятних витрат або незворотних негативних наслідків за несприятливих комбінацій шоків.

Робастне рішення не є окремим інженерним або природоорієнтованим заходом, а є структурованою управлінською конструкцією, яка включає початкові дії, резервні можливості, систему моніторингу, порогові значення та правила переходу до наступного етапу.

Окремий захід не здатен підтримувати всі критичні функції міської території за різних комбінацій шоків. Збільшення поливу може тимчасово зменшити водний стрес рослинності, але не усуває ущільнення ґрунту, не гарантує надходження води до активної кореневої зони та створює залежність від стабільного водо- й енергопостачання. Розширення зливової труби може збільшити відведення води, але не розв'язує проблеми засмічення зливоприймачів, поверхневого спрямування стоку до підвальних входів або підпору нижче за течією.

Тому об'єктом порівняння повинні бути не ізольовані заходи, а портфелі рішень, які поєднують природоорієнтовані заходи, ґрунтово-відновлювальні дії, інженерні рішення, просторове проектування, організаційні процедури, моніторинг наслідків, експлуатаційне обслуговування та резервні дії на випадок перевищення проектної спроможності.

У прийнятті рішень за глибокої невизначеності найбільш робастними часто виявляються не одиничні заходи, а гібридні стратегії, що поєднують інженерні, природоорієнтовані та організаційні компоненти. Водночас результат залежить від вибраних сценаріїв і критеріїв робастності, тому такі висновки не можна механічно переносити між територіями.

Послідовність обґрунтування робастного рішення розглянемо у практичному кейсі для міського парку у великому місті (Сад імені Тараса Шевченка, м. Харків, Україна).

1. Визначення просторових і управлінських меж. На першому етапі визначаємо територію, на яку безпосередньо поширюється рішення, функціонально пов'язані території, джерела зовнішніх впливів, просторовий рівень, на якому проявляється наслідок, власників і балансоутримувачів, суб'єктів, відповідальних за реалізацію й обслуговування, часовий горизонт рішення.

Сад імені Тараса Шевченка розташований у центральній частині Харкова.

Під час реконструкції саду були реалізовані^{24, 25}:

- 1) близько 95 тис. м² газонів;
- 2) близько 57 тис. м² асфальтового та гранітного дорожнього покриття;
- 3) система автоматичного поливу;
- 4) зливово каналізація;
- 5) штучна водойма;
- 6) пішохідні та рекреаційні об'єкти;
- 7) заходи зі збереження старовікових дубів, вік яких у проєктних матеріалах оцінювався у 200–300 років.

Ці дані свідчать про значну внутрішню неоднорідність території. Сад поєднує деревні насадження, газони, відкриті рекреаційні площі, алеї, що інтенсивно використовуються, водний об'єкт та інженерні мережі. За задокументованими площами газонів і дорожнього покриття останнє становить приблизно 37,5% їхньої сумарної площі. Це не частка запечатування всього саду, оскільки проєктні дані не охоплюють усіх типів поверхонь, але показник підтверджує істотну роль твердих покриттів у водному й тепловому режимі території.

В офіційному переліку природно-заповідного фонду ботанічна пам'ятка природи «Сад ім. Т. Г. Шевченка» має площу 0,75 га²⁶. Цю величину не слід помилково використовувати як загальну площу всього саду, вона характеризує площу охоронного об'єкта у відповідному реєстрі.

Для Саду імені Тараса Шевченка формальна межа парку недостатня для аналізу водного режиму, оскільки необхідно враховувати надходження стоку з твердих поверхонь, систему поливу, дренаж і розподіл рекреаційного навантаження.

2. Визначення критичних функцій. Рішення має оцінюватися не за кількістю реалізованих заходів, а за здатністю підтримувати визначені функції.

Для паркового кейсу такими функціями є доступність ґрунтової вологості²⁷; підтримання деревної рослинності; терморегуляція; збереження ґрунтової структури; стабільність поверхні; рекреаційний комфорт.

²⁴ Kharkiv Development & Investment. Реконструкція Саду імені Тараса Шевченка [Електронний ресурс]. URL: <https://inkharkiv.com/projects/rekonstruktsiia-sadu-imeni-tarasa-shevchenka/> (дата звернення: 30.07.2026).

²⁵ Харківська міська рада. Новий каскад, озеро і зони відпочинку – у Харкові реконструюють сад Шевченка [Електронний ресурс]. 11.05.2018. URL: <https://city.kharkiv.ua/news/misto/noviy-kaskad-ozero-i-zoni-vidpochinku---u-kharkovi-rekonstruyuyut-sad-shevchenka> (дата звернення: 30.07.2026).

²⁶ Харківська обласна державна адміністрація. Перелік територій та об'єктів природно-заповідного фонду Харківської області станом на 01.10.2021 року [Електронний ресурс]. URL: https://kharkivoda.gov.ua/content/documents/1127/112691/Attaches/perelik_pzf_na_sayt_hoda.pdf (дата звернення: 30.07.2026).

²⁷ Tams L., Paton E., Kluge B. Urban tree drought stress: Sap flow measurements, model validation, and water management simulations. *Science of the Total Environment*. 2024. Vol. 957. Article 177221. DOI: <https://doi.org/10.1016/j.scitotenv.2024.177221>.

Функції мають бути ранжовані за критичністю. Безпечність людей, захист житлових приміщень і збереження старовікових дерев не повинні математично компенсуватися другорядними благоустроєвими перевагами.

3. Встановлення прийнятної та неприйнятної станів. Для кожної критичної функції необхідно встановити бажаний стан; прийнятний мінімум; поріг управлінського реагування; критичний поріг; неприйнятний результат.

Порогом може бути фізичний показник; функціональна втрата; тривалість порушення; площа ураження; кількість користувачів під ризиком; перевищення бюджету; неможливість відновлення в заданий строк.

Наприклад, для парку не досить встановити, що дерева залишилися живими. Необхідно визначити прийнятний рівень збереження листового покриву, транспірації, охолоджувальної функції²⁸ та стану кореневої зони.

4. Ідентифікація функціонального «вузького місця». «Вузьким місцем» є компонент або процес, який першим обмежує збереження критичної функції. Його не можна встановлювати лише за найгіршим значенням окремого індикатора. Потрібно визначити причинний механізм.

Для Саду імені Тараса Шевченка можливий ланцюг має вигляд: рекреаційне навантаження → ущільнення → зменшення макропористості та інфільтрації → недостатнє поповнення вологи в кореневій зоні → водний стрес → зменшення транспірації → ослаблення охолодження.

Якщо фактичне «вузьке місце» визначено неправильно, навіть технічно якісне рішення може не дати очікуваного результату. Оцінювання за одним середнім значенням для всього саду буде методично слабким. Пропонується виділити шість типів функціональних зон (табл. 3).

Для кожної зони доцільно сформувати окремий профіль, а потім узагальнювати результати на рівні саду. Особливо важливим є порівняння зон Z1 і Z3: вони можуть мати подібний деревний покрив, але різний стан кореневмісного ґрунту через близькість до алей та рекреаційне навантаження.

5. Формування реєстру невизначеностей. Невизначеності для такого кейсу можна розділити на чотири групи, а саме зовнішні впливи (тривалість спеки, дефіцит опадів, повторюваність посух), стан системи (глибина ущільнення, доступний кореневий об'єм, стан дерев), управлінська спроможність (доступність води, справність поливу, контроль рекреації) та зовнішні обмеження (перебої електроенергії, воєнні пошкодження). Не всі невизначеності потрібно намагатися усунути додатковими дослідженнями. Частина з них дешевше врахувати через сценарне тестування.

²⁸ Stumpe B., Bechtel B., Heil J. et al. Soil texture mediates the surface cooling effect of urban and peri-urban green spaces during a drought period in the city area of Hamburg (Germany). *Science of the Total Environment*. 2023. Vol. 897. Article 165228. DOI: <https://doi.org/10.1016/j.scitotenv.2023.165228>.

Таблиця 3

**Функціональні зони для функціонально-сценарного профілю Саду
імені Тараса Шевченка**

Код	Функціональна зона	Основні ознаки	Очікувана роль у сценарії
Z1	Старовікові деревні насадження	Зімкнений намет, значні кореневі системи, затінення	Висока кліматорегульовальна цінність; висока критичність збереження коренових зон
Z2	Газони та відкриті галявини	Трав'янистий покрив, інсоляція, рекреаційне використання	Швидко реагують на посуху; індикатор водного дефіциту й витоптування
Z3	Буферні смуги вздовж основних алей	Контакт ґрунту з твердим покриттям, інтенсивний рух	Найімовірніші осередки рекреаційного ущільнення і обмеження кореневого простору
Z4	Вузли високої рекреаційної активності	Входи, пам'ятники, майданчики, місця відпочинку	Локальне витоптування, оголення поверхні, високий антропогенний тиск
Z5	Запечатані й напівпроникні поверхні	Асфальт, граніт, площі, основні доріжки	Нагрівання, формування стоку, обмеження надходження води до ґрунту
Z6	Водойма, каскад, пониження і дренажні вузли	Накопичення або відведення води	Потенційний ресурс перерозподілу води, але необов'язково джерело доступної вологості для дерев

6. Побудова набору реалістичних сценаріїв. Сценарії не мають обмежуватися категоріями «нормальний», «поганий» і «найгірший». Кожен сценарій має відображати конкретну комбінацію сили й тривалості шоку; вихідного стану; попередніх стресів; технічного стану інфраструктури; доступності ресурсів; ефективності управлінського реагування.

Сценарії для парку.

R1 коротка хвиля спеки, достатня вологість ґрунту, справний полив;

R2 тривала спека після дефіциту опадів, полив доступний;

R3 тривала спека, обмеження водопостачання на 50%;

R4 спека, посуха, несправність частини системи поливу;

R5 спека й посуха за високого рекреаційного навантаження;

R6 повторення посушливих сезонів без повного відновлення ґрунту та дерев.

Польові дослідження міських дерев показують, що водний стрес залежить від вологості ґрунту, розміру водозбірної площі та управління водою; збільшення надходження води може зменшувати, але не завжди повністю усувати посуховий стрес. Охолоджувальна ефективність зелених територій також знижується за сухого ґрунту, навіть якщо деревний покрив формально збережений.

Узагальнений функціонально-сценарний профіль саду імені Тараса Шевченка побудовано для шести пріоритетних функцій з урахуванням опірності, відновлюваності та адаптивності.

Таблиця 4

**Узагальнений функціонально-сценарний профіль Саду
імені Тараса Шевченка**

Функція	Поточний потенціал	Опірність до короткої події	Опірність до тривалої події	Відновлюваність	Головне «вузьке місце»
F1 Водний режим	Середній	Середня	Низько-середня	Середня	Ущільнення і просторове відокремлення води від корневих зон
F2 Рослинистість	Середньо-високий	Середньо-висока	Середня або низька локально	Середня	Доступний кореневий простір і ґрунтова волога
F3 Охолодження	Високий у звичайних умовах	Висока через затінення	Середня через втрату транспірації	Середня	Водозабезпечення дерев
F4 Біологічні функції	Невизначений	Середня	Низько-середня	Повільна	Волога, агрегатна структура, органічні надходження
F5 Стабільність поверхні	Середня	Середня	Низька в рекреаційних вузлах	Середня	Оголення ґрунту і неформальні стежки
F6 Рекреаційний комфорт	Високий під наметом	Середньо-високий	Середній	Швидка після закінчення спеки, повільніша після деградації рослинності	Відкриті нагріті поверхні

Сад імені Тараса Шевченка має значний базовий потенціал кліматичної резильєнтності завдяки сформованому деревному намету, ґрунтовим площам, старовіковим деревам і створеній системі поливу. Водночас цей потенціал суттєво обмежений просторовою мозаїкою запечатаних поверхонь, локальним рекреаційним ущільненням і недостатнім надходженням води безпосередньо до активних корневих зон.

7. Формування управлінських альтернатив. Альтернативи мають бути взаємно порівнюваними за складом, строком реалізації та необхідними ресурсами. Неправильно порівнювати, наприклад, один дощовий сад

із повною реконструкцією дренажної мережі без урахування різниці в масштабі та функціях.

Для кожного заходу складається паспорт з інформацією про зміст, особливості та наслідки:

- цільова функція (яку функцію підтримує захід);
- механізм (як саме він змінює систему);
- просторовий масштаб (точка, ділянка, квартал, водозбір);
- строк реалізації (негайний, сезонний, багаторічний);
- капітальні витрати (проектування і будівництво);
- експлуатаційні витрати (обслуговування, вода, енергія, персонал);
- термін служби (очікуваний період працездатності);
- умови провалу (коли захід не забезпечує функцію);
- побічні ефекти (позитивні й негативні);
- оборотність (можливість зміни або демонтажу);
- відповідальний суб'єкт (хто реалізує і підтримує);
- показник результативності (що вимірюється після реалізації).

8. Робастний портфель рішень. Робастне управління не потребує реалізації всіх можливих заходів одночасно. Метод *Dynamic adaptive policy pathways*²⁹ передбачає реалізацію обґрунтованих короткострокових дій, збереження майбутніх можливостей і перехід до наступного заходу після досягнення заздалегідь встановленого порогу.

Для кожної території формується адаптивна траєкторія, що представлена ланцюгом: базове рішення → моніторинг → сигнал зміни → перевірка → додатковий захід → повторна оцінка.

Основний ланцюг впливу для Саду імені Тараса Шевченка пропонується представити так: інтенсивне рекреаційне використання → ущільнення та втрата макропористості → зниження інфільтрації й доступного кореневого об'єму → недостатнє поповнення запасів вологи → посуха → закриття продихів і зменшення транспірації → ослаблення охолоджувальної функції → підвищення температури поверхні → прискорене висушування ґрунту.

Формується позитивний зворотний зв'язок менше доступної вологи → слабше випаровувальне охолодження → вища температура → більший дефіцит вологи. Корені дерев можуть суттєво покращувати інфільтрацію навіть у відносно щільних міських ґрунтах, але ефект залежить від розподілу коренів, глибини ґрунту та ступеня ущільнення³⁰. В окремих експериментальних дослідженнях кореневі системи збільшували швидкість

²⁹ Haasnoot M., Kwakkel J. H., Walker W. E., ter Maat J. Dynamic adaptive policy pathways: A method for crafting robust decisions for a deeply uncertain world. *Global Environmental Change*. 2013. Vol. 23, No. 2. P. 485–498. DOI: <https://doi.org/10.1016/j.gloenvcha.2012.12.006>.

³⁰ Gregory J. H., Dukes M. D., Jones P. H., Miller G. L. Effect of urban soil compaction on infiltration rate. *Journal of Soil and Water Conservation*. 2006. Vol. 61, No. 3. P. 117–124. DOI: <https://doi.org/10.1080/00224561.2006.12435870>.

інфільтрації порівняно з бездеревними ділянками, причому важливими контролюючими показниками були пористість і щільність ґрунту.

Під час посухи теплорегульовальна функція парку складається з двох частин затінення, яке може певний час зберігатися навіть за водного стресу та еватранспіраційного охолодження, яке безпосередньо залежить від доступної вологості.

Дослідження міських зелених територій показують, що в умовах посухи охолоджувальна дія рослинності істотно залежить від водозабезпечення, текстури ґрунту та його гідралічних властивостей; просторові відмінності часто сильніше пов'язані з індексами вологості, ніж лише з кількістю зеленої біомаси.

Тригери (табл. 5) мають відповідати чотирьом вимогам:

- 1) бути вимірюваними;
- 2) реагувати раніше, ніж стан стане незворотним;
- 3) мати визначеного відповідального за спостереження;
- 4) бути безпосередньо пов'язаними з управлінською дією.

Таблиця 5

Робочі тригери для Саду імені Тараса Шевченка

Тригер (сигнал)	Робоча умова	Дія (управлінська реакція)
Дефіцит вологості	вологість кореневої зони нижча за локальний 10-й перцентиль протягом 5–7 діб	пріоритетний адресний полив
Дистанційний водний стрес	стійка негативна аномалія NDMI, а саме NDMI нижчий за середній сезонний рівень більш ніж на 1 стандартне відхилення у двох послідовних сценах	польова перевірка і коригування режиму поливу
Втрата рослинності	NDVI або проєктивне покриття зменшилися більш ніж на 20% від референту	обмеження доступу, відновлення покриття
Перегрівання	температура поверхні ділянки більш ніж на 3°C перевищує медіану затінених ділянок саду	посилення затінення, перегляд покриття й водного режиму
Ущільнення	опір penetрації більш ніж у 1,5 рази вищий за референт, а інфільтрація нижча за 50% референтної	локальне обмеження доступу, локальна діагностика й обережне розущільнення
Слабке відновлення	функція не повернулася до 90% референтного рівня через 30–45 днів	перехід від очікування природного відновлення до керованого втручання, перехід до структурної реконструкції кореневої зони
Втрата покриття (оголення поверхні)	оголений ґрунт займає понад 20% управлінської комірки	перенаправлення потоків відвідувачів і відновлення поверхні

Метою рішення щодо практичного обґрунтування портфеля для Саду імені Тараса Шевченка є підтримка життєздатності дерев, водного режиму ґрунту та охолоджувальної функції парку за поєднання хвилі спеки; тривалої посухи; рекреаційного ущільнення; можливої обмеженості поливної води; перебоїв енергопостачання.

Функціональним «вузьким місцем» є не загальна відсутність поливу, а просторова й часова недоступність води для активних коренів унаслідок ущільнення, запечатування, обмеженого ґрунтового об'єму та нерівномірного надходження води.

Ущільнення міських ґрунтів здатне істотно знижувати інфільтрацію; водночас розвинені кореневі системи можуть частково відновлювати макропористість і збільшувати проникнення води³¹. Це означає, що втручання має захищати корені, а не розглядати ґрунт як однорідний субстрат, який можна механічно розпушити.

Таблиця 6

Порівняння альтернативних варіантів

Альтернатива	Очікуваний результат	Основний ризик	Попередня робастність
Календарний полив без моніторингу	підтримує газони й частину дерев за звичайних умов	перевитрата води, недополив корневих зон, залежність від енергії	низька
Локальне розуцільнення	може покращити інфільтрацію	пошкодження коренів, короткочасний ефект без контролю рекреації	низько-середня
Лише висадження нових дерев	збільшує перспективний намет	не захищає старі дерева, висока смертність саджанців за посухи	низька в короткому горизонті
Моніторинг + адресний полив	краще розподіляє воду	не усуває ущільнення й рекреаційний тиск	середня
Захист корневих зон + адресний полив + контроль потоків	впливає на причину й наслідок	потребує координації благоустрою та експлуатації	висока
Повний адаптивний портфель	поєднує воду, ґрунт, рекреацію, покриття й моніторинг	вищі організаційні вимоги	найвища

³¹ Xie C., Cai S., Yu B., Yan L., Liang A., Che S. The effects of tree root density on water infiltration in urban soil based on a Ground Penetrating Radar in Shanghai, China. *Urban Forestry & Urban Greening*. 2020. Vol. 50. Article 126648. DOI: <https://doi.org/10.1016/j.ufug.2020.126648>.

Рекомендовано такий робастний портфель³² для паркового кейсу.

На першому етапі (один сезон) картування корневих зон старовікових і найбільш уразливих дерев; встановлення 10–20 репрезентативних датчиків вологості замість рівномірного дорогого покриття всієї території; польове вимірювання інфільтрації та опору пенетрації; виділення референтних і рекреаційно навантажених ділянок; перевірка фактичного розподілу поливної води; локальне перенаправлення відвідувачів; збереження органічної підстилки у визначених зонах; адресний полив за вологістю ґрунту, а не лише за календарем.

На другому етапі (після виявлення стабільних осередків вразливості) обережне пневматичне або інше малотравматичне розущільнення після картування коренів; локальна заміна непроникних покриттів; спрямування чистого покрівельного або поверхневого стоку до корневих зон; створення невеликих накопичувачів поливної води; диференційований режим управління газонами; підбір ґрунтопокривних рослин для затінених і витоптуваних ділянок.

На резервному етапі³³ реконструкція окремих корневих просторів; заміна частини покриттів; створення структурних ґрунтових комірок; коригування видового складу насаджень; зміна функціонального режиму ділянок, які неможливо одночасно використовувати для інтенсивної рекреації та збереження коренів.

Серед заходів, що мають бути відхилені без додаткового обґрунтування, визначаємо масове механічне розпушення без картування кореневої зони; збільшення поливу без вимірювання вологості; герметичне покриття пристовбурних зон; заміна старих дерев молодими як формальна компенсація; повне прибирання листя з усіх ділянок; оцінювання результату лише за кольором газону або середнім NDVI.

Запропонована методика не зводить резильєнтність до загального індексу озеленення, стану ґрунту або рівня інженерного оснащення. Вона розрізняє різну часову природу шоків; різні причинні механізми; різні критичні функції; різні типи відновлення; різні вимоги до моніторингу; різні портфелі управлінських рішень.

Робастність повинна бути закріплена не лише в проєктній документації, а й у системі управління. Сучасні дослідження природоорієнтованої політики підкреслюють, що окремі демонстраційні проєкти залишаються

³² Hölscher K., Frantzeskaki N., Collier M. J. et al. Strategies for mainstreaming nature-based solutions in urban governance capacities in ten European cities. *npj Urban Sustainability*. 2023. Vol. 3. Article 54. DOI: <https://doi.org/10.1038/s42949-023-00134-9>.

³³ Davies C., Sheikholeslami D., Laforteza R. Nature-based policy as a pathway to urban sustainability transitions. *Urban Sustainability*. 2026. DOI: <https://doi.org/10.1038/s42949-026-00430-0>.

фрагментарними, якщо природні рішення не інтегровані в інвестиційні, експлуатаційні та міжсекторальні процедури міста.

Повноцінна цифрова модель міста не є обов'язковою умовою початку робастного управління. Для пілотних територій досить системи, яка фіксує ключові функції й тригери. Для парку це 10–20 датчиків вологості на контрастних ділянках; сезонні вимірювання опору пенетрації; інфільтраційні тести; оцінка стану дерев; Sentinel-2 NDVI та NDMI; температура поверхні; картування рекреаційного навантаження; облік обсягів поливу. Моніторинг повинен відповідати управлінському рішення. Збір показників, для яких не визначено порога та відповідної дії, створює дані, але не забезпечує адаптивного управління.

8. Методичні обмеження. Робастний підхід не усуває невизначеності та не гарантує відсутності пошкоджень. Його завдання зменшити залежність рішення від помилкового прогнозу та зробити умови провалу видимими до реалізації.

За визначеного набору сценаріїв, порогів і обмежень вибраний портфель має найменшу схильність до критичного функціонального провалу, прийнятні витрати, допустимі побічні наслідки та можливість адаптивного коригування.

Обґрунтування робастних управлінських рішень для міських територій – це послідовний процес переходу від функціональної діагностики до сценарного стрес-тестування портфелів заходів. Центральним елементом є визначення критичних функцій, причинного «вузького місця», умов провалу альтернатив і правил переходу до наступного етапу.

Апробація методики на прикладі Саду імені Тараса Шевченка показує її придатність для аналізу комбінованого сценарію, у якому хвиля спеки та посуха взаємодіють із попередньо сформованою вразливістю ґрунту внаслідок рекреаційного ущільнення.

Її головною перевагою є коректне розмежування гострого кліматичного шоку, тривалого стресора та хронічного антропогенного тиску, що запобігає механічному об'єднанню різних причин деградації. Це дає змогу побудувати причинний ланцюг «ущільнення – зниження пористості та інфільтрації – дефіцит доступної кореням вологи – водний стрес рослинності – ослаблення евапотранспіраційного охолодження» і пов'язати кожен його ланку з вимірюваними показниками. Важливою перевагою є орієнтація не на загальний стан парку, а на критичні функції, в т.ч. підтримання водного режиму, життєздатності деревної рослинності, терморегуляції, стабільності ґрунтового покриву та рекреаційної придатності.

Функціонально-сценарний профіль також є методично сильнішим за єдиний інтегральний індекс, оскільки не допускає компенсації критичної втрати однієї функції високими значеннями інших. Додатковою перевагою є можливість безпосередньо пов'язати виявлене «вузьке місце» з портфелем робастних заходів, у т.ч. захистом кореневих зон, адресним поливом, регулюванням рекреаційних потоків, локальним відновленням інфільтрації та адаптивним моніторингом. Поєднання польових вимірювань, дистанційного зондування, просторових аналогів і сценарного аналізу робить методику застосовною навіть за неповних даних, але лише за умови чіткого позначення статусу кожної оцінки.

Разом із тим наведений парковий кейс є концептуальною апробацією, а не завершеною емпіричною валідацією методики. Найсуттєвіше обмеження пов'язане з відсутністю тривалих часових рядів вологості ґрунту, інфільтрації, фізіологічного стану дерев, температури поверхні та фактичного поливу до, під час і після шоку. Без таких спостережень неможливо надійно визначити вихідний рівень функціональності, фактичний мінімум під час події та тривалість відновлення, тому оцінки опірності залишаються сценарними. Запропоновані пороги вологості, інфільтрації, температури та втрати рослинного покриву мають робочий характер і повинні калібруватися за локальними референтними ділянками, видами рослинності та сезонними умовами. Методика також поки що не досить формалізує економічну здійсненність, експлуатаційні витрати, доступність поливної води, інституційну відповідальність і можливість реалізації заходів за воєнних та енергетичних обмежень.

Отже, парковий кейс підтверджує логічну цілісність і практичну спрямованість функціонально-сценарного підходу, але його кількісна достовірність залежатиме від сезонного моніторингу, локального калібрування порогів і перевірки того, чи справді запропонований портфель заходів зменшує втрати критичних функцій за різних сценаріїв спеки, посухи та рекреаційного навантаження.

ВИСНОВКИ

У дослідженні обґрунтовано доцільність розгляду міських ґрунтово-ландшафтних систем як відкритих, просторово неоднорідних і багаторівневих соціо-еколого-технологічних підсистем міста.

Запропоновано визначати потенціал екологічної резильєнтності міської ГЛС через її опірність, відновлюваність та адаптивність щодо збереження конкретних критичних функцій за заданих сценаріїв впливу.

Розроблено причинно-функціональний підхід, який пов'язує вихідний стан системи, хронічні тиски і стресори, характеристики шоку,

функціональні втрати, каскадні наслідки, відновлення та управлінську відповідь.

Удосконалено типологію комбінованих шоків шляхом їх розмежування за походженням і механізмом взаємодії на одночасні, послідовні, попередньо зумовлені, каскадні та просторово поєднані впливи.

Визначено, що вибір показників резильєнтності має здійснюватися за принципом «функція – механізм порушення – індикатор», а результати оцінювання доцільно подавати як функціонально-сценарний профіль без необґрунтованого зведення до єдиного інтегрального індексу.

Запропоновано обґрунтовувати робастні управлінські рішення через сценарне стрес-тестування портфелів природоорієнтованих, ґрунтовідновлювальних, інженерних та організаційних заходів із визначеними порогами, тригерами й резервними діями.

Досліджено можливості практичного застосування методики на прикладі Саду імені Тараса Шевченка та встановлено, що ключовим функціональним обмеженням може бути недостатня доступність води для активних кореневих зон унаслідок ущільнення, запечатування та нерівномірного водопостачання.

Визначено, що подальша кількісна валідація методики потребує сезонного моніторингу, локального калібрування референтних і порогових значень, оцінювання витрат та перевірки результативності заходів за реальних кліматичних, воєнних, енергетичних і ресурсних обмежень.

АНОТАЦІЯ

У статті досліджено проблему оцінювання потенціалу екологічної резильєнтності міських ґрунтово-ландшафтних систем до комбінованих кліматичних, урбанізаційно-техногенних, воєнно зумовлених і каскадних шоків. Актуальність дослідження визначається необхідністю переходу від статичного оцінювання екологічного стану території до аналізу здатності системи зберігати, відновлювати та адаптувати критичні функції за умов невизначеності. Запропоновано розглядати міські ґрунтово-ландшафтні системи як відкриті, просторово неоднорідні та багаторівневі соціо-еколого-технологічні підсистеми міста. Розроблено причинно-функціональний методичний підхід, що пов'язує вихідний стан системи, хронічні тиски і стресори, параметри шоку, функціональні втрати, каскадні наслідки, відновлення та управлінську відповідь. Удосконалено типологію комбінованих шоків за походженням чинників і механізмами їх одночасної, послідовної, попередньо зумовленої, каскадної або просторово пов'язаної дії. Запропоновано оцінювати потенціал резильєнтності через опірність, відновлюваність та адаптивність критичних функцій,

а результати подавати у формі функціонально-сценарного профілю, що враховує референтні стани, порогові значення та невизначеність оцінок. Обґрунтовано процедуру формування робастних управлінських рішень на основі сценарного стрес-тестування портфелів природоорієнтованих, ґрунтовідновлювальних, інженерних та організаційних заходів із визначеними тригерами їх перегляду. Практичну придатність підходу проаналізовано на прикладі Саду імені Тараса Шевченка у Харкові, для якого визначено критичні функції, функціональні зони, сценарії спеки й посухи, основні «вузькі місця» та адаптивний портфель заходів. Встановлено, що кількісна валідація методики потребує сезонного моніторингу, локального калібрування порогів та перевірки результативності запропонованих рішень за реальних кліматичних, воєнних, енергетичних і ресурсних обмежень.

Література

1. McPhearson T., Cook E. M., Berbés-Blázquez M. et al. A social-ecological-technological systems framework for urban ecosystem services. *One Earth*. 2022. Vol. 5, No. 5. P. 505–518. DOI: <https://doi.org/10.1016/j.oneear.2022.04.007>.
2. Pickett S. T. A., Cadenasso M. L., Grove J. M., Nilon C. H., Pouyat R. V., Zipperer W. C., Costanza R. Urban ecological systems: Linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. *Annual Review of Ecology and Systematics*. 2001. Vol. 32. P. 127–157. DOI: <https://doi.org/10.1146/annurev.ecolsys.32.081501.114012>.
3. Zscheischler J., Westra S., van den Hurk B. J. J. M. et al. Future climate risk from compound events. *Nature Climate Change*. 2018. Vol. 8. P. 469–477. DOI: <https://doi.org/10.1038/s41558-018-0156-3>.
4. Тітенко Г. В., Баскакова Л. В. Критерії та параметри для розробки моделі урболандшафту. *Вісник Харківського національного університету імені В. Н. Каразіна. Серія «Екологія»*. 2013. № 1070, вип. 9. С. 91–95. URL: http://nbuv.gov.ua/UJRN/VKhNU_2013_1070_9_15 (дата звернення: 30.07.2026).
5. Meerow S., Newell J. P., Stults M. Defining urban resilience: A review. *Landscape and Urban Planning*. 2016. Vol. 147. P. 38–49. DOI: <https://doi.org/10.1016/j.landurbplan.2015.11.011>.
6. Lempert R. J. Robust Decision Making (RDM). Decision Making under Deep Uncertainty: From Theory to Practice / eds. V. A. W. J. Marchau, W. E. Walker, P. J. T. M. Bloemen, S. W. Popper. Cham : Springer, 2019. P. 23–51. DOI: https://doi.org/10.1007/978-3-030-05252-2_2.

7. Hall J. W., Lempert R. J., Keller K. et al. Robust climate policies under uncertainty: A comparison of Robust Decision Making and Info-Gap methods. *Risk Analysis*. 2012. Vol. 32, No. 10. P. 1657–1672. DOI: <https://doi.org/10.1111/j.1539-6924.2012.01802.x>.
8. Zhang C., Wu J., Grimm N. B., McHale M., Buyantuyev A. A hierarchical patch mosaic ecosystem model for urban landscapes: Model development and evaluation. *Ecological Modelling*. 2013. Vol. 250. P. 81–100. DOI: <https://doi.org/10.1016/j.ecolmodel.2012.09.020>.
9. Pickett S. T. A., Cadenasso M. L., Rosi-Marshall E. J. et al. Dynamic heterogeneity: A framework to promote ecological integration and hypothesis generation in urban systems. *Urban Ecosystems*. 2017. Vol. 20. P. 1–14. DOI: <https://doi.org/10.1007/s11252-016-0574-9>.
10. Fan K., Chu H., Eldridge D. J. et al. Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nature Ecology & Evolution*. 2023. Vol. 7. P. 113–126. DOI: <https://doi.org/10.1038/s41559-022-01935-4>.
11. Li F., Liu X., Zhang X., Zhao D., Liu H., Zhou C., Wang R. Urban ecological infrastructure: An integrated network for ecosystem services and sustainable urban systems. *Journal of Cleaner Production*. 2017. Vol. 163, Suppl. P. S12–S18. DOI: <https://doi.org/10.1016/j.jclepro.2016.02.079>.
12. Childers D. L., Bois P., Hartnett H. E. et al. Urban Ecological Infrastructure: An inclusive concept for the non-built urban environment. *Elementa: Science of the Anthropocene*. 2019. Vol. 7. Article 46. DOI: <https://doi.org/10.1525/elementa.385>.
13. Pouyat R. V., Day S. D., Brown S. et al. Urban soils. Forest and Rangeland Soils of the United States Under Changing Conditions / eds. R. V. Pouyat, D. S. Page-Dumroese, T. Patel-Weynand, L. H. Geiser. Cham : Springer, 2020. P. 127–144. DOI: https://doi.org/10.1007/978-3-030-45216-2_7.
14. Shankar M., Ng M., Rogers M. et al. Unearthing the role of soils in urban climate resilience planning. *Nature Sustainability*. 2024. Vol. 7. P. 1374–1376. DOI: <https://doi.org/10.1038/s41893-024-01436-1>.
15. O’Riordan R., Davies J., Stevens C., Quinton J. N., Boyko C. The ecosystem services of urban soils: A review. *Geoderma*. 2021. Vol. 395. Article 115076. DOI: <https://doi.org/10.1016/j.geoderma.2021.115076>.
16. Frene J. P., Pandey B. K., Castrillo G. Under pressure: Elucidating soil compaction and its effect on soil functions. *Plant and Soil*. 2024. Vol. 502. P. 267–278. DOI: <https://doi.org/10.1007/s11104-024-06573-2>.
17. Ribeiro P. J. G., Pena Jardim Gonçalves L. A. Urban resilience: A conceptual framework. *Sustainable Cities and Society*. 2019. Vol. 50. Article 101625. DOI: <https://doi.org/10.1016/j.scs.2019.101625>.

18. Cariolet J.-M., Vuillet M., Diab Y. Mapping urban resilience to disasters – A review. *Sustainable Cities and Society*. 2019. Vol. 51. Article 101746. DOI: <https://doi.org/10.1016/j.scs.2019.101746>.

19. Solokha M., Demyanyuk O., Symochko L. et al. Soil degradation and contamination due to armed conflict in Ukraine. *Land*. 2024. Vol. 13, No. 10. Article 1614. DOI: <https://doi.org/10.3390/land13101614>.

20. Bonchkovskiy O., Ostapenko P., Bonchkovskiy A., Shvaiko V. War-induced soil disturbances in north-eastern Ukraine (Kharkiv region): Physical disturbances, soil contamination and land use change. *Science of the Total Environment*. 2025. Vol. 964. Article 178594. DOI: <https://doi.org/10.1016/j.scitotenv.2025.178594>.

21. Casal-Campos A., Sadr S. M. K., Fu G., Butler D. Reliable, resilient and sustainable urban drainage systems: An analysis of robustness under deep uncertainty. *Environmental Science & Technology*. 2018. Vol. 52, No. 16. P. 9008–9021. DOI: <https://doi.org/10.1021/acs.est.8b01193>.

22. Urich C., Rauch W. Exploring critical pathways for urban water management to identify robust strategies under deep uncertainties. *Water Research*. 2014. Vol. 66. P. 374–389. DOI: <https://doi.org/10.1016/j.watres.2014.08.020>.

23. Yin D., Zhang X., Cheng Y., Jia H., Jia Q., Yang Y. Can flood resilience of green-grey-blue system cope with future uncertainty? *Water Research*. 2023. Vol. 242. Article 120315. DOI: <https://doi.org/10.1016/j.watres.2023.120315>.

24. Kharkiv Development & Investment. Реконструкція Саду імені Тараса Шевченка. URL: <https://inkharkiv.com/projects/rekonstruktsiia-sadu-imeni-tarasa-shevchenka/> (дата звернення: 30.07.2026).

25. Харківська міська рада. Новий каскад, озеро і зони відпочинку – у Харкові реконструюють сад Шевченка. 11.05.2018. URL: <https://city.kharkiv.ua/news/misto/noviy-kaskad-ozero-i-zoni-vidpochinku---u-kharkovi-rekonstruyuyut-sad-shevchenka-> (дата звернення: 30.07.2026).

26. Харківська обласна державна адміністрація. Перелік територій та об'єктів природно-заповідного фонду Харківської області станом на 01.10.2021 року. URL: https://kharkivoda.gov.ua/content/documents/1127/112691/Attaches/perelik_pzf_na_sayt_hoda.pdf (дата звернення: 30.07.2026).

27. Tams L., Paton E., Kluge B. Urban tree drought stress: Sap flow measurements, model validation, and water management simulations. *Science of the Total Environment*. 2024. Vol. 957. Article 177221. DOI: <https://doi.org/10.1016/j.scitotenv.2024.177221>.

28. Stumpe B., Bechtel B., Heil J. et al. Soil texture mediates the surface cooling effect of urban and peri-urban green spaces during a drought period in

the city area of Hamburg (Germany). *Science of the Total Environment*. 2023. Vol. 897. Article 165228. DOI: <https://doi.org/10.1016/j.scitotenv.2023.165228>.

29. Haasnoot M., Kwakkel J. H., Walker W. E., ter Maat J. Dynamic adaptive policy pathways: A method for crafting robust decisions for a deeply uncertain world. *Global Environmental Change*. 2013. Vol. 23, No. 2. P. 485–498. DOI: <https://doi.org/10.1016/j.gloenvcha.2012.12.006>.

30. Gregory J. H., Dukes M. D., Jones P. H., Miller G. L. Effect of urban soil compaction on infiltration rate. *Journal of Soil and Water Conservation*. 2006. Vol. 61, No. 3. P. 117–124. DOI: <https://doi.org/10.1080/00224561.2006.12435870>.

31. Xie C., Cai S., Yu B., Yan L., Liang A., Che S. The effects of tree root density on water infiltration in urban soil based on a Ground Penetrating Radar in Shanghai, China. *Urban Forestry & Urban Greening*. 2020. Vol. 50. Article 126648. DOI: <https://doi.org/10.1016/j.ufug.2020.126648>.

32. Hölscher K., Frantzeskaki N., Collier M. J. et al. Strategies for mainstreaming nature-based solutions in urban governance capacities in ten European cities. *npj Urban Sustainability*. 2023. Vol. 3. Article 54. DOI: <https://doi.org/10.1038/s42949-023-00134-9>.

33. Davies C., Sheikholeslami D., Laforteza R. Nature-based policy as a pathway to urban sustainability transitions. *Urban Sustainability*. 2026. DOI: <https://doi.org/10.1038/s42949-026-00430-0>.

Information about the author:

Titenko Ganna,

Candidate of Sciences in Geography,
Director of the Karazin Institute of Environmental Sciences,
Green Energy and Sustainable Development,
Associate Professor at the Department of Ecology
and Environmental Management
V. N. Karazin Kharkiv National University
4, Svobody Square, Kharkiv, Ukraine

МОНІТОРИНГ ТА СТАН ІНТРОДУКЦІЙНИХ ПОПУЛЯЦІЙ ПРЕДСТАВНИКІВ РОДУ *PULSATILLA* MILL. У КУЛЬТУРФІТОЦЕНОЗАХ

Чеканов Михайло, Куземко Анна, Діденко Інна
DOI <https://doi.org/10.30525/978-9934-26-714-7-20>

ВСТУП

Збереження біорізноманіття рослин є однією з найбільш актуальних проблем сучасності, особливо це стосується рідкісних та зникаючих видів. Підтримання їх генетичного різноманіття здійснюється шляхом збереження цілісності екосистем у природних місцезростаннях (*in situ*) і значно доповнюється технологіями збереження рослин в умовах культури (ботанічних садах та дендропарках (*ex situ*))¹. Вирощування рідкісних видів рослин в умовах інтродукції є одним із основних напрямів збереження їхнього генофонду². Найдієвішим є культивування рідкісних видів, за якого забезпечується висока чисельність особин в умовах, наближених до екологічного оптимуму цих видів. Серед таких шляхів є розмноження рослин у спеціалізованих розсадниках та закладання насаджень у культурфітоценозах і паркових ценозах, створення інтродукційних популяцій^{3,4}.

До видів, які перебувають під загрозою зникнення в Україні, належать види роду *Pulsatilla* Mill. родини Ranunculaceae Juss.: *Pulsatilla grandis* Wend., *P. patens* (L.) Mill., *P. pratensis* (L.) Mill., *P. scherfelii* (Ullep.) Skalický та *P. taurica* Juz.⁵. Мета наших досліджень – здійснити аналіз

¹ Global Strategy for Plant Conservation, GSPC : Richmond, U.K., 2002. 16 с.

² Антонюк Н. Є., Бородіна Р. М., Собко В. Г., Скворцова Л. С. Рідкісні рослини флори України в культурі. Київ : Наукова думка, 1982. 216 с.

³ Мельник В. І. Моделювання інтродукційних популяцій у культурфітоценозах як метод охорони рідкісних видів рослин *ex situ*. Науковий вісник ЧНУ. Біологія. 2002. Вип. 145. С. 25–37.

⁴ Гапоненко М. Б. Інтродукція рослин як метод збереження та збагачення біологічного різноманіття в ботанічних садах та дендропарках. Інтродукція рослин, збереження та збагачення біорізноманіття в ботанічних садах і дендропарках : матеріали міжн. наук. конф. (Київ, 15–17 вересня 2010 р.). Київ, 2010. С. 34–36.

⁵ Червона книга України. Рослинний світ / за ред. Я. П. Дідуха. Київ : Глобалконсалтинг, 2009. 900 с.

стану інтродукційних популяцій *P. patens* та *P. pratensis* у ботанічних садах і дендропарках України, а також спрогнозувати шляхи їх збереження.

1. Методи та матеріали досліджень

Об'єктами наших досліджень були інтродукційні популяції *P. patens* та *P. pratensis ex situ* Національного дендрологічного парку «Софіївка» НАН України (м. Умань, Черкаська область). Згідно з ботаніко-географічним районуванням України він знаходиться у центральній частині Правобережного Лісостепу України, за фізико-географічним районуванням належить до Західноукраїнської лісостепової провінції. За зведеними даними агрометеорологічних спостережень по зоні, клімат характеризується такими рисами: помірно-холодна зима зі значною амплітудою коливання температури повітря в окремі дні, з незначними опадами, невеликим сніговим покривом, іноді із сильними східними вітрами; помірно-тепла весна зі значним зниженням температури повітря в окремі дні, з холодними, інколи сухими вітрами і нерівномірним розподілом опадів; помірно-жарке літо, в окремі роки з посушливим вегетаційним періодом і нерівномірним розподілом опадів, часто у вигляді злив, з переважанням західних вітрів; помірно-тепла осінь, іноді зі значними коливаннями температури наприкінці періоду⁶.

Назви видів наводяться згідно з довідковими системами World Flora Online⁷.

Моніторинг за станом інтродукційних популяцій *P. patens* і *P. pratensis* на досліджуваній території проводився впродовж 2011 р. і дотепер. Результати досліджень висвітлені станом на 2025 р. Щільність визначали на ділянках від 0,25 м² до 8 м² залежно від площі інтродукційної популяції⁸. Визначення категорії життєвості видів здійснювали за даними І. М. Григори та В. А. Соломахи⁹.

На зазначених облікових ділянках оцінювали кількість рослин різних онтогенетичних станів¹⁰. Встановлення належності рослин до того чи іншого онтогенетичного стану здійснювали з урахуванням загальних підходів до періодизації онтогенезу, спираючись на ступінь вираження

⁶ Маринич О. М., Шищенко П. Г. Фізична географія України : підручник. Київ : Знання, 2005. 511 с.

⁷ World Flora Online. Published on the Internet. URL: <http://www.worldfloraonline.org>. (дата звернення: 16.01.2026).

⁸ Методичні рекомендації щодо ведення фенологічних спостережень за рослинами на території природно-заповідного фонду / за ред. Г. В. Драбинюк. РЛП «Приінгульський», 2016. С. 6–7.

⁹ Григора І. М., Соломаха В. А. Основи фітосоціології. Київ : Фітосоціоцентр, 2000. С. 6.

¹⁰ Злобін Ю. А. Популяційна екологія рослин: сучасний стан, точка росту : монографія. Суми : Університетська книга, 2009. С. 108–110.

певних морфоознак. Вікові стани досліджуваних видів ідентифікували на підставі детальних досліджень Л. В. Бакаліни¹¹ та О. В. Чуй¹², О. Ф. Щербакової й К. В. Новосад^{13, 14}, В. В. Птиці¹⁵.

На заключному етапі дослідження онтогенетичної структури була встановлена належність популяції до певної з трьох категорій: інвазійної, якій притаманне переважання особин передгенеративних станів, генеративної (нормальної) – характерне переважання генеративних особин, регресивної – вирізняється переважанням постгенеративних особин.

Статистичне опрацювання та узагальнення даних про онтогенетичну структуру ценопопуляцій досліджуваних видів здійснено із використанням спеціальної комп'ютерної програми ANONS 6, розробленої Ю. А. Злобіним. Для комплексної оцінки вікової структури популяцій використано систему інтегральних онтогенетичних індексів за І. М. Коваленком. Розрахунок включав індекси відновлюваності, старіння і генеративності, а також індекс віковості популяції, що базується на співвідношенні критеріїв старіння та відновлення. Для оцінки процесів самовідновлення популяцій використовували індекс відновлення (Ів), який визначали як співвідношення чисельності прегенеративних особин до кількості генеративних. Значення індексу понад 1 свідчить про активне природне поновлення популяції¹⁶.

2. Досвід інтродукції представників роду *Pulsatilla* l. ex situ

В Україні ідея охорони рідкісних та зникаючих видів рослин *ex situ* виникла ще в 50-х роках минулого століття. М. І. Котов та С. С. Харкевич вперше вказували на необхідність охорони рідкісних та зникаючих видів рослин поза межами природних місцезростань як одне

¹¹ Бакаліна Л. В. Онтогенез і популяційна структура сонів широколистої і чорніючої в екосистемах Канівського природного заповідника. *Заповідна справа в Україні*. 1997. Т. 1, Вип. 2. С. 16–22.

¹² Чуй О.В. Стан ценопопуляцій *Pulsatilla grandis* Wend. та *Pulsatilla patens* (L.) Mill. (Ranunculaceae Juss.) в Західному Опіллі : автореф. дис. ... канд. біол. наук : 03.00.05. Київ, 2016. 21 с.

¹³ Щербакова О. Ф., Новосад К. В. Особливості сезонного розвитку та поліваріантність структури річних квітконосних пагонів *Pulsatilla patens* (L.) Mill. і *P. pratensis* (L.) Mill. в умовах Київського мегаполісу. *Інтродукція рослин*. 2013. № 3. С. 34–42.

¹⁴ Щербакова О. Ф., Новосад К. В. Поліваріантність елементарних модулів у структурі річних квітконосних пагонів *Pulsatilla patens* (L.) Mill. і *P. pratensis* (L.) Mill. (Ranunculaceae). *Український ботанічний журнал*. 2013. Т. 70, № 6. С. 751–761.

¹⁵ Птиця В. В. Біологічні основи збереження раритетних видів рослин *ex situ* на південному сході України : дис. ... канд. біол. наук : 03.00.05. Київ, 2008. 178 с.

¹⁶ Коваленко І. М. Структура популяцій домінантів трав'яно-чагарничкового ярусу в лісових фітоценозах Десянянсько-Старогутського національного природного парку. І. Онтогенетична структура. *Український ботанічний журнал*. 2005. Т. 62. № 5. С. 707–714.

з найважливіших завдань у діяльності ботанічних садів¹⁷. При цьому культивування розглядається як доповнення до заповідного режиму в природних умовах, де зникаючі види розвиваються в оптимальних умовах. *Ex situ* створюється резервний фонд пропагул для подальшої репатріації їх у природу з метою відновлення і відтворення деструктивних популяцій¹⁸.

Згідно з каталогом декоративних трав'янистих рослин ботанічних садів і дендропарків України, за класифікацією Plants of the World Online, в Україні культивується 12 видів роду *Pulsatilla* Mill.¹⁹, з них 5 видів занесені до Червоної книги України²⁰.

Першим осередком інтродукції рослин природної флори є Національний ботанічний сад ім. М. М. Гришка (колишній Центральний республіканський ботанічний сад АН УРСР). У 1950 р. до списку рослин з колекції ввійшла *P. albana* Stev.²¹. С. С. Харкевичем було інтродуковано *P. ucrainica* (Ugrinsky.) Wissjul. У 1997 р. колекція цього ботанічного саду збільшилася і нараховувала 7 видів (*P. alba* Reichenb., *P. cernua* (Thunb.) Bercht. et Opiz, *P. grandis* Wend., *P. taurica* Juz., *P. latifolia* Rupr., *P. grandis* Wenderoth., *P. nigricans* Störck., *P. ucrainica* (Ugrinsky.) Wissjul.²².

У ботанічному саду ім. акад. О. В. Фоміна зростає чотири види роду *Pulsatilla*²³. У Криворізькому ботанічному саду види роду *Pulsatilla* культивуються з 1982 року²⁴, у Кременецькому ботанічному саду – з 2002 р.²⁵.

Загалом, рослини *P. patens* культивуються у восьми ботанічних садах, *P. pratensis* – у 14 (табл. 1, 3).

Дендрологічні парки України є зоною культивування різних видів рослин, часто рідкісних і екзотичних. Рослини *P. patens* зростають у трьох дендропарках, *P. pratensis* – у чотирьох (табл. 2, 4).

¹⁷ Харкевич С. С. Інтродукція на Україні корисних рослин природної флори СРСР / АН УРСР, Центр. респ. ботан. сад; відп. ред. С. С. Харкевич. Київ : Наукова думка, 1972. С. 66.

¹⁸ Собко В. Г., Гапоненко М. Б. Інтродукція рідкісних і зникаючих рослин флори України / за ред. М. А. Кохна. Київ : Наукова думка, 1996. 283 с.

¹⁹ Каталог декоративних трав'янистих рослин ботанічних садів і дендропарків України : довідниковий посібник / за ред. С. П. Машковської. Київ, 2015. 282 с. (електронне видання).

²⁰ Каталог раритетних рослин ботанічних садів і дендропарків України : довідковий посібник / за ред. А. П. Лебеди. Київ : Академперіодика, 2011. 184 с.

²¹ Гришко М. М., Соколовський О. І. Ботанічний сад і його колекції. Київ : В-во АН РСР, 1950, 115 с.

²² Каталог рослин Центрального ботанічного саду ім. М. М. Гришка. Київ : Наукова думка. 436 с.

²³ Ботанічний сад ім. акад. О. В. Фоміна. Каталог рослин. Природно-заповідні території України. Рослинний світ. Вип. 7. Київ : Фітосоціоцентр, 2007. 320 с.

²⁴ Каталог рослин криворізького ботанічного саду / за ред. А. Т. Гревцової. Київ : Фітосоціоцентр. 2000. 164 с.

²⁵ Кременецький ботанічний сад. Каталог рослин. Природно-заповідні території України. Рослинний світ. Вип. 8. Київ : Фітосоціоцентр, 2007. 159 с.

Таблиця 1

Культитивування *P. patens* у ботанічних садах України

№ п/п	Назва ботанічної установи
1	Ботанічний сад ім. акад. О. В. Фоміна Київського національного університету ім. Т. Г. Шевченка
2	Ботанічний сад Львівського національного університету ім. Івана Франка
3	Ботанічний сад Сумського педагогічного університету ім. Василя Стефаника
4	Ботанічний сад Українського державного лісотехнічного університету
5	Ботанічний сад Харківського національного університету ім. В. Н. Каразіна
6	Донецький ботанічний сад НАН України
7	Національний ботанічний сад ім. М. М. Гришка НАН України
8	НДЛ «Ботанічний сад» Навчально-наукового центру «Інститут біології» Київського національного університету імені Тараса Шевченка

Таблиця 2

Культитивування *P. patens* у дендрологічних парках України

№ п/п	Назва ботанічної установи
1	Національний дендрологічний парк «Софіївка» НАН України
2	Дендрологічний парк «Асканія-Нова» Біосферного заповідника «Асканія-Нова» імені Ф. Е. Фальц-Фейна НААН
3	Хоростківський державний дендрологічний парк Подільської дослідної станції Тернопільського інституту агропромислового виробництва УААН

Таблиця 3

Культитивування *P. pratensis* у ботанічних садах України

№ п/п	Назва ботанічної установи
1	Ботанічний сад Дніпропетровського національного університету
2	Ботанічний сад ім. акад. О. В. Фоміна Київського національного університету ім. Т. Г. Шевченка
3	Ботанічний сад Одеського національного університету ім. І. І. Мечникова
4	Ботанічний сад Львівського національного університету ім. Івана Франка
5	Ботанічний сад Сумського педагогічного університету ім. Василя Стефаника
6	Ботанічний сад Ужгородського національного університету
7	Ботанічний сад Харківського національного університету ім. В. Н. Каразіна
8	Ботанічний сад Хмельницького національного університету
9	Ботанічний сад Чернівецького національного університету ім. Юрія Федьковича
10	Донецький ботанічний сад НАН України
11	Національний ботанічний сад ім. М. М. Гришка НАН України
12	Запорізький міський дитячий ботанічний сад
13	Криворізький ботанічний сад НАН України
14	НДЛ «Ботанічний сад» Навчально-наукового центру «Інститут біології» Київського національного університету імені Тараса Шевченка

Таблиця 4

Культивування *P. pratensis* у дендрологічних парках України

№ п/п	Назва ботанічної установи
1	Національний дендрологічний парк «Софіївка» НАН України
2	Дендрологічний парк «Асканія-Нова» Біосферного заповідника «Асканія-Нова» імені Ф. Е. Фальц-Фейна НААН
3	Державний дендрологічний парк «Олександрія» НАН України
4	Сирецький дендрологічний парк загальнодержавного значення Державного комітету України з питань житлово-комунального господарства

Не менш цінними є колекції рідкісних рослин дослідних станцій та ботанічних розсадників. У ботанічному розсаднику Уманського національного університету садівництва культивується два види – *P. grandis* Wend. й *P. pratensis*²⁶. Рослини *P. pratensis* вирощуються на дослідній станції лікарських рослин НААН України (с. Березоточа Лубенського р-ну Полтавської області), у лісовому дослідному господарстві «Парк «Веселі боковеньки» (с. Веселі Боковеньки Долинського р-ну Кіровоградської області)²⁷.

Інтродукційними дослідженнями видів роду *Pulsatilla* займаються вітчизняні вчені. У Національному ботанічному саду ім. М. М. Гришка В. В. Гриценко вказує на зростання рослин *P. pratensis* на ботаніко-географічній ділянці «Степи України» та підбиває підсумки їхньої інтродукції²⁸. О. П. Перебойчук представила список рідкісних і зникаючих видів трав'яних багаторічників флори України (Національний ботанічний сад ім. М. М. Гришка НАН України) для їх подальшого використання в декоративному садівництві²⁹. У праці С. І. Галкіна й Л. В. Калашнікової подано созологічну та біологічну характеристику раритетних видів трав'янистих рослин з колекції дендропарку «Олександрія» НАН України^{30, 31}. Т. В. Криворучко досліджено способи розмноження *P. pratensis* в умовах

²⁶ Каталог рослин ботанічного розсадника Уманського національного університету садівництва. Довідковий посібник / Т. В. Мамчур, Г. А. Чорна, М. І. Парубок та ін. ; за ред. В. П. Карпенка. Умань, 2023. 238 с.

²⁷ Куземко А. А., Діденко І. П., Швець Т. А., Чіков І. В., Джус Л. Л., Чеканов М. М. Рідкісні та зникаючі види колекції трав'янистих рослин Національного дендрологічного парку «Софіївка» НАН України : довідник. Київ, 2015. 188 с.

²⁸ Гриценко В. В. Підсумки інтродукції рослин на ботаніко-географічній ділянці «Степи України» Національного ботанічного саду ім. М. М. Гришка НАН України. *Інтродукція рослин*. 2009. № 4. С. 18–24.

²⁹ Перебойчук О. П. Інтродукція рідкісних і зникаючих видів трав'яних багаторічників флори України для подальшого використання в декоративному садівництві. *Флористичне і ценотичне різноманіття у відновленні, збереженні та охороні рослинного світу* : матеріали міжн. наук.-практ. конф. (Київ, 23–25 квітня 2018 р.). Київ : Видавництво Ліра-К, 2018. С. 154–155.

³⁰ Галкін С. І., Калашнікова Л. В. Актуальні питання збереження рідкісних рослин у дендропарку «Олександрія» НАН України. *Інтродукція рослин*. 2013. № 2. С. 12–17.

³¹ Калашнікова Л. В., Галкін С. І. Таксономічна, созологічна та біологічна характеристика раритетних видів трав'янистих рослин з колекції дендропарку «Олександрія» НАН України. *Інтродукція рослин*. 2017. № 4. С. 19–27).

культури Ботанічного саду Полтавського національного педагогічного університету імені В. Г. Короленка³², Г. Н. Шоль з'ясовано успішність інтродукції *P. pratensis* у Криворізькому ботанічному саду НАН України³³. У Донецькому ботанічному саду В. В. Козуб-Птицю вперше надано наукове обґрунтування біологічних основ збереження *P. pratensis ex situ* на південному сході України, розроблено елементи технології розмноження цього виду партикулами без викопування материнської рослини^{34, 35}.

3. Стан інтродукційних популяцій *P. patens* і *P. pratensis ex situ* Національного дендрологічного парку «Софіївка»

У Національному дендропарку «Софіївка» *P. patens* вирощується з 2000 р., *P. pratensis* – з 2002 р. Інтродукційні популяції сформовані у складі культурфітоценозу на двох колекційних ділянках: № 1 – колекційна ділянка трав'янистих багаторічників (кв. 1). У першій локації загальне проективне покриття трав'яного ярусу становить 70–80%. З ранньовесняних рослин Флористичний склад супутньої рослинності локалітету відображає відносну збереженість природного рослинного покриву та сприяє підтриманню стабільності популяції *P. patens* у межах досліджуваної території *Corylus avellana* L., *Aquilegia vulgaris* L., *Elymus repens* (L.) Gould, *Hemerocallis hybrida* Bergmans, *Vinca minor* var. *alba* Weston, *Vinca minor* L., *Salvia officinalis* L., *Dictamnus albus* L., *Tradescantia virginiana* L., *Epimedium alpinum* L., *Betonica alopecuroides* L.; у локації № 2 – ділянка рідкісних та зникаючих рослин на дослідно-інтродукційній ділянці ім. В. В. Мітіна (кв. 3). У структурі культурфітоценозів виділяється другий ярус із проективним покриттям до 50%. *Rhus typhina* L., *Cornus florida* L., *Crataegus monogyna* Jacq., *Acer platanoides* L., *Forsythia suspensa* (Thunb.) Vahl й *Corylus avellana* L. Трав'яний ярус розріджений (проективне покриття 40%), утворений *Aegopodium podagraria* L., *Elymus repens* (L.) Gould, *Glaucium flavum* Crantz., *Muscari armeniacum* H.J. Veitch., *P. pratensis*, *Sedum acre* L., *Taraxacum officinale* F.H. Wigg., *Trifolium fragiferum* L. й *Viola odorata* L.

Після визначення зовнішньої межі популяційного поля площа досліджуваного виду була оцінена від двох до трьох м². Показники

³² Криворучко Т. В. Деякі аспекти збереження генофонду рідкісних первоцвітів Полтавщини. *Проблеми охорони генофонду природи Полісся*. Луцьк: Надстир'я : збірник наукових праць. 2001. С. 54–55.

³³ Шоль Г. Н. Деякі аспекти збереження рідкісних і зникаючих рослин на урбанізованих територіях. *Інтродукція рослин*. 2000. № 1. С. 55–56.

³⁴ Козуб-Птиця В. В. *Pulsatilla bohemica* (Scalycky) Tzvelev *ex situ* та *in situ*. *Промислова ботаніка*. 2010. Вип. 10. С. 72–76.

³⁵ Птиця В. В. До вивчення репродуктивної здатності деяких лікарських рослин, інтродукованих в Донецькому ботанічному саду НАН України. *Промислова ботаніка*. 2004. Вип. 4. С. 216–221.

популяційної щільності особин *P. patens* на модельних ділянках відрізняються удвічі, що пов'язано з різним ступенем антропогенного впливу. У першій локації спостерігається висока урожайність насіння, що пов'язано зі сприятливими умовами (напівтінь, зволоження ґрунту в період дозрівання плодів, що сприяє формуванню нових генерацій). У другій локації прослідковується зменшення щільності, позаяк на ній здійснюється постійний догляд за трав'янистими рослинами, що призводить до ненавмисного висапання (табл. 5).

Таблиця 5

Характеристика місць зростання *P. patens* ex situ НДП «Софіївка»

№ локації	Площа, яку займає популяція, м²	Чисельність (загальна кількість)	Характеристика місця зростання	Чи є загроза зникнення і яка?	Категорія життєвості виду
1	3	27	культурифіто-ценоз (відкрита сонячна ділянка)	посуха	3а
2	2	14	культурифіто-ценоз (напівтінь)	посуха	3

За категорією життєвості *P. patens* у локації № 1 має бал 3а, тобто вид у фітоценозі проходить повний цикл розвитку, нормально плодоносить та висіває насіння. У локації № 2 – три бали, позаяк вид у фітоценозі проходить повний цикл розвитку, який завершується утворенням плодів і насіння з малоактивним розсіюванням насіння і поширенням плодів (табл. 3). Основною загрозою зникнення, ймовірно, може стати тривала відсутність дощів і нестача вологи у ґрунті, але в умовах культури цю причину можна виправити стаціонарним поливом, що буде сприяти збереженню цього виду.

Оскільки досліджувані види характеризуються тривалим життєвим циклом та тривалим періодом прегенеративного розвитку (5–10 років), стан їхніх популяцій *ex situ* має нехарактерний віковий спектр.

Насіння *P. patens* проростає у серпні, а основна частина – наступного року. Проростки (р) мають один трилопатевий листок, близько 7 мм у діаметрі (у такому стані вони залишаються до першої зими́влі). Рослини, що сходять навесні, за період вегетації утворюють три справжні листки. До вересня вони виростають до 2–3 см і залишаються в ювенільному стані ще протягом двох-трьох років. Щорічно в пагоні розетки утворюється три чи чотири нові листки, які з'являються у другій половині травня. Рослина з одним або двома маленькими листками 2,5 см у діаметрі реєструвалася нами як ювенільна (j) (віком близько одного року). Молоді

особини реєстрували у травні та червні, тобто це були особини, які проросли протягом попереднього вегетаційного періоду та пережили одну зиму. Рослини іматурного стану (ім) розрізняли за збільшенням листових пластинок до 4–5 см у діаметрі. Віргінільні й генеративні особини підраховували з кінця травня до початку вересня. У віргінільних особин (v) добре розвинене вертикальне кореневище з великою кількістю черешків відмерлого листя, листова пластинка досягає 10 см у діаметрі. До кінця цього стану (тривалість 2–3 роки) відбувається розгалуження кореневища і розростання куща. У молодих генеративних особин (g_1) розвиток першого генеративного пагону відбувається на 10–12 рік життя рослини. Тривалість цього вікового стану триває 2–3 роки. Середньовічні генеративні особини (g_2) характеризуються розгалуженням епігеогенного кореневища, що мають три генеративних пагони. Кущ розростається і дає початок новим парціальним кущам. У старих генеративних особин (g_3) каудекс сильно зруйнований, є частини, що відмирають, які можуть ділити рослину на частини. Кількість квітконосів значно скорочується, всихають листя і квітконоси. У субсенільних особин (ss) каудекс зруйнований і візуально вони схожі на іматурні.

Онтогенетичний спектр інтродукційної популяції *P. patens* представлений переважно ювенільними, іматурними, віргінільними та генеративними особинами. Через швидкий перехід проростків у ювенільний стан їхня ідентифікація ускладнена. Сенільних рослин не виявлено, що, ймовірно, пов'язано з тим, що *ex situ* вони ще не досягли цього етапу розвитку (табл. 6, рис. 1).

Аналіз онтогенетичної структури популяції рослин *P. patens* показав, що в обох локаціях переважають особини передгенеративного періоду (57,15–59,25%), а частка генеративних – 22,22–28,57%. Високий відсоток проростків (р), ювенільних (j), іматурних (ім) та віргінільних (v) особин свідчить про ефективне насіннєве поновлення та високу життєздатність популяції (табл. 6), що підтверджується індексом відновлення (59,26–64,29).

P. pratensis у НДП «Софіївка» культивується з 2012 року, які зростають у трьох локаціях: № 1 – колекційна ділянка трав'янистих багаторічників (кв. 1); № 2 – ділянка рідкісних та зникаючих рослин на дослідно-інтродукційній ділянці ім. В. В. Мітіна (кв. 3); № 3 – експозиційна ділянка на території науково-адміністративного корпусу (кв. 2). Інтродукційна популяція загалом займає площу 13 м² (табл. 7). У локації № 1 деревний ярус утворює *Picea abies* (L.) H. Karst., *Corylus avellana* L. і *Sorbus domestica* L., проективне покриття яких становить 10%. У ранньовесняній структурі домінує *Galanthus nivalis* L., *Scilla bifolia* L., *Chionodoxa*

luciliae Boiss., *Ornithogalum umbellatum* L. та *Puschkinia scilloides* Adam. Проективне покриття трав'яного ярусу становить 80%, у ньому домінує *Paeonia tenuifolia* L. та *P. pratensis*. У культурфітоценозі представлені також *Aquilegia caerulea* James., *Aquilegia viridiflora* Pall., *Armeria plantaginea* (All.) Willd., *Helianthemum alpestre* (Jacq.) DC., *H. canum* (L.) Baumg., *H. nummularium* (L.) Mill., *Glaucium flavum* Crantz., *Euphorbia myrsinites* L., *Iris pumila* L., *Oenothera missouriensis* Sims., *Papaver orientale* L., *Sisyrinchium angustifolium* Mill. та *Thymus marschallianus* Willd.

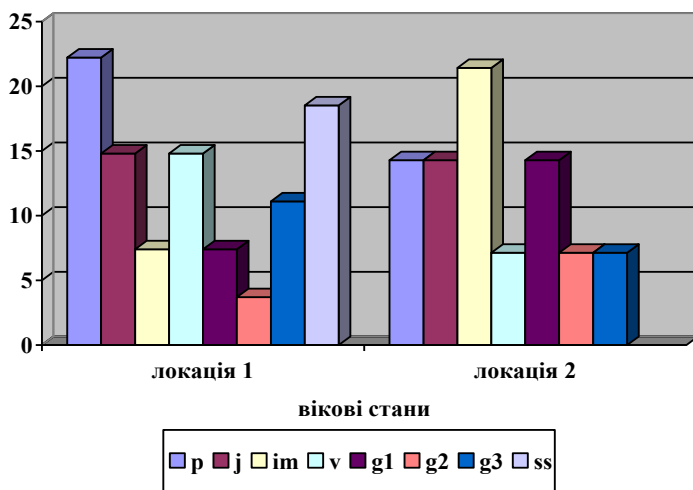


Рис. 1. Вікові спектри популяцій рослин *P. patens* ex situ

Таблиця 6

Онтогенетична структура популяції рослин *P. patens* ex situ

№ локації	Вікові стани								Онтогенетичні індекси			
	p	j	im	v	g ₁	g ₂	g ₃	ss	відновлення	старіння	генера-тивності	віковості
1	22,22	14,81	7,41	14,81	7,41	3,7	11,11	18,52	59,26	29,63	22,22	0,50
2	14,29	14,29	21,43	7,14	14,29	7,14	7,14	0	64,29	14,29	28,57	0,22

У другій локації чисельність особин незначна через високу зімкнутість другого ярусу (50%): *Rhus typhina* L., *Cornus florida* L., *Crataegus monogyna* Jacq., *Acer platanoides* L., *Forsythia suspensa* (Thunb.) Vahl і *Corylus avellana* L. Трав'яний ярус розріджений (проективне покриття 40%), утворений *Aegopodium podagraria* L., *Elymus repens* (L.) Gould, *Glaucium*

flavum Crantz., *Muscari armeniacum* H.J. Veitch., *P. patens*, *Sedum acre* L., *Taraxacum officinale* F.H. Wigg., *Trifolium fragiferum* L. і *Viola odorata* L.

Чисельність у третій локації є найменшою через регулярне викошування цієї ділянки (табл. 7). Трав'яний ярус формують *P. pratensis* і *Phlox subulata* L. Поруч зростають *Picea abies* 'Pendula' і *Thuja occidentalis* L.

Таблиця 7

Характеристика місць зростання *P. pratensis ex situ* НДП «Софіївка»

№ локації	Площа, яку займає популяція, м²	Чисельність (загальна кількість), шт	Характеристика місця зростання	Чи є загроза зникнення і яка?	Категорія життєвості виду
1	8	63	культурифітоценоз (відкрита сонячна ділянка)	—	3а
2	3	14	культурифітоценоз (напівтінь)	—	3
3	2	11	культурифітоценоз (напівтінь)	рекреацій-не навантаження	3

В онтогенетичній структурі *P. pratensis ex situ* НДП «Софіївка» виявлено сім вікових станів.

Проростки (р) мають короткий трилопатевий листок, пластинка якого має клиновидну форму, яка зверху вкрита волосками. Ювенільні особини (j) однопагінні, 5–7 см заввишки з 3–5 простими трійчаторозсіченими, в обрисі широкояйцевидними листками. У іматурних рослин (im) наявний розетковий пагін першого порядку з 4–5 пальчаторозсіченими довгочерешковими листочками. Віргінільні особини (v) однопагінні, утворюють розетку 4–5 довгочерешкових тричі перисторозсічених листків в обрисі видовжено-яйцевидних з лінійними загостреними часточками. Листова пластинка 9–15 см завдовжки, 6–10 см завширшки, черешок 7–10 см завдовжки. Молоді генеративні рослини (g₁) мають 2–5 розеткових і напіврозеткових пагонів, каудекс нерозгалужений, незруйнований. Листкова пластинка продовгувато-яйцевидна в обрисі, тричі перисторозділена з лінійними або вузько-лінійними загостреними часточками. Генеративні пагони густоопушені, 15–25 см заввишки. У середньовікових генеративних особин (g₂) чітко виражений багатоголовий каудекс 8–13 см у діаметрі, утворений за рахунок руйнування кореневища і утворення осей 2–4 порядків. Листова пластинка 19–35 см завдовжки, 12–15 см завширшки, черешок 30–35 см завдовжки. У НДП «Софіївка» кущі компактні (40–60 см у діаметрі) та утворюють по 20–25 генеративних

пагонів. Старі генеративні (g_3) рослини мають зруйнований каудекс, який розпадається на однокі партікули. Розеткові пагони переважають над напіврозетковими, старі частини рослини над молодими. Квіткові пагони у більшості таких кущів недорозвинені й, не зацвівши, засихають. Субсенільні особини (ss) мають зруйновані партікули з пригніченими розетковими пагонами. Листки зменшуються у розмірі й візуально схожі на іматурні рослини. Сенільних особин (s) виявлено не було, що ймовірно пов'язано з тим, що рослини *ex situ* ще не досягли цього онтогенетичного стану.

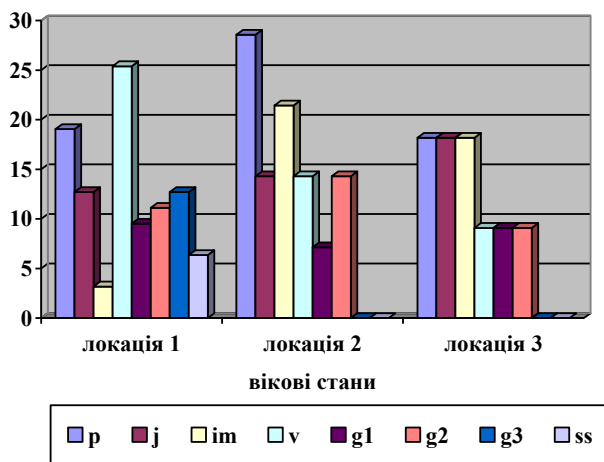


Рис. 2. Вікові спектри популяцій *P. pratensis ex situ* НДП «Софіївка»

За результатами підрахунку особин різних онтогенетичних станів *P. pratensis* та їхнього співвідношення усі досліджувані популяції віднесено до інвазивного типу з переважанням предгенеративних рослин (60,32–78,58%). Відсутність особин станів (g_3) та (ss) у локаціях 2 і 3 не свідчить про деградацію популяцій, оскільки рослини *ex situ* ще не досягли відповідного генеративного та сенільного стану.

Віковий спектр у досліджених локаціях – лівосторонній (з переважанням молодих особин). Це свідчить про успішне виживання сходів, що пов'язане з відсутністю задерніння ґрунту (рис. 2).

Високі показники відновлення (60,32–78,57%) та генеративності (21,43–33,33%) під час оцінки інтегральної онтогенетичної структури свідчать про стійкість інтродукційних популяцій *P. pratensis* (табл. 8).

Таблиця 8

Онтогенетична структура інтродукційних популяцій *P. pratensis*

№ локації	Вікові стани								Онтогенетичні індекси			
	p	j	im	v	g ₁	g ₂	g ₃	ss	відновлення	старіння	генеративності	віковості
1	19,05	12,70	3,17	25,4	9,52	11,11	12,7	6,35	60,32	19,05	33,33	0,32
2	28,57	14,29	21,43	14,29	7,14	14,29	0	0	78,57	0	21,43	0
3	18,18	18,18	18,18	9,09	9,09	9,09	0	0	72,73	9,09	27,27	0,13

ВИСНОВКИ

За результатами досліджень *ex situ* в Національному дендрологічному парку «Софіївка» НАН України з'ясовано, що рослини *P. patens* й *P. pratensis* виявляють високу життєздатність та успішне самовідновлення з індексами (59,26–64,29%) та (60,32–78,57) відповідно. Це свідчить про успішне проростання насіння та виживання сходів. Висока життєздатність молодих рослин *P. pratensis* забезпечується відсутністю задерніння ґрунту в умовах культивування. В усіх досліджуваних популяціях чітко переважають рослини передгенеративного (молодого) періоду. Їхня частка становить 57,15–59,25% для *P. patens* та 60,32–78,58% для *P. pratensis*, тому їх віднесено до інвазивного типу (динамічні популяції, що активно заселяють територію). Достатня частка генеративних особин (22,22–28,57% у *P. patens* та 21,43–33,33% у *P. pratensis*) у поєднанні з масовим молодим поповненням свідчить про високу стійкість та успішну адаптацію обох видів *ex situ*.

АНОТАЦІЯ

Збереження біорізноманіття рослин є однією з найбільш актуальних проблем сучасності, особливо це стосується рідкісних та зникаючих видів. Підтримання їх генетичного різноманіття здійснюється шляхом збереження цілісності екосистем у природних місцезростаннях і значно доповнюється технологіями збереження рослин в умовах культури (ботанічних садах та дендропарках). Нами проаналізовано досвід інтродукції видів роду *Pulsatilla* Mill. *ex situ* в Україні. З'ясовано стан інтродукційних популяцій у культурфітоценозах Національного дендрологічного парку «Софіївка» НАН України. Оскільки досліджувані види характеризуються тривалим життєвим циклом та тривалим періодом прегенеративного розвитку (5–10 років), стан їхніх популяцій *ex situ* має нехарактерний віковий спектр. Рослини *P. patens* і *P. pratensis* проявляють високу життєздатність та успішне самовідновлення. Успішне виживання молодих рослин *P. pratensis* забезпечується відсутністю задерніння ґрунту в умовах культури. В усіх досліджуваних популяціях

переважають рослини передгенеративного (молодого) періоду, тому їх віднесено до інвазивного типу (динамічні популяції, що активно заселяють територію). Достатня частка генеративних особин у поєднанні з масовим молодим поповненням свідчить про високу стійкість та успішну адаптацію обох видів *ex situ*.

Література

1. Global Strategy for Plant Conservation, GSPC : Richmond, U.K., 2002. 16 с.
2. Антонюк Н. Є., Бородіна Р. М., Собко В. Г., Скворцова Л. С. Рідкісні рослини флори України в культурі. Київ : Наукова думка, 1982. 216 с.
3. Мельник В. І. Моделювання інтродукційних популяцій у культурфітоценозах як метод охорони рідкісних видів рослин *ex situ*. Науковий вісник ЧНУ. Біологія. 2002. Вип. 145. С. 25–37.
4. Гапоненко М. Б. Інтродукція рослин як метод збереження та збагачення біологічного різноманіття в ботанічних садах та дендропарках. Інтродукція рослин, збереження та збагачення біорізноманіття в ботанічних садах і дендропарках : матеріали міжн. наук. конф. (Київ, 15–17 вересня 2010 р.). Київ, 2010. С. 34–36.
5. Червона книга України. Рослинний світ / за ред. Я. П. Дідуха. Київ : Глобалконсалтинг, 2009. 900 с.
6. Маринич О. М., Шищенко П. Г. Фізична географія України : підручник. Київ : Знання, 2005. 511 с.
7. World Flora Online. Published on the Internet. URL: <http://www.worldfloraonline.org>. (дата звернення: 16.01.2026).
8. Методичні рекомендації щодо ведення фенологічних спостережень за рослинами на території природно-заповідного фонду / за ред. Г. В. Драбинюк. РЛП «Приінгульський», 2016. С. 6–7.
9. Григора І. М., Соломаха В. А. Основи фітосоціології. Київ : Фітосоціоцентр, 2000. С. 6.
10. Злобін Ю. А. Популяційна екологія рослин: сучасний стан, точка росту : монографія. Суми : Університетська книга, 2009. С. 108–110.
11. Бакалина Л. В. Онтогенез і популяційна структура сонів широколистого і чорніючого в екосистемах Канівського природного заповідника. Заповідна справа в Україні. 1997. Т. 1. Вип. 2. С. 16–22.
12. Чуй О. В. Стан ценопопуляцій *Pulsatilla grandis* Wend. та *Pulsatilla patens* (L.) Mill. (Ranunculaceae Juss.) в Західному Опіллі : автореф. дис. ... канд. біол. наук : 03.00.05. Київ, 2016. 21 с.
13. Щербаківа О. Ф., Новосад К. В. Особливості сезонного розвитку та поліваріантність структури річних квітконосних пагонів *Pulsatilla*

patens (L.) Mill. і *P. pratensis* (L.) Mill. в умовах Київського мегаполісу. *Інтродукція рослин*. 2013. № 3. С. 34–42.

14. Щербаківа О. Ф., Новосад К. В. Поліваріантність елементарних модулів у структурі річних квітконосних пагонів *Pulsatilla patens* (L.) Mill. і *P. pratensis* (L.) Mill. (Ranunculaceae). *Український ботанічний журнал*. 2013. Т. 70, № 6. С. 751–761.

15. Птиця В. В. Біологічні основи збереження раритетних видів рослин *ex situ* на південному сході України : дис. ... канд. біол. наук : 03.00.05. Київ, 2008. 178 с.

16. Коваленко І. М. Структура популяцій домінантів трав'яночагарничкового ярусу в лісових фітоценозах Деснянсько-Старогутського національного природного парку. І. Онтогенетична структура. *Український ботанічний журнал*. 2005. Т. 62. № 5. С. 707–714.

17. Харкевич С. С. Інтродукція на Україні корисних рослин природної флори СРСР / АН УРСР, Центр. респ. ботан. сад; відп. ред. С. С. Харкевич. Київ : Наукова думка, 1972. С. 66.

18. Собко В. Г., Гапоненко М. Б. Інтродукція рідкісних і зникаючих рослин флори України / за ред. М. А. Кохна. Київ : Наукова думка, 1996. 283 с.

19. Каталог декоративних трав'янистих рослин ботанічних садів і дендропарків України : довідниковий посібник / за ред. С. П. Машковської. Київ, 2015. 282 с. (електронне видання).

20. Каталог раритетних рослин ботанічних садів і дендропарків України : довідковий посібник / за ред. А. П. Лебеди. Київ : Академперіодика, 2011. 184 с.

21. Гришко М. М., Соколовський О. І. Ботанічний сад і його колекції. Київ : в-во АН РСР, 1950, 115 с.

22. Каталог рослин Центрального ботанічного саду ім. М. М. Гришка. Київ : Наукова думка. 436 с.

23. Ботанічний сад ім. акад. О. В. Фоміна. Каталог рослин. Природно-заповідні території України. Рослинний світ. Вип. 7. Київ : Фітосоціоцентр, 2007. 320 с.

24. Каталог рослин Криворізького ботанічного саду / за ред. А. Т. Гревцової. Київ : Фітосоціоцентр. 2000. 164 с.

25. Кременецький ботанічний сад. Каталог рослин. Природно-заповідні території України. Рослинний світ. Вип. 8. Київ : Фітосоціоцентр, 2007. 159 с.

26. Каталог рослин ботанічного розсадника Уманського національного університету садівництва : довідковий посібник / Т. В. Мамчур, Г. А. Чорна, М. І. Парубок та ін. ; за ред. В. П. Карпенка. Умань, 2023. 238 с.

27. Куземко А. А., Діденко І. П., Швець Т. А., Чіков І. В., Джус Л. Л., Чеканов М. М. Рідкісні та зникаючі види колекції трав'янистих рослин Національного дендрологічного парку «Софіївка» НАН України : довідник. Київ, 2015. 188 с.

28. Гриценко В. В. Підсумки інтродукції рослин на ботаніко-географічній ділянці «Степи України» Національного ботанічного саду ім. М. М. Гришка НАН України. *Інтродукція рослин*. 2009. № 4. С. 18–24.

29. Перебойчук О. П. Інтродукція рідкісних і зникаючих видів трав'яних багаторічників флори України для подальшого використання в декоративному садівництві. *Флористичне і ценотичне різноманіття у відновленні, збереженні та охороні рослинного світу* : матеріали міжн. наук.-практ. конф. (Київ, 23–25 квітня 2018 р.). Київ : Видавництво Ліра-К, 2018. С. 154–155.

30. Галкін С. І., Калашнікова Л. В. Актуальні питання збереження рідкісних рослин у дендропарку «Олександрія» НАН України. *Інтродукція рослин*. 2013. № 2. С. 12–17.

31. Калашнікова Л. В., Галкін С. І. Таксономічна, соціологічна та біологічна характеристика раритетних видів трав'янистих рослин з колекції дендропарку «Олександрія» НАН України. *Інтродукція рослин*. 2017. № 4. С. 19–27.

32. Криворучко Т. В. Деякі аспекти збереження генофонду рідкісних первоцвітів Полтавщини. *Проблеми охорони генофонду природи Полісся. Луцьк: Надстир'я* : збірник наукових праць. 2001. С. 54–55.

33. Шоль Г. Н. Деякі аспекти збереження рідкісних і зникаючих рослин на урбанізованих територіях. *Інтродукція рослин*. 2000. № 1. С. 55–56.

34. Козуб-Птиця В. В. *Pulsatilla bohemica* (Scalycky) Tzvelev *ex situ* та *in situ*. *Промислова ботаніка*. 2010. Вип. 10. С. 72–76.

35. Птиця В. В. До вивчення репродуктивної здатності деяких лікарських рослин, інтродукованих у Донецькому ботанічному саду НАН України. *Промислова ботаніка*. 2004. Вип. 4. С. 216–221.

Information about the authors:

Chekanov Mykhailo,

Junior Research Fellow Herbaceous Plants Section
The National Dendrological Park 'Sofiyivka'
of the National Academy of Sciences of Ukraine
12 A, Kyivska Street, Uman, Ukraine

Kuzemko Anna,
Doctor of Biological Sciences, Senior Research Fellow
M. G. Kholodny Institute of Botany
of the National Academy of Sciences of Ukraine
2, Tereshchenkivska Street, Kyiv, Ukraine

Didenko Inna,
Candidate of Biological Sciences, Senior Researcher
The National Dendrological Park ‘Sofiyivka’
of the National Academy of Sciences of Ukraine
12 A, Kyivska Street, Uman, Ukraine

THE DIVERSITY OF NATURAL OAK FOREST PHYTOCENOSSES IN TYPICAL STEPPE RAVINES OF THE DNIPROPETROVSK REGION

Yakovlieva-Nosar Svitlana, Bessonova Valentina

DOI <https://doi.org/10.30525/978-9934-26-714-7-21>

INTRODUCTION

In Ukrainian steppe zone, natural forests cover only small areas and are found primarily in ravines, gullies, and river valleys, where the topography creates a more favorable water regime and mitigates the effects of the arid climate. It is precisely the ravine (gully) forests that serve as the main centers of natural woody vegetation in the steppe and play an important role in maintaining the ecological balance of the landscapes. They perform soil conservation, water regulation, climate regulation, and erosion control functions, and also help preserve the gene pool of many plant and animal species^{1, 2, 3}.

Thanks to the diversity of ecological conditions created by the complex topography, slope aspect, and variations in moisture levels and soil cover⁴, ravine forests are characterized by high floristic richness⁵. Their composition includes a mix of non-steppe, steppe, and meadow species, as well as rare plants with varying conservation statuses^{6, 7}. In this regard, beam forests are

¹ Бельгард О. Л. Лісова рослинність південного сходу України. Київський державний університет ім. Т. Г. Шевченка. Київ : Вид-во КДУ ім. Т. Г. Шевченка, 1950. 263 с.

² Божко К. М. Еколого-біологічна характеристика північного варіанта байрачних лісів південно-східної України. *Вісник Дніпропетровського університету. Серія : Біологія. Екологія*. 2007. Вип. 15, Т. 1. С. 3–8.

³ Yakovlieva-Nosar S., Bessonova V. State of coenopopulations of *Quercus robur* L. growing in ravines located in the Dnieper river's rapids section (recreation zone of the city of Zaporizhzhya), Ukraine. *Forestry ideas*. 2021. Vol. 27, № 1 (61). P. 256–270.

⁴ Bessonova V., Yakovlieva-Nosar S. Comparison of transpiration activity of *Quercus robur* L. and *Acer campestre* L. trees under different conditions of moisture supply in the Viiskova ravine. *Ukrainian Journal of Forest and Wood Science*. 2024. 15(3). P. 148–164.

⁵ Яковлева-Носарь С. О. Видове різноманіття дендрофлори байрачних лісів (зона рекреації міста Запоріжжя). *Науковий вісник НЛТУ України*. 2022, т. 32, № 5. С. 13–18.

⁶ Яковлева-Носарь С. О. Рідкісні види трав'янистих рослин степових ділянок балки Генералка (острів Хортиця). *Рослини та урбанізація* : матеріали XIII міжнародної науково-практичної конференції (Дніпро, 1 лютого 2024 р.). Дніпро, 2025. С. 93–95.

⁷ Яковлева-Носарь С. О. Рідкісні види трав'яних рослин степових схилів балки Широка (острів Хортиця). *Рослини та урбанізація* : матеріали XIV міжнародної науково-практичної конференції (Дніпро, 3 лютого 2025 р.). Дніпро, 2025. С. 156–157.

considered important centers of biodiversity in the steppe zone and natural refuges for forest flora⁸.

Amid the significant transformation of steppe ecosystems due to human activities, the role of ravine forests is growing substantially. Not only do they serve as centers for the conservation of natural vegetation, but they also support the ecological stability of landscapes while performing important ecosystem functions⁵.

That is why studying the floristic composition, the structure of plant communities, and the current state of ravine forests is a pressing task, and the results of such studies are crucial for developing measures for their protection, sustainable use, and conservation.

In light of the above, the objective of this study is to analyze the structure of oak communities growing under various forest conditions across five forest tracts in the Dnipropetrovsk region.

The subject of this study is the silvicultural-assessment and botanical-phytocenotic characterization of naturally occurring oak associations in the surveyed forest tracts.

1. Research objects and methods

The study focused on natural oak phytocenoses in five ravine forests – the tracts “Zhucha Balka”, “Domashnia Balka” and “Rezynova Balka” (Fig. 1), located on the right bank of the Dnipro river, and the “Yatsevo” and “Dibrova” tracts, located on its left bank (Fig. 2). They belong to the ravine forests of the southern variant of the Northern Steppe and constitute the forest-park sections of the green belt forests of the city of Dnipro. The coordinates of the surveyed areas are given in Table 1.

The “Zhucha Balka” forest tract belongs to the Mykilske Forestry of the Dnipro Forest Enterprise and is located in the Solonyanskyi District of Dnipropetrovsk Oblast (Fig. 1, 1). It borders the village of Mykilsk-na-Dnipri to the south. It is part of the “Dnipro Rapids” Regional Landscape Park. In this forest tract, trial areas (TA) were established in compartment 13: TA1 – stand 1, TA2 – stand 2 (on the border with stand 1), TA3 – stand 19.

The “Domashnia Balka” tract is also located within the territory of the Mykilske Forestry (Fig. 1, 2) on the border with the lands of the Viyskova Village Council in the Solonyanskyi District. To the north, the ravine borders the village of Mykilske-on-the-Dnieper. The tract is part of the “Dnieper Rapids” Regional Landscape Park. The ravine opens into the Dnieper River valley

⁸ Біотопи степової зони України / Ред. академік НАН України Я. П. Дідух. Київ – Чернівці : ДрукАРТ, 2020. 392 с.

opposite the former famous Nenasyetsky Rapids. Within this forest tract, trial areas were located in compartment 15: TA1 – stand 2; and compartment 16: TA2 – stand 3.

Table 1

Coordinates of the studied ravines

No.	Name of the forest tract	Coordinates	
		ravine tops	mouth of the ravine
Mykilske Forestry (Right Bank)			
1.	“Zhucha Balka”	48°12'30.2"N 35°08'48.9"E	48°12'47.5"N 35°10'55.6"E
2.	“Domashnia Balka”	48°12'11.2"N 35°07'58.5"E	48°11'13.4"N 35°10'28.0"E
3.	“Rezynova Balka”	48°07'46.5"N 35°00'48.6"E	48°06'51.9"N 35°02'05.6"E
Liubymivske Forestry (Left Bank)			
4.	“Yatsevo”	48°20'18.1"N 35°13'39.5"E	48°19'44.3"N 35°10'17.1"E
5.	“Dibrova”	48°18'41.0"N 35°13'22.0"E	48°18'00.7"N 35°10'44.8"E

The “Rezynova Balka” tract is located on the border between the Dnipropetrovsk and Zaporizhzhia regions; specifically, the northern main part, including the side valleys, lies within the Dnipropetrovsk region, while the administrative boundary between the two regions runs along the thalweg of the southern main part (Fig. 1, 3). This part of it is also called “Balka Kantserka”. The tract belongs to the Mykilske Forestry. Trial areas were established in the northern main section, which is 2.25 km long. They were located as follows: in compartment 40: stand 2 (TA1) (the large right-hand ravine); in compartment 41: stand 1 (TA2) – the large middle ravine, stand 4 (TA3); and in compartment 42: stand 3 (TA4).

The “Yatsevo” tract is a forest reserve of national significance covering an area of 175 ha⁹. It is part of the Liubymivske Forestry, located on the left bank of the Dnipro River (Fig. 2, 1). In this ravine, trial areas were established within compartment 18: stand 3 (TA1), stand 6 (TA2); and compartment 19: stand 2 (TA3).

The “Dibrova” tract is also located within the territory administered by the Liubymivske Forestry, south of the “Yatsevo” tract (Fig. 2, 2). One trial area was surveyed; it was established in the central part of compartment 22, in stand 11.

Each of the established trial areas covered an area of 900 m².

⁹ Манюк В. В., Манюк В. В. Природно-заповідний фонд Дніпропетровщини. Дніпро, 2010. 116 с.

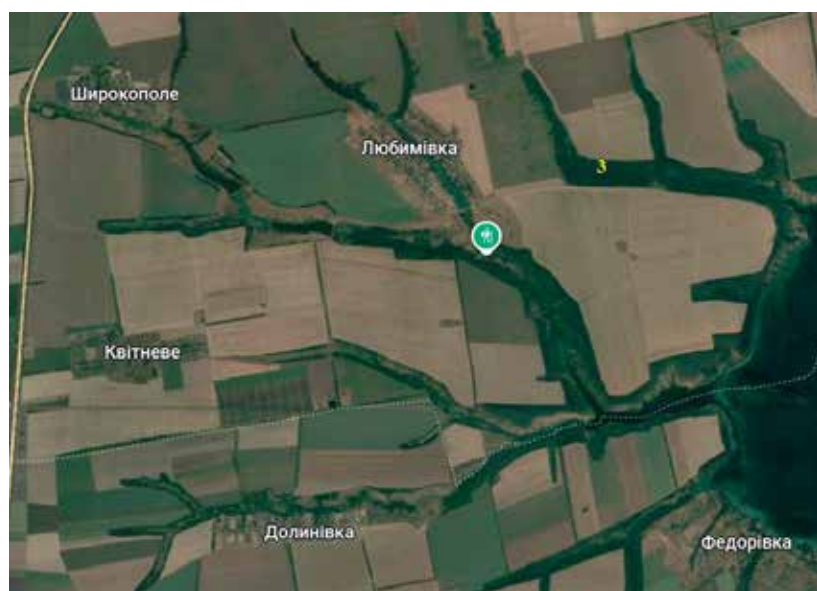


Fig. 1. Location of the studied right-bank tracts: 1 – “Zhucha Balka”, 2 – “Domashnia Balka”, 3 – “Rezynova Balka”



**Fig. 2. Location of the surveyed left-bank tracts:
1 – “Yatsevo”, 2 – “Dibrova”**

Tree height was determined using a Suunto PM-5/1520 optical altimeter. The stem diameter was measured at a height of 1.3 m using a Codimex S-1 tree caliper.

The mean tree height was calculated using the following formula:

$$h_m = h_i \cdot G_i / \Sigma G, \text{ m}, \quad (1)$$

where h_m is the mean tree height, m; h_i is the mean height of the i -th diameter class, m; G_i is the sum of cross-sectional areas of trees in the i -th diameter class, cm^2 ; and G is the total sum of cross-sectional areas of all trees, cm^2 .

The average cross-sectional area of the tree stand, g_m , was calculated using the following formula:

$$g_m = G/N, \text{ cm}^2, \quad (2)$$

where g_m is the mean basal area of the stand, cm^2 ; G is the total basal area of the stand, cm^2 ; and N is the number of trees.

The mean stand diameter (d_m) was determined from the mean stem cross-sectional area using the following formula:

$$d_m = 2 g_m \sqrt{\pi}, \text{ cm.} \quad (3)$$

The primary quantitative characteristic of a forest stand is its growing stock volume (M , m^3/ha). The growing stock volume of a forest stand is equal to the sum of the volumes of all trees comprising the stand.

The stem volume of an individual tree was calculated using the following formula:

$$V = ghf, \text{ m}^3, \quad (4)$$

where V is the stem volume, m^3 ; g is the stem cross-sectional area at breast height (1.3 m), m^2 ; h is the stem height, m; and f is the form factor. The form factors proposed by M. Y. Tkachenko (1952) were applied using a form quotient (q_2) of 0.68.

The classes of aesthetic value, pedestrian accessibility, stand stability, and recreational degradation stage were determined according to the¹⁰ for the forest park sections of green-belt forests.

2. Characteristics of oak forest phytocoenoses in the Right-Bank tracts

2.1. “Zhucha Balka”

The fresh oak forest with *Aegopodium podagraria* (TA1) is located in the thalweg and the lower part of a south-facing slope. The average canopy closure of the stand ranges from 0.75 to 0.80. The stand is characterized by a horizontally closed canopy. The aesthetic value class is 2, the pedestrian accessibility class is 3, the stand stability class is 2, and the recreational degradation is first stage.

The *Quercus robur* individuals are of vegetative origin, with some trees reaching up to 95 years of age. Their mensurational characteristics are presented in Table 2.

The trees are generally healthy, exhibiting good growth and development, while damage caused by pests, diseases, or adverse environmental factors is minimal. The stand is capable of maintaining its structure and ecological functions without a significant reduction in productivity.

The herbaceous layer is mainly confined to canopy openings within the forest stand. The species composition of the spring synusia is dominated by *Ficaria verna*, *Corydalis solida* (Fig. 3 A, B), *C. cava*, *Anemone ranunculoides*, *Gagea*

¹⁰ Інструктивно-методичні вказівки щодо здійснення лісовпорядкування. Державне агентство лісових ресурсів України, ВО «Укрдержліспроект». Ірпінь, 2024. 161 с.

lutea. The summer synusia is primarily composed of *Aegopodium podagraria*, *Viola mirabilis* (Fig. 3 C, D), *Lamium maculatum*, *Stellaria holostea*, *Poa nemoralis*, *Galium odoratum*, *Carex pilosa*, *Salvia nemorosa*, and other species.

Table 2

**Forest mensuration characteristics of the dominant tree species
Quercus robur on the trial areas in the “Zhucha Balka” tract**

Parameters		Phytocoenoses		
		TA1	TA2	TA3
FST		CL ₂	CL ₁	CL ₁₋₂
Composition		10Qr	10Qr + Gt	10Qr
Average	H, m	19.5	17.2	19.2
	D, cm	32.5	22.6	32.2
N, pcs/ha		232	253	225
M, m ³ /ha		162.6	174.8	162.3
Bonitet		III–IV	III–IV	III–IV

Notes: FST – forest site type; CL – clay-loam soil; Qr – *Quercus robur*; Gt – *Gleditsia triacanthos*

The forest site conditions are classified as CL₂ (fresh, mesophilic). Moisture is supplied by both atmospheric-transit precipitation and groundwater.

The dryish oak forest with *Poa angustifolia* (TA2) is located on the middle part of a south-facing slope. The average canopy closure of the stand is 0.70–0.75. The stand is characterized by a horizontally closed canopy. The aesthetic evaluation class is 2, the pedestrian accessibility class is 3, the stability class is 3, and the stand is at the first stage of recreational digression.

This is a pure oak stand with scattered *Gleditsia triacanthos* individuals that originated from the adjacent compartment 3, where a pure *Gleditsia triacanthos* plantation was established over an area of 0.4 ha using artificial forest regeneration. The *Quercus robur* trees are approximately 75 years old. Their mensurational characteristics are presented in Table 2.

The stand is slightly weakened but remains viable. Partial dieback of individual branches is observed in the crowns of some *Quercus robur* trees, along with signs of leaf damage caused by insect pests.

As the stand density is approximately 0.8 (0.78 on average), the projective cover of the herbaceous layer ranges from 30 to 60%. In spring, *Corydalis solida*, *Gagea minima* (Fig. 4 A), *Anemone ranunculoides*, *Ficaria verna*, *Corydalis marschalliana*, and other species appear. The summer synusia is dominated by grasses and xerophytic forbs, including *Poa angustifolia*, *Festuca valesiaca*, *Carex praecox*, *Galium verum*, *Achillea millefolium*, *Salvia nutans*,

Thymus marschallianus, *Artemisia austriaca*. *Hypericum perforatum* occurs locally (Fig. 4 B), while *Fragaria viridis* is also present, *Origanum vulgare* occurs only sporadically.

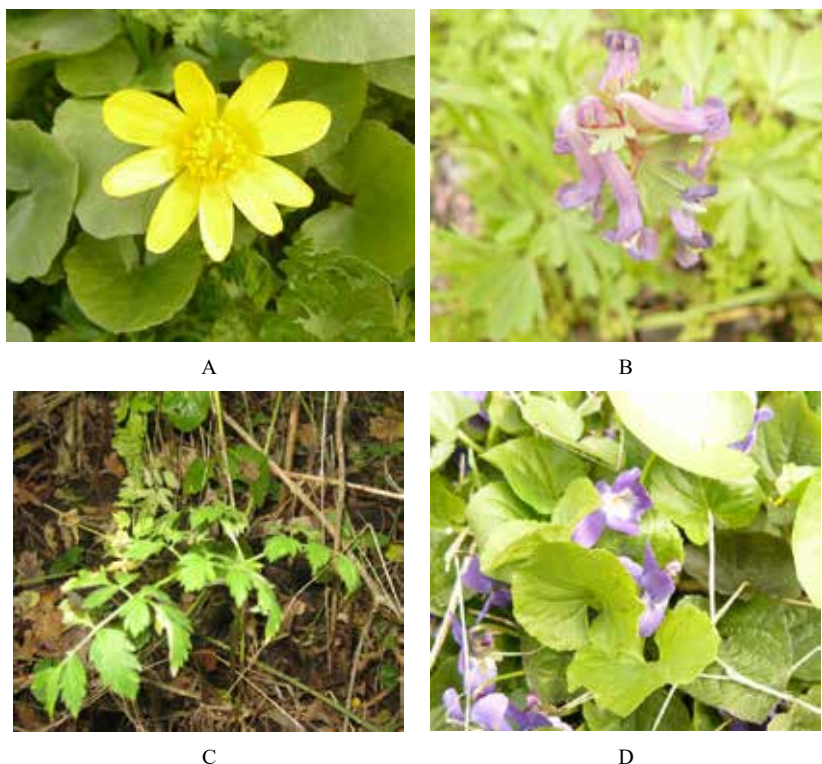


Fig. 3. Typical herbaceous plants of the spring synusia: A – *Ficaria verna*; B – *Corydalis solida*, and summer synusia: C – *Aegopodium podagraria*, D – *Viola mirabilis*

Source: developed by the authors

The forest site conditions are classified as CL₁ (dryish, mesoxerophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

The slightly fresh oak forest with *Polygonatum odoratum* (TA3) is located on the middle part of a northeast-facing slope. The average canopy closure of the stand is 0.75–0.80. The stand is characterized by a horizontally closed canopy. The *Quercus robur* trees are of vegetative origin, with an average age of 90 years, while some individuals reach 100 years. The mensurational

characteristics of this principal species are presented in Table 2. Most *Quercus robur* trees (more than 75–80%) are healthy, possessing dense green crowns and exhibiting normal growth. However, a small proportion of trees (up to 5–7%) are slightly weakened, with isolated dead branches in the upper part of the crown and signs of insect damage.



A



B

Fig. 4. Typical herbaceous plants of the dryish oak forest:

A – *Gagea minima*, B – *Hypericum perforatum*

Source: developed by the authors

No accompanying tree species were recorded in the stands on the surveyed trial areas within the “Zhucha Balka” tract. The only exception was TA2, where, as noted above, *Gleditsia triacanthos* occurred with a minor proportion of less than 5%.

The aesthetic evaluation class is 2, the pedestrian accessibility class is 3, the stability class is 2, and the stand is at the first stage of recreational digression.

The herbaceous layer contains a higher proportion of mesophytic species than the dryish oak forest. The spring synusia is represented mainly by *Corydalis solida*, *C. marschalliana*, *Scilla bifolia*, *Gagea lutea*, *G. minima*, *Anemonoides ranunculoides*, *Ficaria verna*, *Viola odorata*, while *Pulmonaria obscura* occurs locally. The core of the summer synusia is formed by *Polygonatum odoratum*, *Asparagus officinalis* (Fig. 5), *Poa angustifolia*, *Carex pilosa*. In some places, *Aegopodium podagraria*, *Galium odoratum*, *Fragaria vesca*, *Viola odorata* are present; *Hypericum perforatum* and *Origanum vulgare* occur on certain parts of the slope, whereas *Chelidonium majus* is found beneath the forest canopy.

The forest site conditions are classified as CL₁₋₂ (slightly fresh, xeromesophilic). Moisture is supplied primarily by atmospheric-transit precipitation.



A



B

Fig. 5. Typical herbaceous plants of the slightly fresh oak forest:

A – *Polygonatum odoratum*, B – *Asparagus officinalis*

Source: developed by the authors

2.2. “Domashnia Balka”

The fresh lime-ash-field maple-oak forest with *Aegopodium podagraria* (TA1) is located in the lower part of a north-facing slope adjacent to the thalweg. The *Quercus robur* trees are of vegetative origin. They are approximately 90–95 years old, and their mensurational characteristics are presented in Table 3, while those of the accompanying tree species are given in Table 4.

The average canopy closure of the stand is 0.80–0.85. The stand is characterized by a horizontally closed canopy. The aesthetic evaluation class is 2, the pedestrian accessibility class is 3, the stability class is 2, the stand is at the first stage of recreational digression.

The stand is somewhat weakened, with isolated signs of damage and stress, but overall it remains stable. Some trees exhibit slightly thinned crowns (5–10% defoliation), while dieback of individual branches is observed, particularly in the upper part of the crown. There are also isolated trees affected by fungal diseases or infested with stem-boring insects.

The herb layer is poorly developed and confined mainly to canopy gaps. The spring synusia is dominated by *Ficaria vernalis*, *Corydalis solida*, *C. cava*, *Viola mirabilis*, *V. nemoralis*. The summer synusia is dominated by

Aegopodium podagraria, with a projective cover of 60–65%. Other species include *Galium aparine*, *Geranium robertianum*, *G. sylvaticum*, *Urtica dioica* (Fig. 6, A), *Glechoma hederacea* (Fig. 6, B), and *Carex pilosa*. *Pulmonaria obscura* occurs occasionally, whereas *Asarum europaeum* is encountered only rarely.

Table 3

Mensurational characteristics of the dominant species *Quercus robur* on trial areas in the “Domashnia Balka” tract

Parameters		Phytocoenoses	
		TA1	TA2
FST		CL ₂	CL ₂
Composition		6Qr2Ac1Fe1Tc	9Qr1Ap+Rp+Bp
Average	H, m	19.5	18.2
	D, cm	30.6	30.1
N, pcs/ha		186	239
M, m ³ /ha		115.4	136.4
Bonitet		III–IV	IV

Notes: FST – forest site type; CL – clay-loam soil; Qr – *Quercus robur*; Ac – *Acer campestre*; Fe – *Fraxinus excelsior*; Tc – *Tilia cordata*; Ap – *Acer platanoides*; Rp – *Robinia pseudoacacia*; Bp – *Betula pendula*

The forest site conditions – CL₂ (fresh, mesophilic). Moisture regime is atmospheric-transitional.



**Fig. 6. Herb-layer species of the fresh oak forest:
A – *Urtica dioica*, B – *Glechoma hederacea***

Source: developed by the authors

Table 4

**Mensurational characteristics of associated tree species on trial areas
in the “Domashnia Balka” tract**

TAs	FST	Associated tree species	Average		N, pcs/ha	M, m ³ /ha
			H, m	D, cm		
TA1	CL ₂	Ac	18.3	28.1	77	38.3
		Fe	21.4	34.3	23	19.1
		Tc	19.2	28.3	120	19.1
TA2	CL ₂	Ap	19.1	34.3	20	15.4
		Rp	14.8	19.6	2	0.15
		Bp	8.3	15.2	1	0.10

Note: tree species abbreviations as in Table 3

The fresh maple-oak forest with *Aegopodium podagraria* (TA2) occurs in the lower part of a north-facing slope. The common oak trees are of vegetative origin, with an average age of 85–90 years, while some individuals reach 100 years of age. The mensurational characteristics of *Quercus robur* are presented in Table 3, and those of associated tree species are given in Table 4. This oak forest contains individuals of *Robinia pseudoacacia* and *Betula pendula*. *Robinia pseudoacacia* has spread from adjacent forest stands where this species was established through forest plantations, in particular from stand 1 of the same forest compartment 16.

In the 1950s–1970s, large-scale experimental afforestation of gullies and ravines was carried out in the Dnipropetrovsk region. Researchers from the Institute of Forestry often included *Betula pendula* in mixed planting schemes as a buffer or temporary species¹¹. Over time, during the process of species replacement, *Quercus robur* displaced most of its associated species; however, individual birch trees, especially under fresh and favourable conditions in the lower parts of slopes, have survived to the present day.

The average canopy closure of the stand is 0.75–0.8. It is characterized by horizontal canopy closure. The aesthetic evaluation class is 2, the recreational accessibility class is 3, the stability class is 2, the first stage of digression.

The shrub layer is formed by *Crataegus monogyna* and *Cornus sanguinea*, with a canopy closure of 0.40.

The main herbaceous species comprising the spring synusia of this phytocoenosis are *Corydalis solida*, *C. marschalliana*, *Ficaria verna*, *Gagea lutea*, *G. minima*, *Anemonoides ranunculoides*, *Viola odorata*, *V. hirta* (Fig. 7, A), with *Pulmonaria mollis* occurring locally. The summer synusia is composed

¹¹ Гордієнко М. І., Гойчук А. Ф., Гордієнко Н. М. Штучні ліси в дібровах / ред. М. І. Гордієнко. Житомир : Полісся, 1999. 592 с.

of *Aegopodium podagraria*, *Galium aparine*, *Glechoma hederacea*, *Chelidonium majus*, *Anthriscus sylvestris*, *Brachypodium sylvaticum* (Fig. 7, B), *Carex spicata*, *Elymus caninus*, *Viola hirta*, *Alliaria petiolata*, with *Ballota nigra* and *Carex muricata* occurring locally.



Fig. 7. Herbaceous plants of the fresh maple-oak forest:
A – *Brachypodium sylvaticum*, B – *Viola hirta*

Source: developed by the authors

The forest site conditions – CL₂ (fresh, mesophilic). Moisture regime is atmospheric-transitional.

2.3. “Rezynova Balka”

The slightly fresh field maple–tatar maple-oak forest with wild pear and *Poa angustifolia* (TA1) located on the middle part of a north-facing slope. Individual specimens of *Quercus robur* are up to 100 years old and are of seed origin. The main forest inventory characteristics of this species are presented in Table 5, while those of the associated tree species are given in Table 6.

The average canopy closure of the stand is 0.60–0.65, with the presence of semi-open areas. The stand is classified as aesthetic class 2, pedestrian accessibility class 3, stability class 2, the first stage of recreational digression.

The sparse shrub layer is composed of *Crataegus monogyna*, *Rosa canina*, *Euonymus europaea* (along the forest edge and in more open sites), as well as *E. verrucosa* (Fig. 8) beneath the forest canopy. In some places, the forest edge is formed by *Prunus spinosa*. Natural regeneration of *Acer tataricum* and *A. campestre* is present, together with occasional self-sown individuals of *Quercus robur*, which are confined to canopy gaps.

The herb layer of the spring synusia is primarily composed of *Corydalis solida*, *Gagea bulbifera*, *Anemone ranunculoides*, *Ficaria verna*, *Viola odorata*, *Iris pumila*, and others. The summer synusia is represented by *Poa nemoralis*, *Brachypodium sylvaticum*, *Melica picta*, *Stellaria holostea*, *Galium aparine*, *Viola hirta*, *Carex spicata*, *Anthriscus cerefolium*, *Chaerophyllum temulum*, *Alliaria petiolata*, and *Agrimonia eupatoria*. In more shaded and moist habitats, *Glechoma hederacea* and *Chelidonium majus* occur.

Table 5

Mensurational characteristics of the dominant species *Quercus robur* on trial areas in the “Rezynova Balka” tract

Parameters		Phytocoenoses			
		TA1	TA2	TA3	TA4
FST		CL ₁₋₂	CL ₁	CL ₁	CL ₁₋₂
Composi-tion		4Qr3At2Ac1Pc	8Qr2Ac+Pc	4Qr2Ac2Um2Pc	7Qr2Ac1Pc+Um
Average	H, m	17.5	17.2	16.5	17.7
	D, cm	26.6	29.8	30.3	28.6
N, pcs/ha		96	225	65	154
M, m ³ /ha		40.8	123.0	36.0	78.8
Bonitet		IV	IV	IV	IV

Notes: FST – forest site type; CL – clay-loam soil; Qr – *Quercus robur*; At – *Acer tataricum*; Ac – *Acer campestre*; Pc – *Pyrus communis*; Um – *Ulmus minor*

Table 6

Mensurational characteristics of associated tree species on trial areas in the “Rezynova Balka” tract

TAs	FST	Associated tree species	Average		N, pcs/ha	M, m ³ /ha
			H, m	D, cm		
TA1	CL ₁₋₂	At	10.9	21.9	163	30,2
		Ac	16.9	25.8	50	20,0
		Pc	13.5	21.8	44	10,0
TA2	CL ₁	Ac	14.2	27.8	80	30,6
		Pc	12.4	23.7	2	0,4
TA3	CL ₁	Ac	15.4	27.6	45	18,4
		Um	15.1	22.1	69	18,3
		Pc	13.6	23.9	66	18,3
TA4	CL ₁₋₂	Ac	15.6	24.3	74	22,2
		Pc	13.8	21.9	47	11,0
		Um	13.8	21.1	1	0,2

Note: tree species abbreviations as in Table 5



A



B

Fig. 8. Understory shrub species:
A – *Euonymus europaea*, B – *E. Verrucosa*

Source: developed by the authors

The forest site conditions are classified as CL₁₋₂ (slightly fresh, xeromesophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

The dryish tatar maple–oak forest with pear and *Carex* (TA2) is located on the middle part of a south-facing slope.

The average canopy closure is 0.70–0.75. The stand is characterized by a horizontally closed canopy. The aesthetic value class is 2, the pedestrian accessibility class is 3, the stability class is 4, the stand is at the first stage of recreational degradation.

The trees are in a more weakened condition than those on the other sample plots described above. Some individuals exhibit sparse crowns, reduced annual growth, and dieback of individual branches. Standing dead trees are present, and damage caused by pests and diseases is also observed.



**Fig. 9. *Viola matutina* and *Veronica persica*
in the steppe-like section of the ravine**

Source: developed by the authors

The spring synusia is dominated by *Gagea lutea*, *Corydalis solida*, *Anemonoides ranunculoides*, *Scilla bifolia*, *Viola odorata*, *Ficaria verna*, *Gagea minima*. The herb layer of the summer synusia is primarily composed of *Carex humilis*, *Melica picta*, *Poa angustifolia*, *Festuca valesiaca*, *Elytrigia intermedia*, *Polygonatum odoratum*, *Stellaria holostea*, *Galium aparine*, *Viola hirta*, *Veronica chamaedrys*, *Campanula bononiensis*, *Brachypodium pinnatum*, *Agrimonia eupatoria*, *Clinopodium vulgare*, *Origanum vulgare*, *Fragaria viridis*, *Asparagus officinalis*. On this trial area, *Viola matutina* and *Veronica persica* were also recorded (Fig. 9), although they are neither diagnostic (indicator) nor characteristic species of this type of oak forest. Their presence

does not reflect the ecological specificity of these oak forests, as *Viola matutina* is a relatively plastic species that occurs not only in oak forests but also along forest edges, in shrublands, open woodlands, and other semi-open habitats. Likewise, *Veronica persica* may occur locally in natural ravine oak forests but is not a component of the characteristic nemoral floristic assemblage.

The forest site conditions are classified as CL₁ (dryish, mesoxerophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

The dryish elm-tatar maple-oak forest with wild pear and *Dactylis glomerata* (TA3) is located in the middle (closer to the upper) part of the north-facing slope in the main northern section of the gully.

The pedunculate oak trees are of vegetative origin. They are 85–88 years old. Their mensurational characteristics are presented in Table 5, while those of the associated tree species are given in Table 6.

These are semi-open stands. The average canopy closure is 0.60–0.65. The stand is assigned to aesthetic class 2, pedestrian accessibility class 3, and stability class 4, and is at the first stage of recreational digression. It provides good accessibility for recreational visitors.

The shrub layer consists of *Euonymus verrucosa* and *Crataegus monogyna*, with occasional *Rosa canina*, while *Prunus spinosa* occurs along the forest edge. Shrub canopy closure is 0.3, occasionally reaching 0.4.

The spring synusia includes *Ficaria verna* and, in more shaded sites, occasional *Corydalis solida*, *C. cava*, *C. marschalliana*, *Gagea lutea*, *G. minima*, *Viola odorata*, *V. hirta*, *Stellaria holostea*, *Iris pumila*. The summer synusia is represented mainly by *Dactylis glomerata*, *Poa nemoralis*, *Melica picta*, and *Brachypodium sylvaticum*, with occasional *Carex praecox*, *Elytrigia intermedia*, *Asparagus officinalis*.

The forest site conditions are classified as CL₁ (dryish, mesoxerophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

The slightly fresh field maple-oak forest with wild pear and *Stellaria holostea* (TA4) occupies the middle section of the north-facing slope.

The mature pedunculate oak trees are of vegetative origin. They are 95–100 years old.

Semi-open stands have developed here. The average canopy closure is 0.60–0.65. The stand is assigned to aesthetic class 2, pedestrian accessibility class 3, and stability class 2, and is at the first stage of recreational digression.

The stand is sufficiently stable and in good physiological condition. Damage is minor, the tree crowns are generally well developed.

The species composition of the shrub layer is practically the same as in TA3; however, its canopy closure averages 0.4 and reaches 0.5 in some parts of the trial area.



A

B

Fig. 10. *Ornithogalum boucheanum* (A) in the slightly fresh field maple–oak forest and *Salvia nemorosa* in the steppe patches of the tract

Source: developed by the authors

The main species of the spring synusia include *Corydalis solida*, *Anemone ranunculoides*, *Ficaria verna*, *Gagea pusilla*, *Viola odorata*, *Stellaria holostea*, *Ornithogalum boucheanum* (Fig. 10, A). The summer synusia is represented mainly by *Stellaria holostea*, *Melica picta*, *Poa nemoralis*, *Brachypodium sylvaticum*, *Geum urbanum*, *Carex muricata*, *Galium aparine*, *Agrimonia eupatoria*, *Hypericum perforatum*, *Campanula rapunculoides*, *Viola hirta*, *Anthriscus sylvestris*, *Chaerophyllum temulum*, *Ballota nigra*, *Alliaria petiolata*. In open, steppe-like habitat, *Salvia nemorosa* occurs (Fig. 10, B).

The forest site conditions are classified as CL₁₋₂ (slightly fresh, xeromesophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

3. Characteristics of oak forest phytocoenoses in the Left-Bank tracts

3.1. “Yatsevo”

The fresh linden–field maple–oak forest with elm and *Stellaria holostea* (TA1) is located in the lower part of the north-facing slope and in the thalweg.

Some pedunculate oak trees within the trial area are about 100 years old. The mensurational characteristics of this species are presented in Table 7. The characteristics of the associated tree species in this ravine forest are given in Table 8.

Table 7

Mensurational characteristics of the dominant species *Quercus robur* on trial areas in the “Yatsevo” tract

Parameters		Phytocoenoses		
		TA1	TA2	TA3
FST		CL ₂	CL ₁	CL ₂
Composition		7Qr2Ac1Tc+Um	10Qr	7Qr2Ac1Tc
Average	<i>H</i> , m	20.7	19.1	22.5
	<i>D</i> , cm	36.9	34.0	41.5
<i>N</i> , pcs/ha		98	130	97
<i>M</i> , m ³ /ha		99.3	107.1	138.5
Bonitet		IV	IV	III

Note: tree species abbreviations as in Table 3 and 5

Figure 11 shows pedunculate oaks within the trial area, including a multi-stemmed individual.

The trees are relatively sparsely distributed, forming semi-open stands. Small glades occur in some places. The average canopy closure is 0.60–0.65. The stand is assigned to aesthetic class 2, pedestrian accessibility class 3, and stability class 2, and is at the first stage of recreational digression. The stand is in good condition, with well-developed trees and only a small proportion showing damage caused by insects or diseases (up to 5 % of all trees within the trial area).

Table 8

Mensurational characteristics of associated tree species on trial areas in the “Yatsevo” and “Dibrova” tracts

TAs	FST	Associated tree species	Average		N, pcs/ha	M, m³/ha
			H, m	D, cm		
“Yatsevo” tract						
TA1	CL₂	Ac	18.5	29.7	48	28.4
		Tc	19.8	27.6	25	14.1
		Um	20.2	27.3	1	0.2
TA3	CL₂	Ac	20.0	38.1	38	39.5
		Tc	20.7	42.1	15	19.8
“Dibrova” tract						
TA	CL₁,₂	Ac	13.9	27.8	30	12.3

Notes: Ac – Acer campestre; Tc – Tilia cordata; Um – Ulmus minor

The shrub layer consists mainly of *Frangula alnus* and *Euonymus europaeus*, while patches of *Prunus spinosa* occur along the forest edge. The shrub canopy closure is 0.6.

The characteristic herbaceous species of the spring synusia in this type of oak forest include *Corydalis solida*, *C. cava*, locally forming patches of *Anemone nemorosa*, *Ficaria verna*, *Gagea lutea*, *G. minima*, *Viola mirabilis*, *V. odorata*, and occasional *Pulmonaria obscura*. The summer synusia is dominated by *Stellaria holostea*, *Melica nutans*, *Pulmonaria obscura*, *Viola mirabilis*, with occasional *Geum urbanum*. Along the forest edge, *Alliaria petiolata* occurs, while the lower, more humid part of the site is characterized by *Asarum europaeum*, *Aegopodium podagraria*, *Glechoma hederacea*, and, locally, *Carex pilosa* and *Polygonatum multiflorum*.

The forest site conditions – CL₂ (fresh, mesophilic). Moisture regime is atmospheric-transitional.



Fig. 11. Pedunculate oak trees within the trial area (A) and a multi-stemmed oak tree (B)

Source: developed by the authors

The dryish oak forest with *Carex* (TA2) is located on the middle part of the south-facing slope and is represented by a pure pedunculate oak stand. The average age of the oak trees is 95–98 years. Semi-open stands have developed

within the trial area. The average canopy closure is 0.55–0.60. The stand is assigned to aesthetic class 2, pedestrian accessibility class 3, stability class 2, is at the first stage of recreational digression.

The shrub layer consists of *Crataegus monogyna*, *Rosa canina*, *Euonymus verrucosa*, while patches of *Prunus spinosa* occur locally along the forest edge.

The herbaceous species are typical of this forest type. The spring synusia is represented by early-flowering spring ephemeroids and nemoral species, whereas the summer synusia is dominated by mesoxerophytic and forest-edge species characteristic of the middle parts of south-facing slopes.

The forest site conditions are classified as CL₁ (dryish, mesoxerophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

The fresh linden–field maple–oak forest with *Carex pilosa* (TA3) occurs in the lower part of the north-facing slope. Some pedunculate oak trees are nearly 100 years old, while the average age is 90 years. The mensurational characteristics of the dominant tree species are presented in Table 7, and those of the associated tree species are given in Table 8. The trial area is characterized by a horizontally closed stand, with an average canopy closure of 0.70–0.75. The stand is assigned to aesthetic class 2, pedestrian accessibility class 3, stability class 2, is at the first stage of recreational digression. A few standing dead pedunculate oak trees occur near the trial area.

The shrub layer is composed of *Euonymus europaeus*, *E. verrucosus*, *Crataegus monogyna*, *Rosa canina*, *Sambucus nigra*, *Frangula alnus*, with occasional *Swida sanguinea*. The shrub canopy closure is 0.60–0.65.

The most characteristic herbaceous species of this type of oak forest are *Carex pilosa*, *Stellaria holostea*, *Asarum europaeum*, *Aegopodium podagraria*. The species composition of the herbaceous layer is similar to that recorded in TA1.

The forest site conditions – CL₂ (fresh, mesophilic). Moisture regime is atmospheric-transitional.

We investigated the mensurational characteristics of *Quercus robur* and calculated the vitality indices of its stands in the natural oak forests located in the mouth and middle sections of the Yatsevo tract¹². The stand vitality index (Ln) indicates that the stands in the thalweg, both in the mouth and in the middle section of the ravine, are healthy, with values of 86.3 and 84.3, respectively. In contrast, the stand in the upper part of the slope is somewhat weakened, with an Ln value of 76.7. The analysis of the mensurational characteristics and vitality of the oak phytocoenoses indicates that more hygrophilous site conditions are

¹² Бессонова В. П., Яковлева-Носарь С. О. Таксаційні показники та життєвий стан *Quercus robur* L. за різних лісорослинних умов гирла та середньої частини урочища Яцево (Дніпропетровська область). *Питання степового лісознавства та лісової рекультивації земель*. 2023. Т. 52. С. 3–17.

more favorable for the development of pedunculate oak stands than xerophilous conditions.

During the same period, the study in this ravine was expanded by establishing additional trial areas in the southern side ravine of the tract¹³. The mensurational characteristics of *Quercus robur* and the associated tree species were analyzed under hygromesophilous, xeromesophilous, and xerophilous conditions, corresponding to the distribution of the oak forests along the slope elevation gradient. The oak phytocoenoses also differed in stand density, expressed as the number of trees per hectare. The results showed that the mensurational characteristics and vitality of the trees are influenced not only by moisture conditions but also by stand density. Thus, under xerophilous conditions, the height and basal area of pedunculate oak were greater than under xeromesophilic conditions, where the number of trees per hectare, including associated species, was excessively high. A similar pattern was observed for tree vitality. The highest values of these parameters were recorded in oak forests under hygromesophilic conditions.

3.3. “Dibrova”

The slightly fresh field maple–oak forest with *Viola mirabilis* (TA) is located in the lower part of the north-facing slope, closer to the thalweg. The oak trees are of vegetative origin, with some individuals being 90–95 years old. The forest mensurational characteristics of the dominant tree species are presented in Table 9, while those of the accompanying species, field maple, are given in Table 8. The presence of a small number of the North American introduced species, green ash, may be attributed to the dispersal of its seeds from the surrounding erosion-control artificial forest plantations established around the ravine.

Semi-open spaces have formed within the sample plot. The aesthetic value class is 2, the pedestrian accessibility class is 3, the stability class is 2, the site is at the first stage of recreational digression.

The principal representatives of the herbaceous layer are similar to those of the slightly fresh field maple–oak forest in the previously described ravine, Yatsevo tract, but with the predominance of *Viola mirabilis*, which flowers from mid-April to mid-May and subsequently remains in the vegetative stage.

The forest site conditions are classified as CL_{1,2} (slightly fresh, xeromesophilic). Moisture is supplied primarily by atmospheric-transit precipitation.

¹³ Яковлєва-Носарь С. О., Бессонова В. П. Таксаційні показники та життєвий стан *Quercus robur* L. за різних лісорослиних умов південного приярка урочища Яцево (Дніпропетровська область). *Наукові доповіді НУБіП України*. 2023. № 4(104).

Table 9

**Mensurational characteristics of the dominant species *Quercus robur*
on trial area in the “Dibrova” tract**

Parameters		Phytocoenoses
FST		TA
Composition		CL ₁₋₂
		8Qr2Ac+Fp
Ave-range	H, m	13.7
	D, cm	32.3
N, pcs/ha		92
M, m ³ /ha		48.8
Bonitet		V

Notes: Qr – *Quercus robur*; Ac – *Acer campestre*; Fp – *Fraxinus pennsylvanica*

In our previous publication¹⁴, we described a study of vegetation on seven sample plots located in the central part of the Viyskovyi ravine, which also belongs to the southern variant of ravine forests. The tract is situated within the Mykilske Forestry. It was found that the tree species composition of the stands varies depending on the forest site conditions and the orographic features of the terrain. It should be noted that the elevation difference within the ravine is 50–55 m (thalweg – 50 m a.s.l.; upper edge of the slopes – 100 m a.s.l.). This ecotopic heterogeneity promotes the formation of a considerable diversity of oak forest phytocoenoses within the ravine, differing in their floristic composition. In this ravine, the following oak forest phytocoenoses were identified: slightly fresh oak forest with *Aegopodium podagraria*; slightly fresh field maple oak forest with linden and *Aegopodium podagraria*; fresh linden–field maple oak forest with wild pear and *Aegopodium podagraria*; tatar maple–field maple oak forest with linden, wild pear and *Aegopodium podagraria*; ash–elm–field maple oak forest with *Viola suavis*; slightly fresh oak forest with *Aegopodium podagraria* and *Geranium robertianum*; and dry field maple–tatar maple oak forest with wild pear and *Agropyron pectinatum*. It was established that an optimal proportion of the dominant and accompanying tree species is an important prerequisite for the stable functioning and high productivity of forest phytocoenoses. The obtained results indicate the high conservation value of the Viyskovyi ravine and provide grounds for recommending its inclusion in the network of protected areas of Ukraine.

¹⁴ Яковлева-Носарь С. О., Бессонова В. П. Різноманіття дібровних фітоценозів основної частини байраку Військовий (Дніпропетровська область). *Сільське господарство та лісництво*. 2025, т. 36, № 2. С. 189–204.

CONCLUSIONS

1. The species composition of the dendroflora and the herbaceous plants forming the core of the spring and summer synusia was determined on trial areas in five tracts of the Dnipropetrovsk region: “Zhucha Balka”, “Domashnia Balka”, “Rezynova Balka”, “Yatsevo, and “Dibrova”. A forest inventory description of the investigated natural stands is provided, including forest site conditions, canopy closure, stand composition, and site quality class (bonitet). For the dominant and accompanying tree species, the main forest inventory characteristics were determined, including mean tree height and diameter, tree density, and stem wood volume per hectare. The aesthetic value, pedestrian accessibility, and stand stability classes, as well as the stage of recreational digression, were also determined.

2. A diversity of oak forest phytocoenoses growing under different forest site conditions was identified within the trial areas: fresh oak forest with *Aegopodium podagraria*, dryish oak forest with *Poa angustifolia*, slightly fresh oak forest with *Polygonatum odoratum*, fresh linden-ash-field maple-oak forest with *Aegopodium podagraria*, fresh Norway maple-oak forest with *Aegopodium podagraria*, slightly fresh field maple-tatar maple-oak forest with wild pear and *Poa angustifolia*, dryish tatar maple-oak forest with pear and *Carex*, dryish elm-tatar maple-oak forest with wild pear and *Dactylis glomerata*, slightly fresh field maple-oak forest with wild pear and *Stellaria holostea*, fresh linden-field maple-oak forest with elm and *Stellaria holostea*, dryish oak forest with *Carex*, fresh linden-field maple-oak forest with *Carex pilosa*, slightly fresh field maple-oak forest with *Viola mirabilis*.

3. Associated tree species are present in all studied phytocoenoses, except for the trial areas located in the “Zhucha Balka” tract and trial area 2 in the “Yatsevo” tract.

4. It was recorded on the trial areas that most pedunculate oak (*Quercus robur*) trees are 80–95 years old, with some individual specimens reaching 100 years of age. Their average height is approximately 19 m, and the average diameter is 30 cm. The stands are predominantly classified as site bonitet class IV, indicating moderate productivity of oak stands, which is typical of the forest-growing conditions of the steppe zone.

5. The vitality of oak stands is higher under conditions of better moisture availability, particularly in the thalweg and the lower part of the north-facing slope. In general, the analyzed natural oak forests are characterized by an aesthetic evaluation class of 2, a pedestrian accessibility class of 3, a stability class of 2, and the first stage of recreational digression.

6. The analysis of the floristic composition of the natural oak forest phytocoenoses in the surveyed ravines demonstrated that ravine forests in both

the right-bank and left-bank parts of the Dnipropetrovsk Region are important centers for the conservation of the natural phytodiversity of the steppe zone. Despite the long-term anthropogenic transformation of the surrounding landscapes, these forest ecosystems have retained a considerable number of characteristic nemoral species and provide habitats for many rare plant species, including those listed in regional conservation lists.

7. The species composition of the vegetation in the studied oak forests is shaped by a complex of ecological factors, among which slope aspect, slope steepness, the topographic position of the sample plots, hydrothermal regime, and soil conditions are of primary importance. The diversity of habitat types within the ravines results in the formation of a mosaic of plant communities, ranging from relatively dry oak forests on the upper parts of south-facing slopes to fresh oak forests in the lower parts of the slopes and thalwegs, thereby substantially increasing the overall floristic diversity of the ravine forests.

8. The ravine oak forests of the Dnipropetrovsk Region, located on both banks of the Dnipro River, form a unified regional complex of forest vegetation within the steppe zone. They support a high level of floristic diversity, contribute to the conservation of a wide range of ecological plant groups, and enhance the stability of forest ecosystems.

9. The obtained results confirm the important conservation role of the ravine oak forests of the Dnipropetrovsk Region as refugia of forest flora within the steppe zone. Their continued study, regular monitoring, and effective protection are essential for conserving the region's biodiversity, maintaining the ecological stability of landscapes, and preserving natural populations of native plant species.

SUMMARY

Natural oak phytocoenoses of five ravine forests located on the right and left banks of the Dnipro River were investigated. These forests belong to the Mykilske and Liubymivske Forestry of the Dnipro Forestry Enterprise. The studied tracts are ravine forests representing the southern variant of the Northern Steppe and form part of the forest-park section of the green belt surrounding the city of Dnipro.

The species composition of the dendroflora and herbaceous layer, which forms the basis of the spring and summer synusiae, was determined. Forest mensurational characteristics of the stands were assessed, including the forest site type, canopy closure, species composition, site quality class (bonitet). For the dominant and associated tree species, the main mensurational parameters were determined, including mean height, mean stem diameter, tree density, and stemwood volume per hectare. The aesthetic evaluation, pedestrian

accessibility, stability classes, and the stage of recreational digression were also established.

A variety of ecotopes are formed within the surveyed ravines, resulting in a high phytocoenotic diversity of vegetation. The investigation of the floristic composition of the natural oak phytocoenoses demonstrated the high conservation value of the ravine forests of the Dnipropetrovsk Region. These forests play an important role in preserving the phytodiversity of the steppe zone by maintaining populations of characteristic nemoral and rare plant species.

Bibliography

1. Бельгард О. Л. Лісова рослинність південного сходу України. Київський державний університет ім. Т. Г. Шевченка. Київ : Вид-во КДУ ім. Т. Г. Шевченка, 1950. 263 с.
2. Божко К. М. Еколого-біологічна характеристика північного варіанта байрачних лісів південно-східної України. *Вісник Дніпропетровського університету. Серія : Біологія. Екологія*. 2007. Вип. 15. Т. 1. С. 3–8.
3. Yakovlieva-Nosar S., Bessonova V. State of coenopopulations of *Quercus robur* L. growing in ravines located in the Dnieper river's rapids section (recreation zone of the city of Zaporizhzhya), Ukraine. *Forestry ideas*. 2021. Vol. 27, № 1 (61). P. 256–270.
4. Bessonova V., Yakovlieva-Nosar S. Comparison of transpiration activity of *Quercus robur* L. and *Acer campestre* L. trees under different conditions of moisture supply in the Viiskova ravine. *Ukrainian Journal of Forest and Wood Science*. 2024. 15(3). P. 148–164. <https://doi.org/10.31548/forest/3.2024.148>.
5. Яковлєва-Носарь С. О. Видове різноманіття дендрофлори байрачних лісів (зона рекреації міста Запоріжжя). *Науковий вісник НЛТУ України*. 2022, т. 32, № 5. С. 13–18. <https://doi.org/10.36930/40320502>.
6. Яковлєва-Носарь С. О. Рідкісні види трав'янистих рослин степових ділянок балки Генералка (острів Хортиця). *Рослини та урбанізація : матеріали XIII міжнародної науково-практичної конференції (Дніпро, 1 лютого 2024 р.)*. Дніпро, 2024. С. 93–95.
7. Яковлєва-Носарь С. О. Рідкісні види трав'янистих рослин степових схилів балки Широка (острів Хортиця). *Рослини та урбанізація : матеріали XIV міжнародної науково-практичної конференції (Дніпро, 3 лютого 2025 р.)*. Дніпро, 2025. С. 156–157.
8. Біотопи степової зони України / Ред. академік НАН України Я. П. Дідух. Київ – Чернівці : ДрукАРТ, 2020. 392 с.
9. Манюк В. В., Манюк В. В. Природно-заповідний фонд Дніпропетровщини. Дніпро, 2010. 116 с.

10. Інструктивно-методичні вказівки щодо здійснення лісовпорядкування. Державне агентство лісових ресурсів України, ВО «Укрдержліспроєкт». Ірпінь, 2024. 161 с. URL: https://lisproekt.gov.ua/fileadmin/user_upload/Instruktivno-metodichni_ukazivki-druk.pdf.

11. Гордієнко М. І., Гойчук А. Ф., Гордієнко Н. М. Штучні ліси в дібровах / ред. М. І. Гордієнко. Житомир : Полісся, 1999. 592 с.

12. Бессонова В. П., Яковлева-Носарь С. О. Таксаційні показники та життєвий стан *Quercus robur* L. за різних лісорослинних умов гирла та середньої частини урочища Яцево (Дніпропетровська область). *Питання степового лісознавства та лісової рекультивациі земель*. 2023. Т. 52. С. 3–17. <https://doi.org/10.15421/442301>.

13. Яковлева-Носарь С. О., Бессонова В. П. Таксаційні показники та життєвий стан *Quercus robur* L. за різних лісорослинних умов південного приярка урочища Яцево (Дніпропетровська область). *Наукові доповіді НУБіП України*. 2023. № 4(104). [https://doi.org/10.31548/dopovidi4\(104\).2023.012](https://doi.org/10.31548/dopovidi4(104).2023.012).

14. Яковлева-Носарь С. О., Бессонова В. П. Різноманіття дібровних фітоценозів основної частини байраку Військовий (Дніпропетровська область). *Сільське господарство та лісництво*. 2025. Т. 36, № 2. С. 189–204. <https://doi.org/10.37128/2707-5826-2025-2-17>.

Information about the authors:

Yakovlieva-Nosar Svitlana,

Candidate of Biological Sciences,

Associate Professor at the Department of Agricultural Technologies

Municipal Institution of Higher Education “Khortytsia National Educational and Rehabilitational Academy” of Zaporizhzhia Regional Council
59, Naukove Mistechko Street, Zaporizhzhia, Ukraine

Bessonova Valentina,

Doctor of Biological Sciences,

Professor at the Department of Gardening and Landscape Design

Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Izdevniecība “Baltija Publishing”
Avotu iela 8 k-1 - 25, Rīga, LV-1011
E-mail: office@baltijapublishing.lv

Iespiests tipogrāfijā SIA “Izdevniecība “Baltija Publishing”
Parakstīts iespiešanai: 2026. gada 9. oktobrī
Tirāža 300 eks.