Національний університет біоресурсів і природокористування України

БІОЛОГІЧНІ СИСТЕМИ:
Теорія та Інновації

Том 17, № 2
2026

Київ 2026

ISSN 2706-8382
e-ISSN 2706-8390

Засновник:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 13 від 21 травня 2026 р.)

Державна реєстрація: Ідентифікатор медіа R30-02292.
Рішення Національної Ради України
з питань телебачення і радіомовлення
№ 1795, протокол № 31 від 21.12.2023 р.

Журнал включено до категорії «Б» Переліку наукових фахових видань України,
відповідно до якого можуть публікуватися результати дисертаційних робіт на здобуття наукових ступенів доктора та кандидата біологічних наук зі спеціальностей
(наказ Міністерства освіти і науки України № 409 від 6 березня 2020 року):
091 – Біологія; 101 – Екологія; 162 – Біотехнології та біоінженерія; 202 – Захист і карантин рослин

Журнал представлено у міжнародних наукометричних базах даних, репозитаріях та пошукових системах:
Open Ukrainian Citation Index, University of Hull Library, University of Oslo Library, WorldCat, Crossref, Національна бібліотека України імені В.І. Вернадського, BASE, EBSCO, Google Академія, Ulrichsweb Global Serials Directory, UCSB Library, Dimensions, Litmaps, AGRIS, CABI

Адреса редакції:
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 13, м. Київ, Україна
E-mail: bs@bioscience.com.ua
https://bioscience.com.ua/uk

National University of Life and Environmental Sciences of Ukraine

BIOLOGICAL SYSTEMS:
Theory and Innovation

Vol. 17, No. 2
2026

Kyiv 2026

ISSN 2706-8382
e-ISSN 2706-8390

Founder:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 13 of May 21, 2026)

State registration: Media identifier R30-02292.
Decision of the National Council of Television
and Radio Broadcasting of Ukraine
No. 1795, Minutes No. 31, dated 21.12.2023.

The journal is included in category "B" of the List of scientific professional publications of Ukraine, in which the results of dissertations for the degree of doctor and candidate of biological sciences can be published in the following specialties
(Order of the Ministry of Education and Science of Ukraine No. 409 dated 06.03.2020):
0511 – Biology; 0521 – Environmental sciences; 0588 – Interdisciplinary programmes and qualifications involving natural sciences, mathematics and statistics; 0712 – Environmental protection technology

The journal is presented in the international scientometric databases, repositories and scientific systems:
Open Ukrainian Citation Index, University of Hull Library, University of Oslo Library, WorldCat, Crossref, Vernadsky National Library of Ukraine, BASE, EBSCO, Google Scholar, Ulrichsweb Global Serials Directory, UCSB Library, Dimensions, Litmaps, AGRIS, CABI

Editors office address:
National University of Life and Environmental Sciences of Ukraine
03041, 13 Heroiv Oborony Str., Kyiv, Ukraine
E-mail: bs@bioscience.com.ua
https://bioscience.com.ua/en

Редакційна колегія

Kowiyou Yessoufou	PhD in Botany, Professor, University of Johannesburg, South Africa
Andrii Gryganskyi	PhD in Biology, AeroVironment Inc., USA
Volodymyr Radchuk	PhD, Senior Researcher, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
Eva Szkwarek	Doctor of Biological Sciences, Associate Professor, Maria Skłodowska-Curie University of Lublin, Poland

Зміст/Contents

Received: 20.12.2025 Revised: 18.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 10-30

UDC 628.472:504.5

DOI: 10.31548/biologiya/2.2026.10

Unauthorised landfills as a source of heavy metals: The influence of waste morphological composition

Serhii Leliushok*
Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0009-0002-8576-8950

Olena Naumovska
PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
Scientific Research Department of the Park-Monument of Landscape Art
of the National Importance "Mezhyhiria"
07354, 19 Ivan Franko Str., Novi Petrivtsi Village, Ukraine
https://orcid.org/0000-0002-5938-8471

Abstract. Against the background of an increasing number of local uncontrolled landfills in Ukraine, the issue of their impact on the state of soils has become especially urgent, due to the high mobility of toxic elements and their long-term preservation in the environment. This study aimed to investigate the connection between the morphological composition of waste on unauthorised landfills and the degree of heavy metal content in soils of technogenically transformed territories. The standardised technique of sampling of municipal solid waste and soils was used, in accordance with national standards of Ukraine and international standards. Determination of the morphological composition of municipal solid waste was carried out by fractional sorting into the following morphological fractions: plastics, paper and cardboard, glass, metal, organic, textiles, building materials, and other mixed fractions. Soil sampling was carried out at a depth of 0-20 cm, where the maximum accumulation of anthropogenic metals takes place. Determination of the concentration of Cd2+, Cu2+, and Zn2+ was carried out by atomic absorption spectrometry, which provided a high accuracy of quantitative analysis. Statistical processing of the results consisted of correlation analysis, determination of the coefficients of determination and creation of regression models to establish the relationship between the share of separate morphological fractions and the degree of soil pollution. Additionally, the environmental risk was estimated by calculating the accumulation index, the contamination factor and the modified potential ecological risk index. The obtained results testified to the statistically significant relationship between the increased concentrations of heavy metals in the soil cover and the presence in the waste stream of such morphological fractions, as metal scrap, fine-dispersed plastics, electronic and electrical waste, building materials with paints, varnishes and other mixtures based on polymers. The highest mobility to soils was shown by Pb2+ and Cd2+, which correlated with the morphological fractions that included used batteries, small metal items and electrical and electronic

Suggested Citation:
Leliushok, S., & Naumovska, O. (2026). Unauthorised landfills as a source of heavy metals: The influence of waste morphological composition. *Biological Systems: Theory and Innovation*, 17(2), 10-30. doi: 10.31548/biologiya/2.2026.10.

*Corresponding author (sergei.lelyushok@gmail.com)

waste. The organic morphological fraction is not a direct source of heavy metals; however, it contributes to their mobilisation during the mineralisation process and formation of organic acids

Keywords: concentration factor; pollution index; mobile forms of elements; adsorption; leachates

INTRODUCTION

Illegal dumping is one of the most serious environmental problems in today's urbanised society. The environmental risk posed by illegal dumping sites is not only due to the stock of waste but also to the absence of artificial impermeable layers, leachate collection systems and monitoring systems, which allows the interaction of the waste with the soil profile and the hydrological network. In urban and periurban environments, the dumping sites are frequently located in micro depressions, gullies, roadsides and along the boundaries of agrocenoses, that is, areas that receive surface run-off and occasional inundation, which facilitates the flow of pollutants to the soil and water bodies. This implies that the environmental risk does not stop at the spatial limits of the dump, but extends to other areas with different land uses. In the physical disintegration of waste and biodegradation of the organic fraction, and after rainfall, the leachates are generated. Leachates can be enriched with heavy metal soluble and colloidal fractions and may flow through the soil and into the groundwater. This mechanism transfers contaminants from the waste to the environment, expanding the spatial scale of the risk and the complexity of remediation actions.

W. Ahmad *et al*. (2021) pointed out that soil pollution in the surrounding areas of dumping sites usually shows higher concentrations compared to the background values and poses higher environmental and health risks. Recent environmental studies have evidenced that the movement of leachate is one of the key sources of metal pollution in the soil and water of dumping sites. Thus, J.K. Drall *et al*. (2025) in an integrated study on an uncontrolled dumping site (soil and groundwater study and application of pollution indexes) concluded that the mobility of leachate components causes metal pollution in the environmental components and suggests the need for control of infiltration, monitoring and minimising the dispersal of leachates. Notably, the influence of leachate is not limited to abiotic compartments. J. Chen *et al*. (2025) studied the environmental impacts of illegal dumping (slag and sewage sludge) and found that heavy metal pollution was associated with significant changes in the structure of the bacterial soil community, such as a decrease in bacterial diversity and changes in the relative abundance of the bacterial groups. The authors indicated that the illegal dumping acts as a disturbance factor of the soil microbiome and consequently could affect the soil processes of matter transformation.

The morphological composition of municipal solid waste (MSW), the percentage distribution of organic matter, polymeric materials, metals, glass, textiles, and construction debris, determines not only the physical properties of a landfill (such as porosity and permeability) but also the chemical processes occurring within it. For example, the predominance of the polymer fraction promotes the prolonged persistence of organic and inert components, while also potentially enhancing the accumulation of metallic impurities through adsorption and the mechanical release of fillers and additives from plastics. Conversely, a substantial proportion of batteries, cables, printed circuit boards, and metal fragments directly increases the risk of Pb^{2+} and Pb^{2+} ions entering soils and leachate. I. Hölzle *et al*. (2022) concluded that environmental control is a critical factor in the risk assessment and planning of landfill management measures. Q. Wang *et al*. (2021) found that the morphological composition of waste is closely related to the species and contents of elements that are likely to be dissolved or extracted. A. Podlasek *et al*. (2023), discussing the pollution potential of MSW landfill leachate (case studies and worldwide overview), stated that leachate properties are influenced by the percolation of atmospheric precipitation through the waste mass and the set of physicochemical and biological processes occurring in the landfill body. With regard to morphological composition, they pointed out the large variability of leachate and the need to take into account the context of waste formation and transformation conditions when evaluating environmental risk. Another issue is the chemical bioavailability and mobility of metals in the soil, which depend on multiple factors, such as pH, content of organic matter, sorption and desorption processes, redox conditions, and the presence of complexing agents. The morphological structure of waste indirectly affects these factors. For example, the organic component accelerates the formation of low-molecular-weight organic acids during decomposition, which increases the solubility and mobility of some metals. The polymeric component can serve as a matrix for the adsorption or dispersion of particles with metallic impurities. Therefore, the correct interpretation of the content of metals in soils requires simultaneous analysis of both the morphological structure of waste and soil properties, as indicated by K. Godyń *et al*. (2023).

This article aimed to investigate the effect of the morphological composition of unauthorised landfills on the accumulation of heavy metals (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}) in the upper soil horizon of technogenically impacted areas, to reveal statistically significant correlations, and to develop practical recommendations for monitoring and remediation. The scientific novelty of the research is the mandatory consideration of the actual morphological structure of waste as an independent factor in the interpretation of soil properties, while in previous studies, this issue was considered separately or only partially. The proposed approach is of practical importance for regional waste management programmes and ecologically optimal planning of remediation activities.

MATERIALS AND METHODS

The research was carried out on two landfill sites in different physiographic zones of Ukraine, which made it possible to comprehensively study the regional features of anthropogenic pressure on the soil environment. Site 1 was placed in Nizhyn district, Bakhmach territorial community, Chernihiv region (51°09'45"N, 32°42'14"E) at a distance of 17 km from the town of Bakhmach. The area of technogenically disturbed land is 2.25 ha. Soil samples were collected twice: in spring 2025 (the third ten-day period of April) and again in summer 2025 (the third ten-day period of August), which permitted evaluation of the seasonal changes in soil ecological state. Site 2 was placed in Uman district, KniazhoKrynytsia territorial community, Cherkasy region (49°06'22"N, 29°44"18"E), 4 km from the village of Kniazha Krynytsia. The landfill area was 0.2 ha. The soil samples collection was done in the same order and terms as at Site 1: in spring 2025 (the third ten-day period of April) and repeated in summer 2025 (the third ten-day period of August), which allowed comparison of spatial and temporal transformations of soil properties. Both sites are characterised by chernozem soils. According to regional features, in Nizhyn district, chernozems prevail (together with marsh soils in the floodplains), whereas in Cherkasy region, typical chernozems prevail (together with podzolised and dark grey podzolised soils), with typical and degraded chernozems being a significant part of the regional soil cover. Climatic characteristics of the regions are presented according to the data of Climate-Data.org (n.d.).

Site 1:

- climate type: humid continental with warm summers (Dfb according to the Köppen-Geiger classification);
- mean annual air temperature: approximately 8.3°C;
- mean annual precipitation: approximately 671 mm/year; for Nizhyn district, a range of 550-660 mm/year is reported, with a maximum during the warm season.

Site 2:

- climate type: humid continental with warm summers (Dfb);
- mean annual air temperature: 9.6°C;
- mean annual precipitation: approximately 618 mm/year.

Land use of the area surrounding Site 1 is characterised by a mixed economy structure with agriculture being a leading component and forest and shelterbelt components. The district is also characterised as a district where the leading sectors are agricultural production and forestry (Baranovska *et al*., 2000). Therefore, the surrounding areas are classified as agricultural landscapes (arable land/fields) with elements of forest vegetation and floodplain areas. Cherkasy region is characterised by a very high degree of land cultivation. According to the data of R. Podzerei (2024), in some regions of Ukraine, among them the Cherkasy region, the ploughed lands' share may be about 90%. Thus, for areas surrounding Site 2, intensive agricultural (arable land) use is the prevailing land use type, which enhances the relevance of production soils risk evaluation. Rationale for the selection of these sites and regions:

1. Representativeness of two different natural and climatic situations within the forest-steppe/transitional zone of Ukraine: Chernihiv region is characterised by a lower mean annual temperature and (according to reference data) higher or comparable precipitation in comparison with Uman district. This results in different soil moisture regimes and different conditions for contaminants migration.

2. A common soil basis, chernozem soils, was preserved at both sites, but a higher bulk density is observed at Site 2, which allows comparing the possible effects of compaction and permeability on contaminants migration without changing the soil type.

3. Types of anthropogenic land use: Site 1 is agricultural, with a significant proportion of forest and floodplain areas in the district; Site 2 has a very high proportion of cultivated land and intensive agricultural land use.

4. The similar sampling scheme (same seasons and sampling dates: third ten-day periods of April and August 2025) guarantees the comparability of the sites in terms of seasonal changes in the region (climate, land use), without any methodological differences.

Waste samples were collected from each landfill. The optimal mass of a single sample was 7 kg of dry or mixed waste. The selected sample sizes provide a representative sample of the total mass of waste, taking into account the diversity of fractions. The samples were selected manually, that is, the fractions were separated based on visual and tactile assessment, type of material, structure, density, type of packaging and other characteristics. Three 7 kg composite samples were collected at each Site 1 and Site 2. To reduce methodological error and the effect of point waste accumulation, the sampling was carried out at several points, spread over the area of the landfill body. Randomisation was provided as follows:

1. Before sampling, the visible surface of the landfill was divided into three conditional sectors;

2. One sample was taken from each sector;

3. Samples were not taken from obviously anomalous places (for example, recent local dumping of a single fraction, separate accumulations of construction waste, large objects).

To maintain representativeness at the stage of sample preparation for sorting and laboratory analysis, the quartering method was used:

1. The 7 kg composite sample was placed on a clean sheet or tray and mixed as much as possible for the most uniform distribution of fractions;

2. A conical pile of waste was formed, levelled to a uniform thickness and divided into four equal sectors;

3. Two opposite quarters were removed, and the remaining two were combined.

The sample was sorted into standard fractions by hand; each fraction was weighed, and its proportion (%) of the total sample mass was determined. No waste transportation was carried out, since the morphological composition was determined directly in the field on the day of sampling. In this research, the characterisation of solid waste flows in the study areas was carried out by analysing the morphological composition of the samples taken, identifying the main components that make up the material composition of the waste. The fractions were determined as follows: paper and cardboard, iron, rubber waste, organic, plastic waste, textiles, glass, wood, and polyethene. This morphological classification system allows the analysis of both the material parts of interest (paper, plastics, glass, metals) and the parts that influence the intensity of biological processes and the risk to the environment (organic matter, rubber, textiles, wood). The obtained data on the composition of the wastes were used as the basis for the study of the seasonal dynamics and interpretation of the patterns of formation of technogenic pressure, in particular, for the accumulation of heavy metals in the surrounding soils. The following waste components were distinguished: polymeric materials (plastics), rubber, organic fraction, paper and cardboard, wood, as well as inert fractions, including glass. The classification is in accordance with ASTM D5231-92 (2016). After manual sorting, each fraction was weighed separately, and the mass percentage of each fraction to the mass of the total sample was calculated:

$$\%\,\text{fraction} = \frac{m_{\text{fraction}}}{m_{\text{total sample}}} \times 100\%, \qquad (1)$$

where m_{fraction} is the mass of the fraction after sorting, is the mass of the total sample.

Before weighing, the samples were dried (or their humidity was standardised) in order to prevent the distortion of the results due to humidity differences, especially in the organic fraction. The soils were sampled in technogenically transformed areas adjacent to the unauthorised landfills at a depth of 5-20 cm, corresponding to the root zone and the layer most intensively affected by pollutants. The spatial scheme of sampling was determined by the morphology of the landfill, relief, and the presumed direction of contaminant spread. Three directionally oriented profiles were chosen for each site to ensure representativeness and trace the gradient of pollution. At Site 1, samples were collected in the north, north-east, and south-east directions. Three point-like samples were taken in each direction at a distance of 5 m from the body of the landfill, and then merged into a combined sample. The same scheme was applied at Site 2, where the sampling was performed in the west, north, and south directions; three pointlike samples were taken in each direction at a distance of 5 m. A control (background) point was chosen at a distance of about 200 m from the landfill boundary in an area not affected by direct technogenic impact. The control site is represented by the absence of economic activity signs, which makes it possible to use these samples for comparative analysis. The statistical processing of the data was performed in Microsoft Excel 2019 using the built-in functions and tools of descriptive statistics. Descriptive statistics for each variable, including mean, minimum, maximum and standard deviation, were calculated. Spearman's rank correlation was used to determine the correlation between heavy metal concentration and morphological composition of the waste. Differences and correlation coefficients were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Site 1 is a technogenically transformed territory with a long history of intensive anthropogenic impact. The site was formed due to an uncontrolled deposition of MSW of diverse morphological composition. The morphological structure of the landfill surface is represented by anthropogenic relief of mounds and local depressions filled with leachate. The natural water regime is broken, and the infiltration of atmospheric precipitation is disturbed, causing the formation of anaerobic conditions in the soil profile. Main waste fractions are represented by polymers, polyethene, organic residues, glass, paper, wood, rubber and metal, which indicates the complex nature of the pollution. Soil cover within the landfill and in the adjacent area is subject to complex degradation caused by the migration of leachate and the accumulation of heavy metals (Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}), organic acids, ammonium nitrogen, phenolic compounds and polymeric substances. According to U. Wydro *et al*. (2022), the leachate of landfills significantly changes the physical, chemical, biological and biochemical properties of soils, which manifests itself in the transformation of the microbial structure and changes in the enzymatic activity of soils. Similar processes were studied by M. Malovanyy *et al*. (2023) and H. Kachmar *et al.* (2023), who highlighted the importance of leachate as a factor for the spread of pollutants and soil degradation of adjacent shelterbelts.

The vegetation cover on the landfill is characterised by fragmentation and mosaic distribution. Within the landfill body, vegetation is virtually absent, whereas on its periphery, zones of secondary vegetation develop. These are mainly represented by grass species of the *Poaceae* family, as well as ruderal and invasive species from the *Asteraceae*, *Amaranthaceae*, and *Chenopodiaceae* families. Dominant species are represented by scentless mayweed (*Tripleurospermum inodorum*), common ragweed (*Ambrosia artemisiifolia*), dock (*Rumex* spp.), stinging nettle (*Urtica dioica*), creeping thistle (*Cirsium arvense*), couch grass (*Elytrigia repens*), and common reed (*Phragmites australis*). The listed species indicate the initial stages of secondary succession and the high ecological plasticity of the flora under conditions of toxic and physical stress. Grasses and some ruderal species have a dual ecological function; on the one hand, they ensure partial stabilisation of the soil surface, reducing the risk of erosion, and on the other, they are actively involved in the bioaccumulation of heavy metals in the root system and aboveground mass. This is in line with the research of L. Štofejová *et al*. (2021) and M.T. Samadi *et al.* (2024), who showed the high potential of couch grass and common reed for metal phytostabilisation. Data of A. Cakaj *et al.* (2023) about the role of representatives of the families *Poaceae* and *Asteraceae* as bioindicators of degraded soils are also confirmed. A particular environmental threat is posed by the presence, in the immediate vicinity of the landfill, of agrocenoses, in particular soybean (*Glycine max*) and wheat (*Triticum* L.) crops. As shown in the studies of X. Liu *et al.* (2024) and S. Razanov *et al.* (2022), these crops are capable of accumulating heavy metals in their biomass, thereby creating a risk of their entry into the food chain. The dichotomy between natural ruderal vegetation of the landfill and agrogenic soils calls for a comprehensive approach to environmental status assessment. Therefore, Site 1 is a point of multifactor anthropogenic pollution affecting soil, biota, surface and ground water, and atmospheric air simultaneously. The anthropogenic relief formation, soil cover degradation, microbiological activity inhibition and a peculiar ruderal flora development give evidence for drastic changes in natural processes. The data obtained prove the relevance of this site as a reference zone of maximal anthropogenic impact for further soil-ecological and microbiological investigations.

Site 2 is a technogenically transformed territory demonstrating persistent manifestations of intensive anthropogenic impact, which developed as a consequence of long-term uncontrolled storage of household, construction, polymeric, organic, as well as hazardous and medical waste. The morphological heterogeneity of waste and the absence of any engineering infrastructure have led to the formation of a multi-component focus of chronic environmental risk. The landfill surface has a disturbed structure and anthropogenic microrelief with mounds, compacted areas, charred soil and ash layers. Periodic waste burning is one of the major factors that induce changes in the soil cover. The thermal impact causes the destruction of the structural-aggregate state of the soil, humus horizon degradation and organic matter destruction. According to G. Pathak *et al*. (2024), combustion of mixed municipal and polymeric waste results in ash, aerosol and leachate formation with an increased content of heavy metals, polycyclic aromatic hydrocarbons, dioxins and furans, which predominantly accumulate in the surface soil horizon. The soil environment of the landfill is characterised by high variability of chemical composition caused by mechanical contamination, pyrogenic loading and infiltration of waste decomposition products.

The accumulation of heavy metal cations (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}) and organic toxicants has been registered, which may cause secondary pollution of the soil-water system. As was underlined by J. Szulc *et al*. (2024), uncontrolled landfills are persistent sources of metals, dust and potentially toxic compounds capable of long-term soil retention. The microbiological status of the landfill soil is characterised by signs of imbalance: inhibition of mesophilic and cellulolytic microflora, decrease in enzymatic activity and increase in the share of micromycetes and bacteria tolerant to toxic and thermal stress. The changes in the balance of the main trophic groups of microorganisms indicate loss of the soil system's capacity for self-regulation, which agrees with the data by M. Zhan *et al.* (2025) on α-diversity decrease of microbiota under conditions of metal and organic stress. The vegetation cover of the landfill is undergoing a profound transformation. In areas of direct waste accumulation, an almost complete degradation of the soil-vegetation complex is observed. The peripheral part is presented with fragmented ruderal and synanthropic coenoses dominated by species of the *Asteraceae* and *Amaranthaceae* families and invasive species. The prevalence of common ragweed (*Ambrosia artemisiifolia*), stinging nettle (*Urtica dioica*), burdock (*Arctium* spp.), and mallow (*Malva* spp.) indicates the involvement of degraded and technogenically affected ecotopes. Woody and shrub vegetation has signs of chronic stress (partial necrosis of crowns, decrease of assimilating surface, necrotic damage of leaves). Ecological danger is presented by the presence of animal biological and medical waste, which, in the course of decomposition, contributes to the formation of toxic gaseous products (ammonia, hydrogen sulphide, phenolic compounds) and the development of pathogenic microflora. The combination of organic, polymeric and hazardous waste creates a long-term toxicological and ecological tension, which complicates the natural process of rehabilitation. Thus, Site 2 functions as a pyrogenic-technogenic pollution focus, where mechanical soil degradation, chemical pollution, thermal influence and microbiological destabilisation are combined. Their interactions lead to a deep transformation of the soil-plant system, a decrease in the ecological stability of the territory and the appearance of potential threats to the adjacent agroecosystems and natural ecosystems.

The analysis of morphological (fractional) composition of MSW is the basis for understanding both the potential environmental impact and the prospects for further disposal, recycling or neutralisation. The morphological composition characterises which of the fractions is dominated (organic, polymeric, papercardboard, glass, metal, textile, construction, hazardous and others) and what is the mass proportion of each of them in the total mass of waste. This is important when studying the pollution of soils, since different fractions have different release rates of potentially toxic compounds (heavy metals, organic compounds), different decomposition abilities, filtration and formation of leachate. For the representativeness and standardisation of the results, it is necessary to plan the sampling, sorting, fraction separation and calculation of mass. Particular attention should be paid to the mass of the sample, the number of repetitions, representativeness, weighing and documentation. The morphological composition of the waste is the initial characteristic, which determines the potential for the formation of leachate and the conditions for the migration of pollutants. Thus, polymeric and rubber components can serve for a long time inside the landfill body as a medium for the accumulation of impurities, and the organic component contributes to the activation of microbiological processes and the change in physicochemical parameters (pH conditions, solubility of compounds). Glass, along with paper and cardboard and wood, demonstrates the potential of waste as a resource and the different rates of processing of the various components over time. Overall, this allows for a more justified association between the composition of waste and the spatio-temporal features of soil pollution and clarifies the causes of high concentrations of heavy metals in territories affected by these facilities.

In the framework of the study, the morphological composition of waste in Site 1 was investigated for the spring season of 2025 in order to establish the structure of the main components and their proportion in the total waste flow. This made it possible to determine which components predominate in the waste and what proportion of the materials can be recycled as secondary raw materials or need separate processing. The data obtained are necessary for the environmental evaluation of the level of anthropogenic load, for the prediction of possible environmental risks, and for the justification of priority measures of waste management. The results of the morphological analysis serve as a basis for comparison of seasonal dynamics and for the development of recommendations for the optimisation of sorting complexes and reduction of the volume of burial. Figure 1 shows the morphological composition of the waste at Site 1.

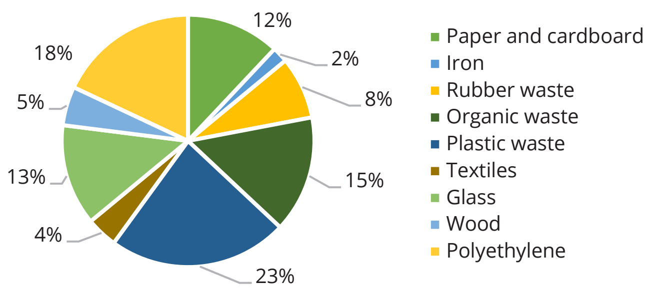

Figure 1. Average morphological composition of collected waste samples at Site 1, %, spring 2025
Source: developed by the authors

The composition of waste presented in Figure 1 is characterised by the dominance of polymeric and packaging fractions. The greatest part is represented by plastic waste (23%), which determines the general structural pattern of the waste. A significant part is also played by polyethene (18%), while the proportion of organic waste is 15%. A significant role is played by glass (13%) and paper and cardboard (12%), which, together with the aforementioned fractions, make up a significant part of the total waste. The sum of the polymeric components (plastic waste 23% + polyethylene 18%)

is 41% and accounts for almost half of the entire morphological composition. This indicates a prevalence of materials typical of packaging and having a long persistence in the environment. The significant proportion of paper and cardboard (12%), along with the polymeric fractions, also indicates the significant role of packaging materials in the structure of the waste. At the same time, the presence of an organic fraction (15%) indicates a significant contribution of biogenic components to the structure of the waste in this period. The least significant are the fractions that make up the secondary component of the morphological composition. These include rubber waste (8%), wood (5%), textiles (4%), and iron (2%). Overall, these components account for 19% of the structure and, therefore, contribute less than the first component. However, these are necessary to evaluate the diversity of the material composition and the potential complexity of the waste management. The lowest percentage of iron (2%) denotes the lowest presence of the metallic fraction in the waste in the spring of 2025 at this site. In the summer of 2025, the morphological composition of the waste in Site 1 was also determined, allowing the characterisation of the real distribution of fractions in the collected samples for this period. Figure 2 shows the morphological composition of the waste at Site 1.

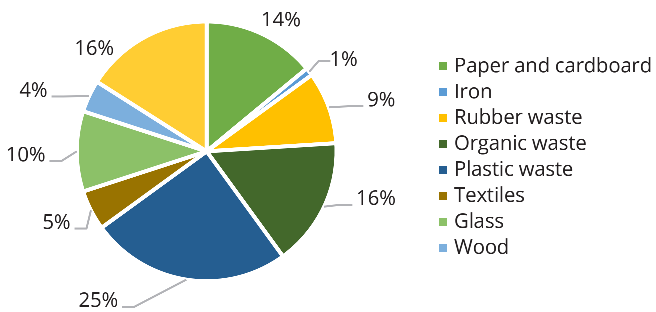

Figure 2. Average morphological composition of collected waste samples at Site 1, %, summer 2025
Source: developed by the authors

The morphological composition of the collected samples of waste in Site 1 (summer 2025) is marked by the prevalence of the polymeric fraction. The highest percentages are of plastic waste (25%) and polyethene (16%), which together represent 41% of the total composition. A large percentage is also occupied by the organic waste (16%), which constitutes a distinct and significant biodegradable fraction. The paper and cardboard (14%) also have a high expression and are part of the main structural fractions. Among the other components, fractions of inert and anthropogenic materials are found. The glass represents 10%, which indicates a stable presence of the mineral (inert) fraction in the samples. The rubber waste represents 9% and constitutes a moderate percentage of materials with slow decomposition, and that require specific management approaches. Lower percentages are registered for the textiles (5%) and wood (4%), which together represent 9% of the morphological composition. The lowest fraction in the summer structure is iron (1%), which indicates the lowest expression of the metallic fraction. In general, the percentages of paper and cardboard (14%), glass (10%), iron (1%), plastic waste (25%), and polyethene (16%) together add 66% and characterise the waste with an expressive presence of components with the potential to be recovered as secondary raw materials. The organic fraction (16%) is highlighted as a significant fraction that requires specific management within the waste management systems. The remaining components, rubber (9%), textiles (5%) and wood (4%) complete the general profile and denote the heterogeneity of the morphological composition in the summer season. In the spring of 2025, the morphological composition of the waste in Site 1 was marked by a high percentage of polymers, since plastic waste and polyethene represented 23% and 18%, respectively (totalising 41%). In the summer of 2025, the total percentage of the polymeric fraction remained the same (41%); however, an internal redistribution was registered, with the percentage of the plastic fraction increasing to 25% (+2%) and the polyethene fraction decreasing to 16% (-2%). The percentage of the organic fraction increased slightly from 15% to 16% (+1%), and paper and cardboard from 12% to 14% (+2%). On the contrary, glass showed a drop from 13% to 10% (-3%), being the most significant decrease among inert fractions. Concerning the secondary fractions, a small variation was observed during the summer: rubber waste increased from 8% to 9% (+1%), textiles from 4% to 5% (+1%), while wood decreased from 5% to 4% (-1%). The metallic fraction was reduced in both periods and decreased again, from 2% to 1% (-1%). The share of principal materials with potential for recovery (paper and cardboard, glass, iron, plastic, and polyethene) was reduced from 68% in spring to 66% in summer, showing a slight decrease due to the drop in glass and metallic fractions. However, the general trend is the same: a constant dominance of polymers in the waste composition at Site 1 in both seasons.

In the context of preparing this article, a study of the morphological composition of waste at Site 2 was carried out for the spring season of 2025, allowing for the quantification of the structure of the main fractions that make up the waste flow. The determination of the proportions of the polymeric, organic, rubber, and inert fractions is necessary to understand the real composition of the waste and the environmental impact that its stockpiling can generate. The data collected served as a basis for evaluating materials with recovery potential through sorting, as well as identifying fractions that require specific management strategies. The results of the spring analysis of Site 2 were also used as raw material for subsequent seasonal comparisons and for supporting actions focused on mitigating the environmental impact of waste. A.E. Peter *et al.* (2019) analysed the composition of both fresh and aged waste in an open-air dump: the fresh MSW contained ~36% of biowaste (food, garden, and organic waste), and around 30% of recyclable materials (plastics, paper, cardboard, and metals). The aged waste showed a prevalence of plastics (25-33%) with high energy value, while the organic matter was practically decomposed. This is related to the state of the dump after several years of operation, where the plastics and polyethene (43% together) are predominant, and the organic fraction reaches only 12%. In this case, the organic fraction is much lower, which, according to F.A. Osra *et al.* (2021), may be related to the age of the dump, the decomposition of organic matter, or the specific composition of the waste, which is mainly packaging, plastics, and inorganic material. Figure 3 shows the morphological composition of the waste at Site 2 (spring 2025).

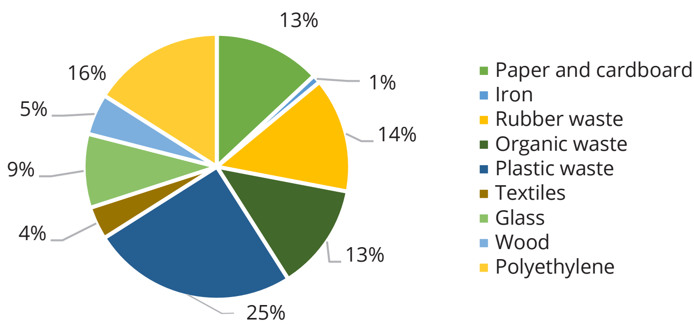

Figure 3. Average morphological composition of collected waste samples at Site 2, %, spring 2025
Source: developed by the authors

The morphological composition of the collected samples of waste, presented in Figure 3, is characterised by the predominance of the polymer fraction. The greatest proportion is represented by plastic waste (25%) and polyethene (16%), which together account for 41% of the morphological composition. A significant share is occupied by rubber waste (14%), which indicates the rather high content of elastomeric materials in the waste. The organic share is 13%, which corresponds to the share of biodegradable components. Among the fractions of materials traditionally considered recyclable, paper and cardboard occupy 13%, and glass 9%, which indicates a rather high content of packaging and inert components in the composition of the waste. The share of wood is 5%, and that of textiles is 4%, which together make up 9% of the morphological composition. The smallest share is occupied by iron (1%), which indicates the rather low content of the metal fraction. In general, the morphological composition of the waste at Site 2 in the spring of 2025 is shifted towards polymeric materials, which make the greatest contribution to its structure. Simultaneously, rubber and organic shares (14% and 13%, respectively) remain significant, which affects the environmental hazard and need for management. The paper and cardboard shares and the glass share (13% and 9%, respectively) indicate the presence of the fractions which can potentially be recovered. On the whole, the morphological composition is rather complex but with a clear dominance of the polymer group in the spring season. In the summer of 2025, a study of the morphological composition of the waste at Site 2 was also carried out with the aim of establishing the real structure of the fractions in the collected samples under the conditions corresponding to the seasonal maximum of waste formation. The data of the summer period at Site 2 are the basis for the seasonal analysis of the morphological structure dynamics and development of practical recommendations for optimising the sorting of waste and reducing the anthropogenic impact on the environment (Fig. 4).

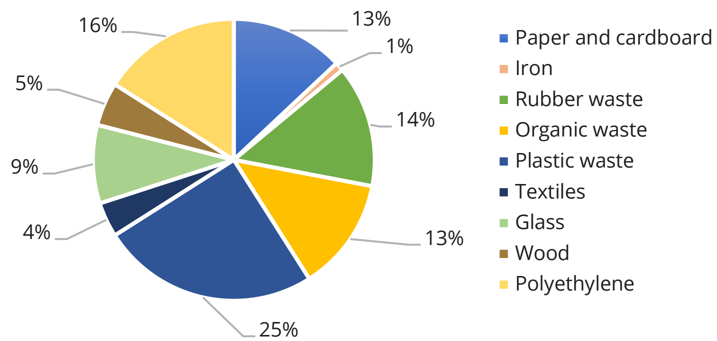

Figure 4. Average morphological composition of collected waste samples at Site 2, %, summer 2025
Source: developed by the authors

The morphological composition of the collected samples of waste at Site 2, presented in Figure 4, is characterised by the dominance of the polymer fraction. The greatest proportion is represented by plastic waste (25%) and polyethene (16%), which together account for 41% of the morphological composition. A significant component is occupied by rubber waste (14%), which indicates a rather high content of elastomeric materials in the waste in the summer season. The share of the organic component is 13%, which corresponds to the share of the biodegradable components. In the list of fractions that could potentially be recovered as secondary raw materials are paper and cardboard (13%) and glass (9%), which indicate the presence of packaging materials and an inert component, typical for mixed waste. The rest of the morphological composition consists of wood (5%) and textiles (4%), which together constitute 9%. The metallic fraction is represented in insignificant quantities (iron is 1%). Briefly, the morphological structure of Site 2 for summer indicates the prevalence of polymers and a significant proportion of rubber and organic components (14% and 13%, respectively). Such a structure is characterised by the high content of slowly degradable materials in nature (polymers and rubber) and a rather significant proportion of biodegradable waste. Paper and cardboard (13%) and glass (9%) form a significant part of the waste, which can be recovered in case of appropriate sorting. In general, the structure is heterogeneous, but with a clear lumping of the main mass in the polymer and related technogenic fractions.

Morphological composition of the studied areas reflects the real structure of the materials entering the landfill body and allows for the identification of the possible sources of pollutants formation in the surrounding environment. The prevalence of polymeric and packaging components, the presence of the organic fraction, as well as the proportion of rubber, textile, wood, and inert materials, determine the intensity of biochemical decomposition processes, formation of leachates, and migration behaviour of substances in the waste-soil system. The morphological structure has a significant impact on the rate of transformation of waste, on acid-base and redox conditions, and on the ability of the medium to accumulate or mobilise toxic substances. In this regard, the morphological structure is considered a key characteristic that determines the subsequent processes of soil contamination. O. Kravchenko *et al.* (2018) characterise the morphological composition of MSW at landfills and dumpsites in Ukraine (the cities of Khmelnytskyi and Kryvyi Rih). The authors demonstrated that the composition of mixed, unsorted waste largely determines the microbiocenosis within the landfill body, and that a high proportion of "bioavailable" organic matter leads to the dominance of bacteria of the genera *Bacillus*, *Micrococcus*, *Sarcina*, and others. However, in samples where polymers and plastics predominate, the activity of such biocenoses decreases. It explains the observed situation: the predominance of plastic and polyethene indicates that the biological activity of the landfill may be low or changed. O.M. Trofymchuk *et al.* (2020) showed that long-term landfills of MSW lead to an accumulation of heavy metals in the soil, with the concentration of copper, lead, nickel, and tin in some cases exceeding the background values by 2-10 times. However, if such a landfill predominantly consists of polymeric, rubber, textile, plastic, and inert wastes, they can serve as potential future sources of pollution, e.g. as a result of the formation of leachates, biogeochemical transformations, and heavy metal migration. According to the presented data (metals 2%, rubber 7%, textiles 5%), there are fractions in the landfill that may contribute to the contamination of soil and groundwater.

The second stage of the research was to assess the accumulation of heavy metals, which are among the most dangerous and durable pollutants that can persist in soils for a long time and penetrate into trophic chains. The various components of waste may serve as direct metal-carriers (especially some components of anthropogenic origin), as well as indirect factors contributing to their enhanced migration due to changes in the physico-chemical parameters of the medium. Therefore, the determination of the content of heavy metals in soils in the areas affected by the studied objects allows quantifying the level of anthropogenic impact and comparing it with the morphological characteristics of the waste in different seasons. This comparison is necessary for understanding the pollution formation mechanisms and for subsequent environmental conclusions and recommendations. Table 1 shows the results of soil analysis at Site 1 in the spring-summer period of 2025. The table shows the parameters obtained from the collected samples for the corresponding season.

Table 1. Content of mobile forms of heavy metals in soils (mg/kg), Site 1, spring-summer 2025

Sample code	MPC, mg/kg	North			North-East			South-East		
		1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3
Spring 2025										
Cu, mg/kg	3.00	0.461	0.681	0.575	0.566	0.715	0.43	0.63	0.387	0.361
Zn, mg/kg	23.00	8.075	5.412	6.875	4.645	1.785	1.86	6.924	8.565	1.91
Cd, mg/kg	0.70	0.898	0.768	0.575	0.505	0.609	0.65	0.49	0.407	0.397
Pb, mg/kg	6.00	1.195	1.53	1.576	1.451	2.19	2.755	1.067	2.115	2.145
Summer 2025										
Cu, mg/kg	3.00	0.354	0.67	0.77	0.611	0.632	0.548	0.63	0.49	0.453
Zn, mg/kg	23.00	8.67	9.625	3.955	5.985	8.405	9.47	2.12	2.125	1.59
Cd, mg/kg	0.70	0.282	0.338	0.433	0.347	0.437	0.492	0.45	0.518	0.35
Pb, mg/kg	6.00	1.91	1.278	2.03	1.89	1.375	0.644	0.95	1.775	1.844

Note: MPC – maximum permissible concentration
Source: developed by the authors

In the spring of 2025, the maximum concentration of Cu^{2+} was observed in sample 2.2 (North-Eastern direction) and was equal to 0.715 mg/kg (for comparison, the remaining values of Cu^{2+} in the spring season were lower and ranged from 0.361 to 0.681 mg/kg). In the summer of 2025, the maximum concentration of Cu^{2+} was recorded in sample 1.3 (North direction) and was equal to 0.770 mg/kg (summer Cu values ranged from 0.354 to 0.770 mg/kg). When comparing the maxima of the seasons, it can be seen that the summer maximum (0.770 mg/kg) exceeded the spring maximum (0.715 mg/kg) by 0.055 mg/kg. At the same time, in the location of the spring maximum (2.2), the summer concentration decreased to 0.632 mg/kg (a change of -0.083 mg/kg). In the location of the summer maximum (1.3), there was an increase in the content of Cu^{2+} from 0.575 mg/kg in spring to 0.770 mg/kg in summer (a change of +0.195 mg/kg), which indicates a seasonal redistribution of this element within the object. The maximum concentration of Zn^{2+} in the spring of 2025 was found in sample 3.2 (South-East direction) and was equal to 8.565 mg/kg (the remaining spring values of Zn^{2+} were in the range from 1.785 to 8.075 mg/kg). In the summer of 2025, the maximum value of Zn^{2+} was observed in sample 1.2 (North direction) with 9.625 mg/kg (range of summer values: 1.590-9.625 mg/kg). A seasonal comparison of peak values shows that the summer maximum exceeded the spring maximum by 1.060 mg/kg; however, at the location of the spring maximum (3.2), Zn^{2+} decreased sharply in summer to 2.125 mg/kg (a change of -6.440 mg/kg). At the location of the summer maximum (1.2), by contrast, Zn^{2+} increased from 5.412 mg/kg in spring to 9.625 mg/kg in summer (a change of +4.213 mg/kg), indicating a summer concentration of peak values in the Northern direction alongside a peak in the southeast.

The maximum value of Cd^{2+} in the spring of 2025 was observed in sample 1.1 (Northern direction) with 0.898 mg/kg (range of other values of Cd^{2+} in the spring: 0.397-0.768 mg/kg). In the summer of 2025, the maximum value of Cd^{2+} was observed in sample 3.2 (South-Eastern direction) with 0.518 mg/kg (range of summer values: 0.282-0.518 mg/kg). A comparison of seasonal maxima indicates that the spring peak of Cd^{2+} was higher than the summer peak by 0.380 mg/kg. At the spring maximum location (1.1), the summer concentration decreased to 0.282 mg/kg (a change of -0.616 mg/kg). At the location of the summer maximum (3.2), Cd^{2+}, in contrast, increased from 0.407 mg/kg in spring to 0.518 mg/kg in summer (a change of +0.111 mg/kg), indicating a seasonal shift in the zone of elevated concentrations from the north towards the south-east. For Pb^{2+}, the maximum value observed in the spring of 2025 was in sample 2.3 (North-Eastern direction) with 2.755 mg/kg (range of other values of Pb^{2+} in the spring: 1.067-2.190 mg/kg). In the summer of 2025, the maximum value of Pb^{2+} was observed in sample 1.3 (Northern direction) with 2.030 mg/kg (range of summer values: 0.644-2.030 mg/kg). A seasonal comparison of maxima shows that the spring peak exceeded the summer peak by 0.725 mg/kg. At the location of the spring maximum (2.3), Pb^{2+} decreased in summer to 0.644 mg/kg (a change of −2.111 mg/kg). At the location of the summer maximum (1.3), Pb^{2+} increased from 1.576 mg/kg in spring to 2.030 mg/kg in summer (a change of +0.454 mg/kg), reflecting a redistribution of the most pronounced Pb^{2+} values across seasons and study sectors.

A significant conclusion is presented in the study by B. Bernardo *et al*. (2025), who examined soils around a landfill during two seasons of the year. They concluded that in the wet period, washing out predominates, which, on the one hand, decreases some indicators of element accumulation in the soils, and, on the other hand, increases the potential mobility of the contaminants, while in the dry period, there is a greater tendency to accumulation and retention. In relation to the object of this study, this means that interseasonal dynamics of moisture content and direction of surface and intra-soil runoffs can change not only the average background contents of Pb^{2+}, but also the redistribution between the sectors. This is also indicated by the pattern described above: the spring maximum is noted in sample 2.3, and the summer maximum in sample 1.3.

Concentrations of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}, which were obtained for the spring-summer period of 2025, can affect the morphological features of the soil cover indirectly, through the changes in soil microbial activity, the intensity of humus formation and the condition of the plant root system. Even without significant exceedances, spatial "peaks" of these elements can cause local differences in the structure (aggregation state) of the soil, its bulk density and in the stability of the surface horizon against degradation. In the areas with relatively higher concentrations of metals, a decrease in the biological activity is more likely, which can be reflected in such morphological features as poorer aggregation, a lower proportion of water-stable aggregates and higher susceptibility to surface sealing. Such an effect, as a rule, has a mosaic nature, corresponding to different degrees of element accumulation in the sectors and points of sampling presented in Table 1. As for the content of MPC, the contents of Cu^{2+} (MPC 3.00 g/kg), Zn^{2+} (MPC 23.00 g/kg) and Pb^{2+} (MPC 6.00 g/kg) in all the points of sampling in the spring and summer periods are below the permissible limits. At the same time, for Cd^{2+} (MPC 0.70 g/kg), the value of 0.898 mg/kg was observed in sample 1.1 in the spring period, which exceeds the indicated standard; the rest of the summer values of Cd^{2+} (0.282-0.518 mg/kg) are below the MPC. Thus, most of the indicators do not exceed the permissible concentrations, although there is a local excess of Cd^{2+} in the spring. From the scientific point of view, such single exceedances are of special interest for the interpretation of possible morphological changes, since they testify to localised areas of potential environmental risk. In Figure 5 below, it can be seen that the excess of MPC is in samples 1.1 and 1.2 in the spring period. The values of Cd^{2+} registered in both samples are higher than the established MPC, which confirms the presence of excesses.

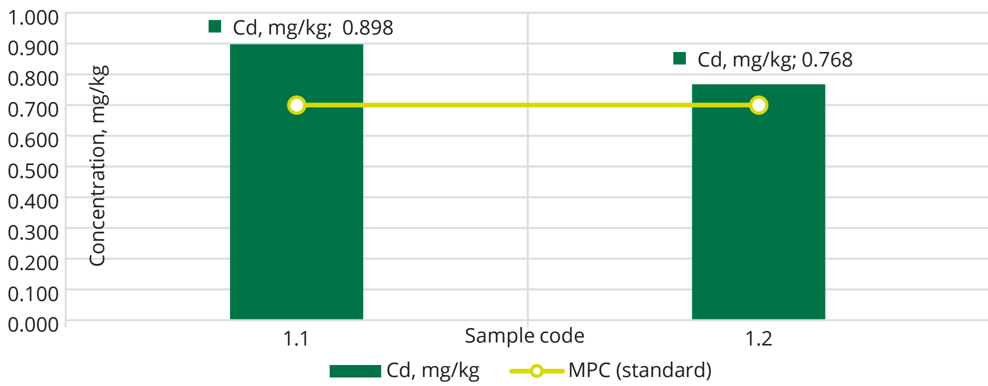

Figure 5. Exceedance of Cd, mg/kg, above the MPC at Site 1, spring 2025
Source: developed by the authors

The comparison of maxima in spring and summer shows that for Cu^{2+} and Zn^{2+}, the summer maxima are higher (0.770 mg/kg vs. 0.715 mg/kg for Cu^{2+} and 9.625 mg/kg vs. 8.565 mg/kg for Zn^{2+}, respectively), whereas the maxima of Pb^{2+} and Cd^{2+} in spring are higher than in summer (2.755 mg/kg vs. 2.030 mg/kg for Pb^{2+} and 0.898 mg/kg vs. 0.518 mg/kg for Cd^{2+}, respectively). This could be due to the seasonal condition of the soil, depending on the moisture content and biological activity. In the summer period, the transformation of organic matter and root activity is more intensive, which results in a change of fixation and mobility of elements within the upper soil horizon. Local summer maxima of Cu^{2+}/Zn^{2+} could be linked to the changes in the conditions of enzymatic activity in the soil, which in turn may affect the structure and aggregate stability of the topsoil. A higher contrast in spring for Pb^{2+} and especially for Cd^{2+} (with a local excess in sample 1.1) indicates the necessity of more detailed consideration of these points concerning morphological (compaction, structure, and aeration) manifestations, even when other metals are within the permissible limits. M. Tziouvalekas *et al.* (2024) found experimentally that the temperature of DTPA extraction influences the mobility and extractability of the elements; with an increase in temperature, the DTPA-extracted element concentration increases (especially for Cu^{2+} and Zn^{2+}, as well as for Pb^{2+}). The authors concluded that temperature may cause systematic biases between batches of determinations and should be taken into consideration when the "bioavailable/mobile" pool is interpreted. Within the framework of the present data, this means that the summer maxima of Cu^{2+} (0.770 mg/kg vs. 0.715 mg/kg in spring) and Zn^{2+} (9.625 mg/kg vs. 8.565 mg/kg) could be influenced not only by the input sources but also by an increase in the share of extractable (labile) forms in the topsoil under conditions of higher temperatures and more intensive transformation of organic matter.

M.S. El-Komy *et al.* (2025) in the study of DTPA-extractable metals found that season (wet and dry periods) and drying and wetting cycles have a significant impact on metal bioavailability and extractability, and stressed that for Cu^{2+}, Pb^{2+}, and Zn^{2+}, ion exchange is one of the key mechanisms controlling metal availability, along with spatial variation of soil characteristics. In the case of the present study, this means that the summer drying and rewetting regime, along with more intensive biological transformation of organic matter and root activity, could cause the shift of Cu^{2+}/Zn^{2+} into more labile extractable forms, which manifests itself as higher summer maxima. The results obtained show the multicomponent composition of the data. Firstly, they serve as a reference point for further monitoring, based on which a comparison of the indicators in subsequent seasons and years can be carried out. Secondly, the distribution of heavy metals over space, which allows tracing areas with high concentrations and assigning specific monitoring points (in particular, for Cd^{2+} in sample 1.1 in spring). Thirdly, the results characterise the environmental risk to soil organisms, vegetation, and the potential entry of pollutants into the trophic chain. Fourthly, the amount of data obtained makes it possible to assess the state of the object at the level of the facility itself and the surrounding territories, which can be used as a basis for developing management and nature protection measures. Thus, the distribution of metals has a mosaic character, maxima for each element are located at different points and sectors, which indicates heterogeneity of the conditions of accumulation. According to the MPCs indicated, the concentrations of Cu^{2+}, Zn^{2+}, and Pb^{2+} during the spring and summer period do not exceed the maximum permissible concentrations at any sampling point, while for Cd^{2++}, a local excess was registered in the spring in sample 1.1 (0.898 mg/kg vs. MPC 0.70 mg/kg). A seasonal shift of maximum values is observed: Cu^{2+} and Zn^{2+} have higher maxima in the summer, while Pb^{2+} and Cd^{2+} have higher maxima in the spring, which may be due to the seasonality of the conditions of migration and fixation of elements in the upper soil horizon. In connection with the potential impact on the morphological properties of the soil, special attention should be paid to points with high or maximum values, especially for Cd^{2+} in sample 1.1 in spring, as a sign of a local strengthening of the anthropogenic factor or soil geochemical features. Table 2 shows the concentrations of mobile forms of heavy metals in soils of Site 2 during the spring and summer period.

Table 2. Content of mobile forms of heavy metals in soils (mg/kg), Site 2, spring-summer 2025

Sampling period/ sample code	MPC, mg/kg	West			North			South		
		1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3
Spring 2025										
Cu, mg/kg	3.00	0.53	0.497	0.463	0.29	0.587	0.609	0.63	0.387	0.361
Zn, mg/kg	23.00	1.185	0.87	1.09	0.825	1.49	0.81	6.924	8.565	1.91
Cd, mg/kg	0.70	0.219	0.412	0.429	0.182	0.445	0.381	0.49	0.407	0.397
Pb, mg/kg	6.00	2.409	2.11	2.245	1.35	0.82	1.635	1.067	2.115	2.145
Summer 2025										
Cu, mg/kg	3.00	0.557	1.25	1.568	0.756	0.518	0.637	36.8	0.366	0.478
Zn, mg/kg	23.00	1.29	3.45	4.47	6.651	1.005	3.828	10.39	1.556	0.837
Cd, mg/kg	0.70	0.518	0.651	0.698	0.756	0.495	0.6255	0.28	0.352	0.38
Pb, mg/kg	6.00	1.595	2.854	2.652	1.79	1.37	1.58	1.415	1.485	1.728

Note: MPC – maximum permissible concentration
Source: developed by the authors

In the spring of 2025, the maximum content of Cu^{2+} (mobile forms) was observed in the Southern direction in sample 3.1, which was 0.63 mg/kg (at other sampling points, the values of Cu^{2+} were in the range of 0.29-0.609 mg/kg). In the summer of 2025, the maximum value of Cu^{2+} was also noted in sample 3.1, where it was 36.8 mg/kg, while the rest of the summer values of Cu^{2+} were in the range of 0.366-1.568 mg/kg. Thus, at the point with the highest values (3.1), the concentration of Cu^{2+} increased from 0.63 mg/kg in the spring to 36.8 mg/kg in the summer (a change of +36.17 mg/kg), which indicates a sharp seasonality at this point. Of the rest, the Western direction shows higher summer concentrations than in the spring, in samples 1.3 (1.568 mg/kg) and 1.2 (1.25 mg/kg), but much lower than the maximum concentration registered in sample 3.1. In the case of Zn^{2+}, the maximum spring 2025 concentration is registered in the Southern direction in sample 3.2 with a value of 8.565 mg/kg. The rest of the samples show spring concentrations between 0.81 and 6.924 mg/kg. For summer 2025, the maximum concentration of Zn^{2+} is registered in sample 3.1 (Southern direction) with a value of 10.39 mg/kg; the rest of the summer concentrations of Zn^{2+} are between 0.837 and 6.651 mg/kg. The maximum summer concentration (10.39 mg/kg) is 1.825 mg/kg higher than the maximum spring concentration (8.565 mg/kg). At the point corresponding to the spring maximum, in sample 3.2, a decrease was observed in summer from 8.565 mg/kg to 1.556 mg/kg (a change of -7.009 mg/kg). At the same time, at the point of the summer maximum, in sample 3.1, Zn^{2+} increased from 6.924 mg/kg in spring to 10.39 mg/kg in summer (a change of +3.466 mg/kg), indicating a seasonal reconfiguration of the spatial distribution of elevated values. In spring 2025, the Cd^{2+} content across all points at Site 2 ranged from 0.182 mg/kg to 0.490 mg/kg: the minimum was recorded in sample 2.1 (Northern direction) at 0.182 mg/kg, while the maximum was recorded in sample 3.1 (Southern direction) at 0.490 mg/kg. For summer 2025, the maximum concentration of Cd^{2+} is registered now in sample 2.1 (Northern direction) with a value of 0.756 mg/kg. The minimum summer concentration is registered in sample 3.1 (Southern direction) with a value of 0.280 mg/kg (the rest of the samples, except 2.1, are between 0.3520.698 mg/kg).

Comparison of seasonal maxima shows an increase in the peak Cd^{2+} level from 0.490 mg/kg (spring, 3.1) to 0.756 mg/kg (summer, 2.1), i.e. by 0.266 mg/kg, indicating a pronounced seasonal and spatial reconfiguration of the "zone of maximum values". At the same time, at the point of maximum spring concentration (3.1), the content of Cd^{2+} decreased from 0.490 mg/kg to 0.280 mg/kg (a change of -0.210 mg/kg), and at the point of maximum summer concentration (2.1), it increased from 0.182 mg/kg to 0.756 mg/kg (an change of +0.574 mg/kg), which directly indicates the redistribution of the main area of high concentrations. Regarding Pb^{2+} for spring 2025, the maximum concentration is registered in the Western direction in sample 1.1 with a value of 2.409 mg/kg. The rest of the spring concentrations of Pb^{2+} are between 0.82 and 2.245 mg/kg. For summer 2025, the maximum concentration of Pb^{2+} is registered in sample 1.2 (Western direction) with a value of 2.854 mg/kg. The rest of the summer concentrations are between 1.37 and 2.854 mg/kg. The maximum summer concentration (2.854 mg/kg) is 0.445 mg/kg higher than the maximum spring concentration (2.409 mg/kg). At the same time, at the point of the spring maximum (1.1), a decrease was observed from 2.409 mg/kg to 1.595 mg/kg (a change of -0.814 mg/kg). On the other hand, at the point of maximum summer concentration (1.2), the content of Pb^{2+} increased from 2.11 mg/kg in spring to 2.854 mg/kg in summer (a change of +0.744 mg/kg), which directly indicates the redistribution of the accumulation intensity in the Western direction.

The registered concentrations of mobile Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} (spring-summer 2025, Site 2) can affect the morphological condition of the soil, first of all, due to changes in biological processes and the stability of aggregates in the upper horizons. Mobile forms of metals are most accessible for migration and sorption complex interaction; thus, they can change humus formation conditions, microbial activity, and root system development. At the morphological level, the difference will manifest as local variation of soil structure, bulk density, surface stability against sealing and compaction, and cracking upon drying. It is worth noting that local "peaks" at individual points can generate a mosaic of morphological features, even if the majority of indicators remain within the limits. In this data set, the most "contrasting" areas relate to the Southern direction (3.1) and the Northern direction (2.1) in the summer. According to the MPC standards presented in the table, no exceedances are noted for most elements at most points; however, there is an exception. For Cu^{2+} (MPC 3.00 mg/kg), the value is 36.8 mg/kg in sample 3.1 in the summer; thus, it significantly exceeds the MPC (for comparison, the maximum Cu^{2+} content in the spring is 0.63 mg/kg, which does not exceed the standard). For Zn^{2+} (MPC 23.00 mg/kg), the maximum values in the spring (8.565 mg/kg, 3.2) and in the summer (10.39 mg/kg, 3.1) do not exceed the permissible level. For Pb^{2+} (MPC 6.00 mg/kg), the maximum in the spring (2.409 mg/kg, 1.1) and the maximum in the summer (2.854 mg/kg, 1.2) also do not exceed the MPC. Figure 6 shows the exceedance of the MPC in sample 3.1 in the summer. The values of Cu^{2+} registered in both samples are higher than the established MPC, which confirms the presence of excesses.

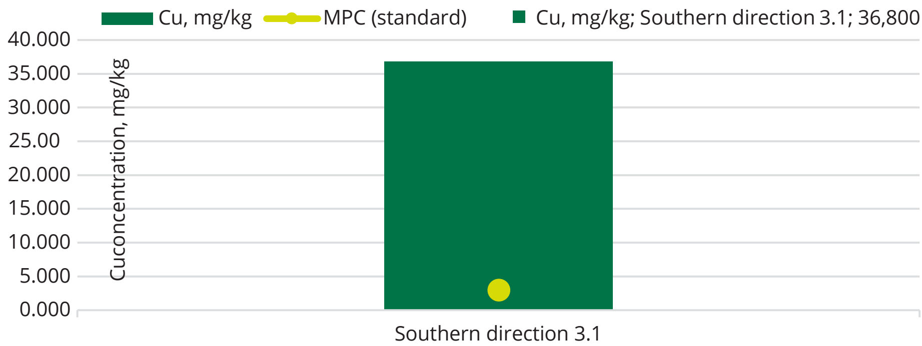

Figure 6. Exceedance of Cu^{2+}, mg/kg, above the MPC (Site 2, summer 2025)
Source: developed by the authors

The sharp increase in the content of the mobile form of Cu^{2+} in the summer in sample 3.1 (up to 36.8 mg/kg) is likely due to a combination of local input of copper and a change in the soil chemical conditions for the transition of copper into more mobile, extractable forms. In the summer, at high temperatures and varying humidity (drying and rewetting cycles), the processes of mineralisation of organic matter and transformation of Cu^{2+} complexes with humic substances are activated, which can temporarily increase the share of mobile compounds. In addition, the local variation often comes from point anthropogenic pollution (input of waste, dust/sludge, metal particles, or wastewater with copper) or redistribution of pollutants by surface flow into microdepressions of the relief, where fine particles are concentrated. L. Filipović *et al.* (2023) showed on a field lysimeter experiment (with a monthly frequency of sampling during two years) performed on the soils of a vineyard slope, that Cu^{2+} concentrations in the leachates are statistically linked to dissolved organic carbon (DOC) concentrations, with a stronger correlation found at the footslope position where the substances tend to accumulate and the redistribution by the flow of water is more pronounced. The authors pointed out in their discussion that the organically complexed forms control the copper mobility: according to the modelling of speciation, the majority of Cu^{2+} in leachate was found as Cu^{2+} – DOC (the sum of humic-complexed forms), whereas the molar fraction of free Cu^{2+} in the aqueous phase is very low (tenths to hundredths of a per cent). This suggests that a seasonal variation in the DOC pool or dissolved organic matter (DOM) might significantly affect the mobile pool of Cu^{2+} in the soil-solution system. In the framework of this research, this statement straightforwardly supports the interpretation of the summer maximum in sample 3.1: if, during the summer period, the input or formation of dissolved organic matter is increased or its composition is changed (e.g. due to the mineralisation of organic matter and drying-rewetting cycles), then part of copper can be mobilised into more mobile, extractable forms due to complexation with DOM/DOC. At the same time, for Zn^{2+} and Pb^{2+}, which do not exceed the MPC values at any points, the morphological effect is generally less pronounced and manifests itself mainly in the form of background seasonal fluctuations without a marked disturbance to the soil profile. In general, it is a combination of "local peaks" with seasonal dynamics that creates conditions for a heterogeneous morphological pattern of the site.

The obtained results allow characterisation of the environmental status of the territory and the spatial distribution of the potential risk. They can be used as a basis for the environmental impact assessment, monitoring of the working environment, and development of scientific models of the migration and accumulation of mobile metal forms in the soil during different seasons. The indicators reflect the possible scheme of the spatial distribution of the contamination and allow identifying objects that require a more detailed morphological description of the soil profile, first of all, at the "contrasting" points. The data indicate a spatial heterogeneity in the distribution of mobile forms of metals. The maximum values of different elements are concentrated in different sectors and points: Cu^{2+} is in sample 3.1 in summer, Zn^{2+} is in sample 3.1 in summer and in sample 3.2 in spring, Cu^{2+} is in sample 2.1 in summer, Pb^{2+} is in sample 1.2 in summer and in sample 1.1 in spring. Zn^{2+} and Pb^{2+} concentrations during the spring-summer period at all points of both sites do not exceed the MPC values. However, Cu^{2+} in sample 3.1 in the summer season exceeds the norm. The most significant seasonal variations were registered for Cu^{2+}: in sample 3.1, the concentration increased from 0.63 mg/kg in spring to 36.8 mg/kg in summer, which significantly changed the environmental interpretation of the site's summer status. The general conclusion that follows from the above data is that, for the assessment of potential morphological transformations, local maxima and their seasonal dynamics are of greater interest than average site concentrations since they determine the areas of priority interest for further study. The comparison of both sites revealed that the risk degree is different: the maximum risk at Site 1 is caused by a sharp increase in the concentration of Cd^{2+} in spring at the Northern direction in samples 1.1 and 1.2 (0.898 mg/kg and 0.768 mg/kg, respectively), while at Site 2, the maximum risk is caused by a sharp increase in the concentration of Cu^{2+} in summer in sample 3.1 (36.8 mg/kg) and the summer maximum of Cd^{2+} concentration in sample 2.1 (0.756 mg/kg). Zn^{2+} and Pb^{2+} concentrations at both sites during both seasons are significantly below the MPC and are subject to local seasonal maxima (Zn^{2+} up to 9.625 mg/kg at Site 1 and up to 10.39 mg/kg at Site 2; Pb^{2+} up to 2.755 mg/kg at Site 1 and up to 2.854 mg/kg at Site 2). The points of priority interest for further monitoring and repeated sampling are 1.1-1.2 (Cd^{2+}, spring) at Site 1, as well as 3.1 (Cu^{2+}, summer) and 2.1 (Cd^{2+}, summer) at Site 2, since they determine the maximum excess of MPC within the framework of the presented data.

Over the spring-summer period of 2025, the morphological structure of wastes at Site 1 is generally represented by the prevalence of the polymer fraction (plastics: 23-25%, polyethylene: 16-18%, the total share is approximately 41%), a considerable share of organic waste (15-16%), and components potentially recoverable (paper/cardboard: 12-14%, glass: 10-13%). Against the background of the recorded morphological structure of waste, the cases of excess of the maximum permissible concentration were registered: in the spring period, the concentration of Cd^{2+} in the Northern direction (samples 1.1: 0.898 mg/kg and 1.2: 0.768 mg/kg) exceeded the MPC (0.70 mg/kg). In summer, a pronounced local excess was registered for Cu^{2+} in sample 3.1 (Southern direction), which is 36.8 mg/kg (MPC 3.00 mg/kg). This fact indicates the formation of a focal zone of high anthropogenic pollution. At Site 2, the morphological composition of waste in the spring and summer of 2025 was the same and consisted of the following: plastics: 25%, polyethylene: 16%, rubber waste: 14%, organic waste: 13%, paper and cardboard: 13%, glass: 9%, wood: 5%, textiles: 4%, ferrous metals: 1%. Such a composition indicates the prevalence of long-lived materials (polymers and rubber) and the presence of a biodegradable fraction, which determines the intensity of the transformation processes in the body of the landfill. The constancy of the morphological composition is a necessary condition for the correct seasonal comparison of the processes of migration and risks of toxicant accumulation in the soils adjacent to the landfill. The likely causes of the revealed excesses of the MPC should be interpreted in the relationship between the morphological composition of the waste and the processes of formation of leachate and migration of contaminants.

The increased content of polymer and rubber fractions can serve as a source or carrier of impurities due to the presence of technological additives, fillers, and pigments, as well as contribute to the sorption/desorption of metals on the surface of materials during degradation. On the contrary, the presence of an organic component (13-16%) contributes to biochemical transformations and the formation of leachate, which changes the physicochemical parameters of the environment (solubility of compounds, mobility of metal forms) and can contribute to their migration and accumulation. Local maxima (of Cd^{2+} in samples 1.1-1.2 and Cu^{2+} in sample 3.1) are associated with point sources of pollution in combination with non-uniform distribution and filtration of moisture in the contaminated areas.

CONCLUSIONS

In the framework of the study, it was found that in 2025, for both landfills, the characteristic feature of the morphological structure of waste is the prevalence of the polymer fraction. At Site 1, in the spring-summer period, the total content of plastics and polyethene in the waste remained at the same level, 41%, and a significant content of the organic fraction (15-16%) and the components, which are potentially recyclable (paper/cardboard and glass), was noted. At Site 2, the morphological composition of waste in the spring and summer was the same in terms of the proportion of fractions, which indicates a constant waste flow and reproducibility of structure. Morphological results validate the high potential of the waste stream both as a resource and environmental threat, as they are mostly composed of long-lifetime materials (polymers and rubber). At Site 1, slight seasonal structural variations were registered (increase of plastics and paper/cardboard and decrease of glass), which might be linked to changes in packaging materials during the spring-summer season. At Site 2, the relatively constant proportions of the main fractions allow considering the morphological profile as a reference characteristic for the interpretation of migration processes and related pollution risks.

The investigation of the soil samples revealed the exceedance of maximum permissible concentrations of heavy metals in certain directions and periods of sampling. For Site 1, in the spring season, MPC exceedance for Cd^{2+} (0.70 mg/kg) was found in the Northern direction in samples 1.1 (0.898 mg/kg) and 1.2 (0.768 mg/kg). In the summer season at the same site, a sharp local MPC exceedance for Cu^{2+} (3.00 mg/kg) was registered in the Southern sample 3.1 (36.8 mg/kg), which indicated the formation of a localised zone of anthropogenic pollution. The reasons for the registered MPC exceedances are related to the morphological features of the waste and processes caused by them in the waste-leachate-soil system. Elevated proportions of the polymer and rubber fractions may act as sources or carriers of impurities and participate in the retention and transport of pollutants, while the organic fraction enhances the formation of leachates and changes the physicochemical conditions that influence the mobility and accumulation of metals in the soil. The local character of the MPC exceedances (for Cd^{2+} in the 1.1-1.2 direction and for Cu^{2+} in sample 3.1) agrees with the spatial heterogeneity of the composition and moisture content of the waste, as well as with the varying intensity of filtration, which controls the focal accumulation of heavy metals. Further investigations are recommended for a deeper analysis of the influence of different waste fractions on heavy metal migration in soils and for the development of efficient measures to mitigate anthropogenic pollution through proper management and treatment of the waste.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Ahmad, W., Alharthy, R.D., Zubair, M., Ahmed, M., Hameed, A., & Rafique, S. (2021). Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. *Scientific Reports*, 11, article number 17006. doi: 10.1038/s41598-021-94616-4.

[2] ASTM D5231-92. (2016). *Standard test method for determination of the composition of unprocessed municipal solid waste*. Retrieved from https://www.eawag.ch/fileadmin/Domain1/Abteilungen/sandec/E-Learning/Moocs/Solid_Waste/Further_resources/Week_1/standard_test_method_composition_unprocessed_msw.pdf.
[3] Baranovska, O.V., Baranovsky, M.O., Smal, V.V., & Smal, I.V. (2000). *Chernihiv region: Nature, population, economy (comprehensive geographical study)*. Nizhyn: Nauka-Servis.
[4] Bernardo, B., Candeias, C., & Rocha, F. (2025). Seasonal dynamics and leachate-induced alteration of soils and associated risk of surface water contamination around the Hulene-B waste dump, Maputo, Mozambique. *Applied Sciences*, 15(21), article number 11663. doi: 10.3390/app152111663.
[5] Cakaj, A., Lisiak-Zielińska, M., Hanć, A., Małecka, A., Borowiak, K., & Drapikowska, M. (2023). Common weeds as heavy metal bioindicators: A new approach in biomonitoring. *Scientific Reports*, 13, article number 34019. doi: 10.1038/s41598-023-34019-9.
[6] Chen, J., Zhang, L., Song, L., Ye, M., Wang, L., Fan, B., Li, B., Yang, Z., Jin, R., & Jia, P. (2025). Effects of illegal solid waste dumping on the structure of soil bacterial communities: A case study in China. *Toxics*, 13(1), article number 20. doi: 10.3390/toxics13010020.
[7] Climate-Data.org. (n.d.). Retrieved from https://en.climate-data.org/.
[8] Drall, J.K., Rautela, R., Jambhulkar, R., Kataria, A.K., & Kumar, S. (2025). Effect of heavy metals contamination due to leachate migration from uncontrolled dumpsites: A comprehensive analysis on soil and groundwater. *Journal of Environmental Management*, 373, article number 123473. doi: 10.1016/j.jenvman.2024.123473.
[9] El-Komy, M.S., Abuzaid, A.S., Fadl, M.E., Drosos, M., Scopa, A., & Abdel-Hai, M.S. (2025). Spatiotemporal variations affect DTPA-extractable heavy metals in coastal salt-affected soils of arid regions. *Soil Systems*, 9(1), article number 26. doi: 10.3390/soilsystems9010026.
[10] Filipović, L., *et al*. (2023). Leached copper correlation with dissolved organic carbon in sloped vineyard soil. *Water*, 15(4), article number 800. doi: 10.3390/w15040800.
[11] Godyń, K., Dutka, B., Rosik-Dulewska, C., Ciesielczuk, T., & Głowacki, M. (2025). Assessment of the possibility of heavy metals elution from municipal solid waste incineration bottom ash into the soil and water. *Desalination and Water Treatment*, 323, article number 101282. doi: 10.1016/j.dwt.2025.101282.
[12] Hölzle, I., Somani, M., Ramana, G.V., & Datta, M. (2022). Heavy metals in soil-like material from landfills – resource or contaminants? *Journal of Cleaner Production*, 369, article number 133136. doi: 10.1016/j.jclepro.2022.133136.
[13] Kachmar, H., Datsko, T., Ivankiv, M., & Dydiv, A. (2023). Effects of lead soil pollution on physical and chemical properties of leached forest-meadow chernozem under conditions of the Western Forest-Steppe of Ukraine. *Bulletin of Lviv National Environmental University. Series Agronomy*, 27, 52-57. doi: 10.31734/agronomy2023.27.052.
[14] Kravchenko, O., Satin, I., Shevchenko, L., & Panchenko, O. (2018). Influence of the morphological composition of solid municipal waste on the species composition of microbiocenoses that are formed in the body of landfill. *Innovations in Biosystems and Bioengineering*, 2(3), 203-209. doi: 10.20535/ibb.2018.2.3.146100.
[15] Liu, X., Zhang, H., Zhang, W., Jia, Q., Chen, X., & Chen, H. (2024). Comparative transcriptome analysis between two different cadmium-accumulating genotypes of soybean (*Glycine max*) in response to cadmium stress. *BMC Genomic Data*, 25, article number 43. doi: 10.1186/s12863-024-01226-w.
[16] Osra, F.A., Ozcan, H.K., Alzahrani, J.S., & Alsoufi, M.S. (2021). Municipal solid waste characterization and landfill gas generation in Kakia landfill, Makkah. *Sustainability*, 13(3), article number 1462. doi: 10.3390/su13031462.
[17] Pathak, G., Nichter, M., Hardon, A., & Moyer, E. (2024). The open burning of plastic wastes is an urgent global health issue. *Annals of Global Health*, 90(1), article number 3. doi: 10.5334/aogh.4232.
[18] Peter, A.E., Shiva Nagendra, S.M., & Nambi, I.M. (2019). Environmental burden by an open dumpsite in urban India. *Waste Management*, 85, 151-163. doi: 10.1016/j.wasman.2018.12.022.

[19] Podlasek, A., Vaverková, M.D., Koda, E., Jakimiuk, A., & Barroso, P.M. (2023). Characteristics and pollution potential of leachate from municipal solid waste landfills: Practical examples from Poland and the Czech Republic and a comprehensive evaluation in a global context. *Journal of Environmental Management*, 332, article number 117328. doi: 10.1016/j.jenvman.2023.117328.

[20] Podzerei, R. (2024). Optimization of agricultural land in Cherkasy region through alternative farming systems. *Public Administration and Law Review*, 1(17), 13-19. doi: 10.36690/2674-5216-2024-1-13.

[21] Razanov, S., Piddubna, A., Gucol, G., Symochko, I., Kovalova, S., Bakhmat, M., & Bakhmat, O. (2022). Estimation of heavy metals accumulation by vegetables in agroecosystems as one of the main aspects in food security. *International Journal of Ecosystems and Ecology Science*, 12(3), 159-164. doi: 10.31407/ijees12.320.

[22] Samadi, M.T., Leili, M., Asgari, G., & Chavoshi, S. (2024). The potential of Phragmites australis to bioaccumulate and translocate heavy metals from landfill leachate. *Journal of Water Process Engineering*, 64, article number 105657. doi: 10.1016/j.jwpe.2024.105657.

[23] Štofejová, L., Fazekaš, J., & Fazekašová, D. (2021). Analysis of heavy metal content in soil and plants in the dumping ground of a magnesite mining factory Jelšava-Lubeník (Slovakia). *Sustainability*, 13(8), article number 4508. doi: 10.3390/su13084508.

[24] Szulc, J., Okrasa, M., Nowak, A., Ryngajłło, M., Nizioł, J., Kuźniar, A., Ruman, T., & Gutarowska, B. (2024). Chemical and microbiological hazards associated with illegal waste dumps in post-industrial areas: A case study from Poland. *Molecules*, 29(7), article number 1496. doi: 10.3390/molecules29071496.

[25] Trofymchuk, O.M., Kuraeva, I.V., Azimov, O.T., Karmazinenko, S.P., & Zlobina, K.S. (2020). Geochemical characteristics for the soil deposits within the municipal waste disposal areas by the environment study results. *Environmental Safety and Natural Resources*, 35(3), 5-21. doi: 10.32347/2411-4049.2020.3.5-21.

[26] Tziouvalekas, M., Noulas, C., Thalassinos, G., Shaheen, S.M., Rinklebe, J., & Antoniadis, V. (2024). Temperature-induced changes in DTPA-extractable trace elements: Predicting the potential impact of climate change on the availability of soil elements. *Chemosphere*, 350, article number 141064. doi: 10.1016/j.chemosphere.2023.141064.

[27] Wang, Q., Ko, J.H., Liu, F., & Xu, Q. (2021). Leaching characteristics of heavy metals in MSW and bottom ash co-disposal landfills. *Journal of Hazardous Materials*, 416, article number 126042. doi: 10.1016/j.jhazmat.2021.126042.

[28] Wydro, U., Wołejko, E., Sokołowska, G., Leszczyński, J., & Jabłońska-Trypuć, A. (2022). Presence of microorganisms in soil and water: Investigating landfill leachate influence on soil microbial biodiversity and cytotoxicity. *Water*, 14(22), article number 3634. doi: 10.3390/w14223634.

[29] Zhan, M., Sun, Y., Lan, H., Zhou, T., Zhao, Y., & Yang, L. (2025). Spatio-temporal distribution of soil microbial communities and nutrient availability around a municipal solid waste landfill. *Frontiers in Microbiology*, 16, article number 1583149. doi: 10.3389/fmicb.2025.1583149.

Несанкціоновані сміттєзвалища як джерело важких металів: вплив морфологічного складу відходів

Сергій Лелюшок

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0009-0002-8576-8950

Олена Наумовська

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
Науково-дослідний відділ парку-пам'ятки садово-паркового мистецтва національного значення «Межигір'я»
07354, вул. Івана Франка, 19, с. Нові Петрівці, Україна
https://orcid.org/0000-0002-5938-8471

Анотація. В умовах зростання кількості локальних, неконтрольованих місць розміщення відходів в Україні проблема оцінки їх впливу на стан ґрунтів набуває особливої актуальності, зважаючи на високий потенціал міграції токсичних елементів та тривалий період їхньої екологічної активності. Метою дослідження був аналіз взаємозв'язку між морфологічною структурою відходів на несанкціонованих сміттєзвалищах та рівнями накопичення важких металів у ґрунтах техногенно навантажених територій. У дослідженні застосовано стандартизовану методику відбору проб твердих побутових відходів та ґрунтів згідно з національними та міжнародними вимогами. Морфологічний склад твердих побутових відходів визначали шляхом фракційного сортування за категоріями: пластик, папір і картон, скло, метали, органічні відходи, текстиль, будівельні матеріали та інші змішані фракції. Відбір ґрунтових зразків здійснювався з глибини 0-20 см, що відповідає зоні найбільшої акумуляції техногенних металів. Концентрації Cd2+, Cu2+ та Zn2+ визначали із застосуванням атомно-абсорбційної спектрометрії, що забезпечило високу точність кількісного аналізу. Для статистичної обробки результатів використано кореляційний аналіз, оцінку коефіцієнтів детермінації та побудову регресійних моделей для визначення зв'язку між часткою окремих морфологічних фракцій та рівнями забруднення ґрунтів. Додатково проведено оцінку екологічного ризику за індексами накопичення, коефіцієнтом забруднення та модифікованим індексом потенційної екологічної небезпеки. Отримані дані виявили статистично значущі зв'язки між підвищеним вмістом важких металів у ґрунтовому покриві та наявністю у складі відходів таких фракцій, як металобрухт, дрібнодисперсний пластик, електронні та електротехнічні компоненти, а також будівельні матеріали, що містять фарби, лаки та інші полімерні суміші. Найбільш інтенсивно до ґрунту мігрують Pb2+ та Cd2+, що корелюють з фракціями старих батарейок, дрібних металевих предметів та електронних відходів. Органічна фракція, хоча і не є прямим джерелом важких металів, сприяє їх мобілізації внаслідок процесів мінералізації та утворення органічних кислот

Ключові слова: коефіцієнт концентрації; індекс забруднення; рухомі форми елементів; адсорбція; фільтрати

Received: 05.01.2026 Revised: 21.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 31-47

UDC 628.4:504.06(282.243.7)

DOI: 10.31548/biologiya/2.2026.31

Efficiency assessment of regional plastic waste collection systems in mountain and border regions: A case study of the Upper Tisza Basin

Ruslan Shvarts*

Postgraduate Student
Uzhhorod National University
88000, 3 Narodna Sq., Uzhhorod, Ukraine
https://orcid.org/0009-0003-2560-3975

Oleg Glukh

Associate Professor
Uzhhorod National University
88000, 3 Narodna Sq., Uzhhorod, Ukraine
https://orcid.org/0000-0002-5725-1981

Abstract. Mountain and border regions face pronounced challenges in organising efficient plastic waste collection due to complex terrain, dispersed settlement patterns and limited infrastructural capacity. These challenges are particularly critical in transboundary river basins, where mismanaged plastic waste generates cumulative downstream environmental risks. The Upper Tisza Basin, shared by Ukraine and several European Union countries, represents a high-risk area for riverine plastic transport and cross-border pollution. The aim of this study was to assess the efficiency of regional plastic waste collection systems in mountain and border conditions and to identify key factors determining system performance. The research was based on a mixed-method approach combining municipal waste statistics, geospatial accessibility analysis, field observations and qualitative assessment of municipal documentation and publicly available operational reports. System efficiency was evaluated using quantitative indicators including plastic collection rate per capita, container coverage, collection frequency and terrain-adjusted logistical performance. The results revealed substantial spatial disparities in collection efficiency. Lowland municipalities achieved plastic collection rates of 9.8-12.3 kg per inhabitant per year, whereas mountainous municipalities collected only 3.2-5.7 kg per inhabitant per year. In mountainous areas, less than 50% of residents had access to collection points within a 300 m radius, compared to over 80% in lowland settlements. Logistical inefficiency in high-altitude municipalities was more than twice as high due to poor road accessibility and elevation changes. Community-driven initiatives, including decentralised collection hubs and river clean-up programmes, increased local collection efficiency by up to 30% in underserved areas. The findings demonstrated that improving plastic waste collection in mountain and border regions requires integrated solutions combining infrastructural optimisation, terrain-sensitive logistics, institutional coordination and community engagement. The proposed assessment framework could be applied to other transboundary mountain regions to enhance environmental safety and sustainable resource use

Keywords: plastic waste management; spatial accessibility; border areas; riverine pollution; collection efficiency; river basin management; environmental safety

Suggested Citation:
Shvarts, R., & Glukh, O. (2026). Efficiency assessment of regional plastic waste collection systems in mountain and border regions: A case study of the Upper Tisza Basin. *Biological Systems: Theory and Innovation*, 17(2), 31-47. doi: 10.31548/biologiya/2.2026.31.

*Corresponding author (ruslan.shvarts@uzhnu.edu.ua)

INTRODUCTION

Efficient plastic waste management is a critical component of environmental safety, particularly in mountain and border regions characterised by dispersed settlement patterns, limited infrastructural capacity and elevated transboundary ecological risks (Bhandari *et al.*, 2021). In such territories, insufficient waste collection systems contribute not only to local environmental degradation but also to the accumulation and downstream transport of plastic waste through shared river basins. This problem is especially significant in the Upper Tisza Basin, where complex terrain, hydrological connectivity and uneven municipal service provision increased the likelihood of riverine plastic leakage beyond administrative and national boundaries. In the Ukrainian upstream section of the basin, within the Zakarpattia region, significant disparities in accessibility, settlement density, and collection infrastructure have led to uneven conditions for separate plastic waste collection. As a result, assessing the efficiency of the system is crucial for understanding environmental safety at the basin level, as noted by C.C. Chen (2010). Recent comprehensive evaluations of the Carpathian watercourses, as identified by M. Liro *et al.* (2023), have highlighted this macroregion's high vulnerability to mismanaged plastic waste, with notable hotspots in Ukraine, Romania, and Hungary.

Recent scientific literature increasingly addressed the challenges of plastic waste management in geographically constrained and environmentally sensitive regions. As highlighted by F.-C. Mihai *et al.* (2022), indicates that rural and mountain communities face ongoing infrastructural deficiencies, limited service coverage, and restricted capacity for implementing circular economy practices, all of which heighten the risk of unmanaged plastic waste accumulation outside urban areas. Meanwhile, recent evaluations of Carpathian watercourses, conducted by M. Liro *et al.* (2023), have confirmed the macroregion's significant vulnerability to mismanaged plastic waste and the formation of hotspots along river corridors, including within Ukraine. These findings supported the relevance of evaluating plastic waste collection efficiency in the Ukrainian section of the Upper Tisza Basin as a mountain-border territory exposed to both local service deficits and downstream environmental risks. The study by U. Leknoi *et al.* (2024) explores sustainable community building through successful waste management initiatives, providing valuable insights into effective practices that can be applied in rural and mountain communities to mitigate plastic waste accumulation. On the other hand, the study by V. Nava *et al.* (2024) investigates how plastic pollution impacts ecosystem processes, including community structure and functional traits in large rivers. This study is highly relevant, as it underscores the broader ecological consequences of mismanaged plastic waste, particularly along river corridors. Previous studies on the Tisza River by T. Kiss *et al.* (2021) have shown that local sources in tributaries play a significant role in the spatial distribution of plastic pollution, highlighting the complexity of transboundary sediment and waste transport. Studies focusing on mountain waste management in the Carpathian region have shown that infrastructure gaps and policy inconsistencies at the municipal level lead to uneven service provision and higher risks of illegal dumping in remote settlements. These findings indicate that mountainous terrain significantly increases logistical complexity and requires differentiated management approaches rather than uniform national strategies.

Despite the growing body of research on waste management, existing studies have mainly focused on urban systems or national-level frameworks, while integrated assessments of plastic waste collection in transboundary mountain regions remain limited. In particular, insufficient attention has been paid to municipal-level plastic collection efficiency in upstream territories where geographical constraints, infrastructural accessibility, institutional capacity and community participation directly influence cross-border riverine pollution dynamics. This research gap highlights the need for a region-specific analytical framework that combines spatial accessibility analysis, quantitative performance indicators and institutional evaluation to better understand how collection inefficiencies translate into environmental safety risks at the basin scale. The present study addressed this gap by conducting a systematic efficiency assessment of plastic waste collection systems within the Ukrainian section of the Upper Tisza Basin. Particular attention was devoted to the interaction between infrastructural distribution, logistical constraints, institutional capacity and community engagement in shaping municipal-level performance. The objective of this research was to determine how geographical and governance-related factors influenced the efficiency of plastic waste collection in a transboundary mountain basin and to identify priority directions for improving environmental safety through enhanced waste management performance.

MATERIALS AND METHODS

Study area. The study was conducted within the Ukrainian section of the Upper Tisza Basin, located in Zakarpattia region in western Ukraine. The analysed territory included municipalities situated within the hydrological boundaries of the Upper Tisza River and its tributaries upstream of the Ukrainian-Hungarian border. Geographically, the study area extended between approximately 47°53′-48°45′ N latitude and 22°08′-24°30′ E longitude. The assessment covered municipalities belonging to Uzhhorod, Mukachevo, Khust, Rakhiv and Tyachiv districts, encompassing both lowland settlements in the western part of the region and mountainous communities located in the Carpathian highlands. Particular attention was devoted to municipalities situated along the Tisza River and its tributaries, including the Uzh, Latorytsia, Rika and Tereblia rivers. These river systems functioned as primary hydrological pathways facilitating the transport of mismanaged plastic waste during flood events.

The study area is characterised by pronounced altitudinal variation ranging from approximately 100 metres above sea level in lowland territories to over 2,000 metres in mountainous zones. Settlement patterns differed substantially between districts: lowland municipalities demonstrated higher population density and more developed road infrastructure, whereas mountainous communities were characterised by dispersed settlements, limited transport accessibility and seasonal road constraints. Seasonal factors were considered in the analysis. Tourist flows in mountain municipalities increased during summer and winter recreational periods, leading to temporary growth in municipal solid waste generation. Additionally, spring flood events significantly influenced plastic waste mobilisation and riverine transport. These seasonal dynamics were incorporated into the interpretation of collection efficiency and leakage risks.

Research design and data collection. A mixed-method research design was applied to assess the efficiency of plastic waste collection systems in the Upper Tisza Basin. The methodological framework combined quantitative analysis of municipal waste indicators with spatial modelling and qualitative institutional assessment. The integration of multiple analytical approaches ensured methodological triangulation and enhanced the reliability of findings. The research design incorporated: statistical analysis of municipal waste management data; geospatial accessibility assessment of collection infrastructure; field observations of collection points and informal dumping sites; document-based qualitative analysis of institutional and operational practices.

Data were collected for the period 2022-2024 from official statistical and administrative sources. Demographic indicators for the study area were derived from the State Statistics Service of Ukraine & the Main Department of Statistics in Zakarpattia Region (2022), including district-level population estimates as of 1 January 2022. Regional contextual data on waste-management conditions, disposal infrastructure and environmental pressures were compiled from the Environmental Passport of Zakarpattia Region for 2022 (2023) and Environmental Passport of Zakarpattia Region for 2023 (2024) and from the Regional Waste Management Plan of Zakarpattia Region to 2030 (Zakarpattia Regional State Administration, 2021). Municipality-level information on collection infrastructure, service frequency and local organisational arrangements was compiled from publicly available reports and official webpages of the selected municipalities and municipal service providers within the study area.

Geospatial data were derived from open-access satellite imagery and digital spatial datasets. Sentinel-2 imagery was accessed through the Copernicus Data Space Ecosystem (n.d.) for land-use verification and visual interpretation. Elevation data were obtained from the Shuttle Radar Topography Mission (SRTM) digital elevation model distributed through the USGS EarthExplorer (n.d.) platform. Road-network and settlement-related spatial data were derived from OpenStreetMap (n.d.) datasets. Spatial processing and analysisCwere performed in QGIS. Spatial analysis was conducted using QGIS software (version 3.x). GIS-based modelling enabled the mapping of official plastic collection points in relation to settlement distribution, road accessibility and elevation gradients. Buffer analysis was applied to estimate service coverage within a 300-metre walking radius. Terrain constraints were assessed through slope analysis derived from the DEM (Digital Elevation Models) dataset.

Field observations were conducted during 2023 in selected lowland and mountainous municipalities of Zakarpattia region, including Uzhhorod urban territorial community, Mukachevo urban territorial community, Rakhiv urban territorial community, Tyachiv urban territorial community and Mizhhiria settlement territorial community. Onsite inspections focused on the physical condition of collection points, evidence of overflow, proximity to watercourses and signs of informal dumping along riverbanks and forest roads. Qualitative assessment was based on document analysis of publicly available municipal reports, official webpages of local authorities and communal service providers, and field observation records from the study area. No interviews, questionnaires or other forms of direct human-subject data collection were conducted in this study. As the analysis relied exclusively on aggregated statistical data and publicly accessible institutional information, no personal data were processed and no additional ethical approval was required.

Community-engagement data were compiled from publicly available materials of civic and project-based waste initiatives active in Transcarpathia during 2022-2024, particularly the Plastic Cup/PET Kupa initiative and the CALL-Action programme (Plastic Cup, 2023; Interreg Danube Region, 2024). Analysed information included publicly reported amounts of collected or processed waste, references to Ecobus and other collection activities, territorial coverage of actions, and the geographic focus of community-based interventions in Uzhhorod, Berehove and other Tisza-adjacent settlements. These materials included official project webpages, public reports of clean-up organisers and online summaries of cross-border river-cleaning actions published during 2022-2024.

Geospatial assessment constituted a core component of the methodological framework. All officially reported plastic waste collection points were mapped within a GIS environment and analysed in relation to settlement distribution, road networks and elevation gradients. Spatial modelling was conducted in QGIS using vector-based municipal boundaries and raster-based elevation data. Service accessibility was evaluated using buffer analysis with a 300-metre walking radius around each collection point. This threshold was applied in accordance with commonly used accessibility standards in municipal waste management studies. Catchment areas were overlaid with population distribution data to estimate the proportion of residents with direct access to plastic collection infrastructure. Terrain-related constraints were quantified through slope analysis derived from the SRTM digital elevation model. Road accessibility was evaluated based on distance to paved roads extracted from OpenStreetMap datasets. These spatial indicators were later incorporated into the logistical accessibility index.

Efficiency indicators and index calculation. To ensure comparability across municipalities with differing geographical and socio-economic characteristics, quantitative indicators were normalised using min-max scaling. Normalisation was performed according to equation (1):

$$X_{norm,i} = \frac{X_i - X_{min}}{X_{max} - X_{min}}, \quad (1)$$

where X_i represented the observed value of the indicator for municipality *i*, and X_{min} and X_{max} denoted the minimum and maximum values across the study area. A Logistical Accessibility Index (LAI) was calculated to reflect terrain-related operational constraints:

$$LAI_i = \sum_{j=1}^{n} w_j \cdot X_{norm,j}, \quad (2)$$

where $X_{norm,j}$ represented normalised terrain-related variables, including elevation gradient and road accessibility. Equal weighting coefficients (*wj*) were applied due to the absence of prior empirical evidence supporting differential weighting. A composite Plastic Waste Collection Efficiency Index (PWCE) was derived by integrating the primary performance indicators:

$$\mathrm{PWCE}_i = \frac{CR_i + CC_i + CF_i + \mathrm{LAI}_i}{4}, \quad (3)$$

where *CR* represented the normalised plastic collection rate per capita; *CC* represented container coverage; *CF* denoted collection frequency; LAI represented logistical accessibility. All four dimensions were weighted equally to avoid introducing subjective bias. The resulting composite index ranged from 0 to 1, where higher values indicated greater collection efficiency. These indices were subsequently used in the Results section to compare performance disparities between lowland and mountainous municipalities.

In addition to municipal efficiency assessment, the integrated analytical framework was used to formulate a conceptual model for evaluating plastic waste collection efficiency in mountain-border regions. The model was derived from the combined interpretation of geospatial accessibility indicators, operational performance metrics, institutional constraints and community-participation factors identified during the study. Equal weighting was retained at the indicator aggregation stage, while interdependencies between dimensions were interpreted qualitatively in the Discussion section, including the influence of seasonal variability (tourism peaks and flood periods) on system performance.

Qualitative analysis. Qualitative analysis complemented the quantitative and spatial assessment by providing contextual interpretation of institutional and operational factors influencing plastic waste collection efficiency. Analytical review of municipal reports, publicly available operational documentation and field observation notes was conducted using thematic categorisation. The qualitative evaluation focused on four principal dimensions: institutional coordination between municipalities and waste operators; financial and logistical constraints affecting service provision; public awareness and participation patterns; interactions between formal waste management systems and community-based initiatives. Thematic coding allowed the identification of recurring structural barriers, including fragmented governance arrangements, limited municipal budgets and infrastructure deficits in mountainous territories. Qualitative findings were used to interpret disparities revealed by quantitative efficiency indicators.

RESULTS AND DISCUSSION

Infrastructure distribution and accessibility

The analysis revealed pronounced disparities in the distribution and accessibility of plastic waste collection infrastructure across municipalities of the Ukrainian section of the Upper Tisza Basin. Higher levels of infrastructure coverage were identified in lowland municipalities of Uzhhorod and Mukachevo districts, where denser settlement patterns and better road connectivity supported more frequent servicing and a greater number of collection points. In contrast, mountainous municipalities of Rakhiv, Tyachiv and Khust districts demonstrated substantially lower infrastructure density, especially in remote settlements located along upper tributaries and valley-based settlement clusters (State Statistics Service of Ukraine & the Main Department of Statistics in Zakarpattia Region, 2022).

GIS-based accessibility assessment (utilising the buffer approach alongside indicator normalisation as described in equation (1) and subsequent index integration) showed that lowland municipalities generally had a substantially larger share of residents living within a 300-metre walking distance of official plastic collection points. In mountainous communities, this proportion was markedly lower due to dispersed settlement patterns, steep slopes and limited road access. Spatial mismatches between container placement and residential distribution were observed most clearly in peripheral villages and hamlets with fragmented street networks (Kwikima & Ngole, 2025). Terrain-related constraints significantly affected operational feasibility. Slope conditions, distance to paved roads and local road quality reduced the practicality of installing and regularly servicing additional collection points in high-altitude settlements. These constraints were reflected in lower values of the logistical accessibility component (LAI, equation (2)), which was later incorporated into the composite plastic waste collection efficiency index (PWCE, equation (3)).

From an environmental safety perspective, uneven infrastructure accessibility increased the risk of unmanaged plastic accumulation near households, roadside areas and watercourses. In municipalities with limited collection coverage, the absence of convenient disposal options created conditions for informal dumping and litter leakage into tributaries of the Tisza River. Such infrastructure deficits therefore represented not only a service performance problem but also a direct factor contributing to riverine plastic pollution and transboundary environmental risk formation (Environmental Passport of Zakarpattia Region for 2022, 2023). Seasonal factors further intensified these disparities. During periods of heavy rainfall and spring flood events, access to remote collection points became less reliable in mountainous areas, which increased the likelihood of delayed collection and local overflow. In tourist-active mountain municipalities, temporary increases in waste generation placed additional pressure on already limited infrastructure, particularly near recreation zones and river-adjacent settlements (Environmental Passport of Zakarpattia Region for 2023, 2024).

Overall, the spatial assessment demonstrated that infrastructure distribution and accessibility constituted foundational determinants of municipal plastic waste collection performance in the Upper Tisza Basin. The observed territorial disparities also indicated that improvements in environmental safety at the basin scale depended on targeted infrastructure planning in underserved upstream mountain communities rather than on uniform expansion measures across all municipalities. Furthermore, the findings suggested that a more localised, context-specific approach to infrastructure development would be crucial for addressing the unique challenges faced by these communities in managing plastic waste effectively.

Collection performance and quantitative indicators

Significant inter-municipal differences were identified in the quantitative indicators of plastic waste collection performance across the Ukrainian section of the Upper Tisza Basin. The comparative assessment showed that municipalities located in lowland districts demonstrated consistently higher collection efficiency than mountainous communities. This pattern reflected the combined influence of infrastructure density, service frequency and terrain-related logistical accessibility. The quantitative indicators presented in Table 1 were calculated using the normalisation and composite-index procedures described in equations (1-3). In particular, equation (1) was applied for min-max normalisation of municipal indicators, equation (2) was used to derive the logistical accessibility index, and equation (3) was used to calculate the composite plastic waste collection efficiency index.

Table 1. Comparative indicators of plastic waste collection efficiency in lowland and mountainous municipalities of the Ukrainian section of the Upper Tisza Basin

Indicator	Lowland municipalities	Mountainous municipalities
Plastic collection rate (kg/inhabitant/year)	9.8-12.3	3.2-5.7
Container coverage (units per 1,000 inhabitants)	12-15	4-6
Population with access to collection points within 300 m (%)	80-85	35-50
Collection frequency (rounds per month)	6-8	2-3
LAI	0.68-0.81	0.29-0.44
Composite PWCE	0.71-0.83	0.34-0.48

Note: indicator values are presented as ranges reflecting inter-municipal variability within each group. LAI was calculated using equation (2); PWCE values were calculated using equation (3) after indicator normalisation according to equation (1). "Lowland municipalities" refer to municipalities predominantly located in Uzhhorod and Mukachevo districts; "mountainous municipalities" refer to municipalities predominantly located in Rakhiv, Tyachiv and Khust districts
Source: developed by the authors based on State Statistics Service of Ukraine & the Main Department of Statistics in Zakarpattia Region (2022)

Table 1 demonstrates that the strongest disparities between municipality groups were observed in container coverage, service accessibility within a 300-metre radius and collection frequency. These parameters directly influenced both the plastic collection rate and the composite PWCE values, indicating that municipal performance depended on the combined effect of infrastructure availability and operational feasibility. From an environmental safety perspective, the lower indicator values recorded in mountainous municipalities indicated a higher probability of unmanaged plastic accumulation and subsequent riverine leakage into the Tisza tributary network. Lowland municipalities achieved higher plastic collection rates per inhabitant and better container coverage, which corresponded to a larger population share with direct access to collection points. In contrast, mountainous municipalities demonstrated lower collection rates and lower service coverage, indicating structural limitations in infrastructure provision and collection logistics. The identified disparities confirmed that insufficient accessibility and lower collection frequency remained key constraints on the performance of plastic waste collection systems in mountainous territories.

Operational frequency also differed substantially between municipality groups. More frequent collection rounds in lowland areas reduced the probability of container overflow and secondary littering near collection points, while lower collection frequency in mountainous communities increased the risk of local plastic accumulation. This difference had direct environmental implications, as delayed collection in settlements located near tributaries increased the likelihood of plastic leakage into river systems during heavy rainfall and flood-related runoff events. The logistical accessibility index (LAI) and the composite PWCE values demonstrated the cumulative effect of terrain constraints on municipal system performance. Lower LAI values in mountainous municipalities reflected poorer road access and more difficult topographic conditions, which reduced service efficiency even where collection points were formally present. Therefore, the results indicated that infrastructure expansion alone would not be sufficient without simultaneous improvements in route planning, seasonal service adaptation and local collection logistics.

Riverine plastic leakage

Field observations conducted in 2023 in Uzhhorod urban territorial community, Mukachevo urban territorial community, Rakhiv urban territorial community, Tyachiv urban territorial community and Mizhhiria settlement territorial community, together with publicly available materials from the Plastic Cup / PET Kupa initiative and the CALL-Action programme, indicated that riverine plastic leakage remained a significant environmental safety problem in the Ukrainian section of the Upper Tisza Basin (Plastic Cup, 2023; Interreg Danube Region, 2024). Plastic waste accumulation was observed along riverbanks, floodplain margins and riparian vegetation, particularly near settlements with limited collection infrastructure and lower service frequency. The most frequently observed fractions included PET bottles, packaging film and disposable food packaging. This pattern was consistent with broader scientific evidence showing that rivers and riparian zones function both as transport pathways and as retention zones for macroplastic accumulation (Gallitelli & Scalici, 2022).

The spatial pattern of leakage hotspots in the observed municipalities corresponded to settlement clusters characterised by insufficient infrastructure accessibility and irregular collection schedules. In such locations, unmanaged plastic waste accumulation near households, roadsides and local drainage pathways increased the probability of transport into tributaries during precipitation events. This pattern strengthened the link between municipal waste collection efficiency and downstream environmental risk formation in the Tisza basin. Seasonal dynamics played an important role in leakage intensity. During spring floods and periods of heavy rainfall, accumulated plastic waste from floodplains and near-channel areas was mobilised and transported downstream. In mountain municipalities with seasonal tourism, temporary increases in waste generation during recreation periods placed additional pressure on local collection systems and contributed to higher plastic accumulation near river-adjacent settlements and informal recreation zones. Publicly available reports and cross-border initiative materials also confirmed the relevance of the Upper Tisza and wider Tisza basin as a hotspot of riverine plastic transport. Peer-reviewed evaluations of citizen science campaigns, such as the PET Kupa initiative, combined with remote sensing, have documented the repeated large-scale removal of macroplastic waste from the Tisza system, emphasising that upstream source-management deficits directly drive downstream pollution dynamics (Di *et al.*, 2025). International academic reviews on riverine litter further highlighted the practical role of coordinated clean-up actions and prevention-oriented basin management for reducing transboundary plastic pollution.

Community clean-up initiatives operating in the region provided additional indirect evidence of persistent leakage pressure by regularly reporting collected volumes, recurring hotspot locations and repeated accumulation after flood events. These observations indicated that clean-up actions, while environmentally beneficial, did not substitute for systematic improvements in municipal collection coverage and service accessibility in upstream settlements. From an environmental protection perspective, riverine plastic leakage in the Upper Tisza Basin represented a cumulative risk mechanism linking local infrastructure deficits with downstream ecological impacts. The findings indicated that targeted improvements in upstream plastic collection systems could reduce plastic transfer into transboundary river channels and strengthen basin-scale environmental safety.

Institutional and socio-economic factors

Institutional and socio-economic conditions substantially influenced the performance of plastic waste collection systems across municipalities of the Ukrainian section of the Upper Tisza Basin. Analysis of municipal documentation, official webpages of local authorities and communal service providers, regional planning materials, and field observation records indicated that mountainous municipalities faced more persistent organisational and service-related constraints than lowland municipalities (Zakarpattia Regional State Administration, 2021; Environmental Passport of Zakarpattia Region for 2022, 2023). In practice, these constraints were reflected in lower service frequency, weaker infrastructure coverage and greater operational instability in remote settlements. Institutional fragmentation further complicated service delivery in geographically dispersed territories. In the observed municipalities, waste collection responsibilities were divided between local authorities, communal enterprises and contracted operators, which reduced consistency in route planning, servicing schedules and monitoring practices. These coordination difficulties were more visible in mountainous municipalities, where long travel distances, road limitations and dispersed settlement structure increased the dependence of service efficiency on well-coordinated operational arrangements. This interpretation was consistent with broader evidence showing that fragmented governance reduces the effectiveness of municipal waste services in territorially complex settings.

Socio-economic differences between lowland and mountainous municipalities also affected the practical functioning of separate collection systems. Infrastructural accessibility remained the dominant determinant of use; however, field observations and document analysis suggested that the visibility of collection points, regularity of servicing and presence of local environmental outreach activities also influenced the practical use of separate collection infrastructure. In municipalities where collection points were sparse or collection rounds were irregular, lower practical utilisation of designated plastic containers was observed, together with more frequent mixed disposal near collection sites (Environmental Passport of Zakarpattia Region for 2023, 2024). The community-participation dimension considered in the qualitative interpretation of results. It was interpreted as a contextual characteristic derived from observable local activity patterns, publicly documented outreach practices and the documented presence of community-driven environmental initiatives within the municipality groups. This approach reduced the risk of overstating behavioural explanations where infrastructural and logistical constraints remained the primary limiting factors.

From an environmental safety perspective, institutional and socio-economic limitations increased the probability that infrastructure deficits would translate into unmanaged plastic accumulation in residential and river-adjacent areas. These factors therefore amplified the environmental consequences of already existing geographical and logistical constraints, especially in upstream mountain municipalities. As a result, the inability to address these limitations could lead to the worsening of plastic pollution, further endangering local ecosystems and complicating future environmental management efforts.

Role of community-driven initiatives

Community-driven initiatives demonstrated a significant complementary role in improving plastic waste collection performance in underserved municipalities of the Ukrainian section of the Upper Tisza Basin. The qualitative and comparative assessment indicated that volunteer clean-up campaigns, local decentralised collection practices and cross-border environmental actions partially compensated for infrastructural and logistical limitations of formal municipal waste services, especially in mountain settlements with difficult access. Concrete examples included activities associated with the Plastic Cup / PET Kupa network and the CALL-Action programme operating in Transcarpathia and the wider Tisza basin (Plastic Cup, 2023; Interreg Danube Region, 2024). Publicly available project materials documented selective waste collection support, Ecobus-related activities, repeated clean-up actions and broader community engagement measures in Uzhhorod, Berehove and other Tisza-connected settlements. These initiatives contributed not only to the direct removal of accumulated waste from river-adjacent areas, but also to the visibility of the plastic-waste problem at community level.

The comparative analysis also indicated that municipality groups where community-led activities were present tended to demonstrate higher average composite efficiency values (PWCE) than municipality groups relying exclusively on formal waste collection services. This pattern was particularly evident in mountainous municipalities, where decentralised local actions improved interim collection, sorting and transfer of plastic waste in areas with irregular municipal servicing. From an environmental management perspective, the role of community initiatives should be interpreted as complementary rather than substitutive. Their practical contribution was greatest where formal infrastructure coverage and service regularity remained insufficient. The quantitative comparison of municipality groups with and without community-driven initiatives is presented in Table 2.

Table 2. Influence of community-driven initiatives on plastic waste collection efficiency in selected municipality groups of the Ukrainian section of the Upper Tisza Basin

Municipality type	Presence of community initiatives	PWCE (average)	Increase in collection efficiency (%)
Lowland	No	0.72	–
Lowland	Yes	0.81	+12
Mountainous	No	0.36	–
Mountainous	Yes	0.47	+30

Note: PWCE – composite Plastic Waste Collection Efficiency Index calculated according to equation (3) after normalisation of component indicators under equation (1); “Presence of community initiatives” indicates the documented presence of volunteer clean-up campaigns, decentralised collection hubs or local environmental outreach activities within the municipality group; “Increase in collection efficiency (%)” shows the relative difference in average PWCE values between municipality groups with and without such initiatives; “–” – no data available
Source: developed by the authors

Table 2 indicated that the association between community-driven initiatives and improved collection efficiency was stronger in mountainous municipalities than in lowland municipalities. This result suggested that local volunteer actions and decentralised collection practices played a compensatory role where formal infrastructure coverage and service frequency remained limited. At the same time, the table also showed that community initiatives complemented rather than replaced formal municipal systems, as the highest efficiency values were observed where community engagement operated alongside more stable collection infrastructure and regular servicing. Overall, the findings confirmed that community-driven initiatives constituted an important adaptive mechanism for improving plastic waste management performance in mountain-border regions. Their greatest practical value was observed in settlements with infrastructural deficits, where local actions reduced leakage risks and supported interim recovery of plastic waste. From an environmental safety perspective, integration of community initiatives into municipal planning represented a feasible and cost-effective direction for reducing riverine plastic pollution in upstream areas of the Upper Tisza Basin.

Influence of geographical and infrastructural constraints on operational efficiency

The results of the present study are consistent with recent evidence showing that waste collection performance in mountain and low-density territories depends not only on the formal availability of infrastructure, but also on the practical relationship between settlement structure, collection mode and route feasibility. A.P.D. Baltrocchi *et al*. (2024), comparing mixed and full door-to-door separate collection systems in two mountainous Italian valleys, demonstrated that collection performance in mountainous areas was strongly affected by territorial configuration and service design. Their results showed that the full door-to-door system achieved better separate-collection performance than the mixed system, indicating that the effectiveness of waste collection in mountain territories cannot be assessed by infrastructure presence alone. This was consistent with the findings of the present study, where municipalities with formally existing collection points still demonstrated lower collection efficiency when accessibility, road conditions and settlement dispersion limited practical service use.

A broader mountainous-region perspective was provided by K. Zhou *et al*. (2024), who developed an integrated framework for assessing environmental risks of domestic waste management systems in mountainous regions. Their study emphasised the combined role of scattered settlements, ecological fragility, limited traffic accessibility and low disposal capacity in generating elevated environmental risks. The Upper Tisza Basin findings were similar in that mountainous municipalities combined weaker infrastructure coverage with more difficult access and greater operational instability. However, the present study extended this perspective by linking these territorial disadvantages specifically to plastic collection efficiency and to riverine leakage risk in an upstream transboundary basin. The geospatial dimension of the present analysis also corresponded to recent GIS-based optimisation research. M.M. Kwikima & F. Ngole (2025), in a case study from Sumbawanga municipality, showed that GIS-based optimisation of collection points and routes substantially improved service coverage and reduced fuel use and driver hours. This supported the methodological relevance of spatial accessibility analysis in the present study, where QGIS-based assessment helped reveal that the weakest-performing municipalities were not simply those with fewer containers, but those where container location, dispersed settlement structure and route difficulty interacted to reduce actual service effectiveness.

Overall, the comparison indicated that geographical and infrastructural constraints should be interpreted as structural determinants of waste collection efficiency, but their practical effects depended on service design, routing logic and territorial configuration. In this respect, the Upper Tisza Basin case contributed a more environmentally specific interpretation by demonstrating that in upstream mountain municipalities these operational inefficiencies also increased the probability of plastic leakage into river corridors with transboundary consequences. Moreover, the study highlighted the need for region-specific solutions that consider the unique challenges of these areas, ensuring more effective waste management strategies to mitigate the cross-border environmental risks.

Riverine leakage and seasonal dynamics

The interpretation of riverine leakage in the present study was consistent with region-specific evidence showing that mountain river systems of the Carpathians are highly exposed to mismanaged plastic waste inputs. M. Liro *et al*. (2023), using high-resolution river-network and mismanaged plastic waste databases for the Carpathian Ecoregion, identified extensive plastic-waste hotspots along Carpathian watercourses, including substantial hotspot lengths in Ukraine. This comparison was important for the Upper Tisza Basin because it confirmed that the observed leakage risk was not an isolated local anomaly, but part of a broader mountain-river pattern in which river corridors function as receivers and conveyors of mismanaged plastic waste.

A more direct transboundary comparison was provided by T. Kiss *et al*. (2021), who analysed microplastic deposition along the Tisza River and its tributaries and found that tributaries transported large amounts of microplastics into the main river, while the most upstream sections showed particularly high contamination associated with lower levels of wastewater management. The present study was consistent with this upstream-downstream logic, but extended it from sediment contamination to municipal collection performance by showing that infrastructural and service deficiencies at municipality level could be interpreted as practical drivers of leakage risk into tributaries and the main Tisza channel. The seasonal dimension identified in the present study was also supported by recent flood-focused river-plastic research. T.H.M. van Emmerik *et al*. (2023) demonstrated that extreme floods strongly amplify plastic mobilisation, transport and retention in river systems, with a substantial share of annual plastic transport occurring during a short flood period. This comparison supported the interpretation that spring floods and high-rainfall episodes in the Upper Tisza Basin were not merely background hydrological conditions, but critical temporal windows during which previously accumulated plastic waste was redistributed from riverbanks, floodplains and near-channel accumulation zones into the active river network. This interpretation is further supported by a recent review E.K. Owowenu *et al*. (2023) showing that precipitation, run-off, flow velocity, channel morphology and riparian trapping strongly influence plastic transport dynamics in riverine systems.

The tourism-related seasonal pressure identified in mountain municipalities also corresponded to broader rural plastic-pollution evidence. F.-C. Mihai *et al*. (2022) argued that rural communities should be understood both as contributors to plastic leakage and as receivers of pollution pressures, and specifically noted the relevance of tourism-related plastic pollution in rural environments. In the Upper Tisza Basin, this perspective helped explain why temporary increases in visitor numbers in river-adjacent recreation areas could intensify plastic accumulation even where permanent population density remained low. Overall, the comparison with recent literature indicated that riverine leakage in the Upper Tisza Basin should be interpreted as a dynamic interaction between municipal service limitations, riparian retention processes and seasonal hydrological remobilisation. In this respect, the present study contributed a municipality-level explanation of why upstream collection inefficiencies in a mountain-border basin could generate cumulative downstream environmental risks with transboundary relevance.

Institutional limitations and governance challenges

The institutional barriers identified in the present study were consistent with broader evidence showing that fragmented governance structures reduced the effectiveness of municipal waste management systems in rural and border regions (Popa, 2022). A similar pattern was observed in the Upper Tisza Basin, where differences in municipal capacity, operator arrangements and local service planning contributed to uneven collection performance. Comparable conclusions have been reported in previous studies of municipalities, which showed that policy inconsistencies and infrastructure deficits, combined with limited local institutional capacity, contributed to territorial inequality in waste service provision (Popa, 2022; Zhang *et al*., 2023). The present study supported this interpretation by demonstrating that municipalities with constrained budgets and organisational fragmentation faced greater difficulty in maintaining regular collection schedules and extending services to remote settlements. The governance-related interpretation of the present findings was consistent with broader research on rural waste service efficiency, where operational inefficiencies were associated not only with accessibility constraints but also with planning and service management limitations. In the Upper Tisza Basin, geographical barriers remained highly influential; however, the results indicated that institutional coordination affected the extent to which these barriers translated into persistent service instability.

The socio-economic dimension discussed in the present study also corresponded to broader evidence indicating that household participation in separate collection depended on the interaction between service accessibility, local outreach practices and the predictability of municipal collection systems (Zhang *et al*., 2023). In this respect, the findings supported the view that behavioural engagement was stronger where local initiatives and visible collection practices encouraged routine participation. At the same time, the present study differed from purely behavioural interpretations by showing that socio-economic and awareness-related factors should be interpreted within the constraints imposed by infrastructure and logistics. In the Upper Tisza Basin, low participation in mountainous municipalities could not be adequately explained without considering sparse collection-point distribution and irregular servicing. This comparison suggested that governance reforms and awareness activities were most effective when implemented alongside improvements in practical service accessibility. Overall, institutional fragmentation and unequal municipal capacity represented major constraints on waste collection efficiency in mountain-border regions. The Upper Tisza Basin case added further evidence that these governance limitations had direct environmental safety implications when they interacted with upstream riverine leakage pathways in a transboundary basin system.

Effectiveness of community-driven initiatives

The results of the present study are consistent with recent evidence showing that community-based waste initiatives can improve local waste-management performance when they are embedded in stable local organisational structures. U. Leknoi *et al*. (2024), analysing a community-led waste-management initiative in a low-income area near Bangkok, demonstrated that successful local action depended on broad participation, practical leadership and context-specific organisational arrangements. Their study showed that waste separation and community-led management could reduce local pollution pressures even under conditions of limited budgets and technological constraints. This was comparable to the Upper Tisza Basin findings, where community-driven initiatives partially compensated for infrastructural and logistical deficits in underserved municipalities.

A broader governance-oriented comparison was provided by S. Wang *et al*. (2025), who analysed 26 community waste-management experiments in China and identified three main pathways to success: committed participants supported by dedicated communities, committed participants backed by local government, and strong grassroots leadership combined with responsible public support. This comparison was particularly relevant to the present study because it confirmed that community initiatives were most effective when they operated together with municipal structures rather than independently of them. In the Upper Tisza Basin, the same pattern was observed: municipalities with documented community engagement achieved better practical waste-recovery performance, but the strongest results were associated with combinations of civic action, regular service provision and more stable infrastructure coverage. Compared with these studies, the Upper Tisza Basin case added a specific environmental-safety dimension. Here, community-driven initiatives are relevant not only because they improved local collection and sorting practices, but also because they reduce plastic accumulation in river-adjacent and upstream settlements where unmanaged waste could later be mobilised into tributaries of the Tisza. In this respect, the present study extends previous evidence by showing that in mountain-border river basins community action may have both a service-supporting function and a preventive role in reducing transboundary leakage risks.

Environmental safety implications and management relevance

The environmental-safety implications identified in the present study are consistent with recent evidence showing that plastic pollution in rivers affects not only water quality and biodiversity, but also broader ecosystem processes. V. Nava *et al*. (2024), analysing plastic pollution in large rivers, demonstrated that biofouled plastics altered community structure, metabolic functioning and oxygen conditions, with potentially profound ecosystem-level consequences. This comparison was relevant to the Upper Tisza Basin because it supported the interpretation that municipal plastic-collection deficiencies in upstream areas should be viewed not merely as a service-performance problem, but as a source of wider ecological disturbance in river systems.

The present findings are also consistent with emerging process-based evidence that plastic pollution can modify the physical functioning of rivers. C.E. Russell *et al*. (2023) showed that plastic in riverbeds was not a passive pollutant, but could alter sediment transport processes, bedform dynamics and the spatial distribution of plastic within fluvial sediments. In the Upper Tisza Basin, this perspective strengthened the management relevance of preventing plastic leakage at source, because repeated accumulation and remobilisation of plastic in upstream channels and riparian zones may intensify both ecological and hydromorphological impacts over time. From a governance perspective, the practical implications of the present study are consistent with recent freshwater-plastic policy research. Y. Shi *et al*. (2024) concluded that effective freshwater plastic governance should integrate source reduction, recycling and substitution measures, monitoring and evaluation, and direct pollution-control actions. This comparison aligns closely with the Upper Tisza Basin findings, which indicated that no single intervention would be sufficient in mountain-border municipalities. Instead, the most environmentally relevant management approach combined terrain-sensitive infrastructure planning, more reliable service schedules, local monitoring of leakage-prone river corridors and integration of community-led initiatives into formal waste-management arrangements. From a broader legal perspective, recent international-law analysis also shows that fragmented regulatory frameworks still hinder coordinated responses to cross-border plastic pollution, which reinforces the need for basin-scale cooperation in transboundary river systems (Shi & Zhang, 2026).

In general, the comparison with the literature confirmed that the effectiveness of plastic waste collection in the Upper Tisza Basin should be interpreted as a preventive mechanism for environmental safety. In this context, improving collection systems in the upstream areas of the river would not only support better efficiency of municipal waste management services but also reduce risks to river ecosystems, decrease the transport of plastic waste downstream, and contribute to more effective transboundary environmental governance. This, in turn, could lead to a reduction in the ecological burden on river ecosystems, contribute to the improvement of water resources, and decrease the negative impact of plastic pollution on biodiversity in transboundary reservoirs.

Conceptual model for assessing plastic waste collection efficiency in mountain-border regions

The findings of the study provided the basis for the formulation of a conceptual model for assessing plastic waste collection efficiency in mountain-border regions under conditions of environmental vulnerability and transboundary riverine risk. Unlike conventional urban-oriented waste management assessments, the proposed model integrated terrain-related accessibility constraints, institutional fragmentation and community-based adaptive mechanisms into a unified analytical structure. The conceptual model was based on four interrelated dimensions identified through the combined quantitative, geospatial and qualitative assessment conducted in the Ukrainian section of the Upper Tisza Basin: infrastructure availability, spatial accessibility, institutional-logistical capacity and community participation support. Infrastructure availability reflected the density and territorial distribution of designated plastic collection points and decentralised collection hubs. Spatial accessibility described the practical reachability of collection infrastructure under conditions of dispersed settlements, slope constraints and road-network limitations. Institutional-logistical capacity captured the regularity of collection services, route feasibility, operator coordination and municipal implementation capacity. Community participation support reflected the presence of local clean-up actions, decentralised recovery practices and outreach activities that improved practical waste recovery in underserved settlements.

At the operational assessment stage, the quantitative indicators integrated into the composite municipal efficiency index (PWCE) were aggregated using equal weights, as no validated empirical basis for differentiated weighting across municipalities was available in the analysed dataset. This approach reduced the risk of introducing subjective weighting bias and ensured methodological transparency. At the same time, the conceptual model recognised that the four dimensions did not function independently. Institutional and logistical weaknesses could reduce the practical effectiveness of available infrastructure, while low spatial accessibility could weaken community participation in routine separate collection despite local awareness efforts. Conversely, active community initiatives could partially compensate for deficiencies in infrastructure and collection frequency in remote settlements. The model also incorporated seasonal variability as a modifying influence on system performance. Tourist peaks in mountain municipalities increased temporary plastic waste generation and placed additional pressure on collection infrastructure, while spring floods and heavy precipitation increased the likelihood of mobilising accumulated plastic waste from floodplains, riverbanks and informal dumping sites. In the proposed model, these seasonal factors acted as amplifiers of existing infrastructure and governance weaknesses rather than independent determinants of efficiency.

A key feature of the model was the explicit linkage between municipal collection efficiency and environmental safety outcomes. In mountain-border river basins, deficiencies in any of the four dimensions could increase plastic leakage into tributaries and main river channels, thereby contributing to cumulative downstream ecological risks and transboundary pollution pressures. For this reason, the model interpreted plastic waste collection efficiency not only as a municipal service-performance characteristic but also as a preventive environmental safety parameter. The proposed conceptual model could support comparative assessment across municipalities and guide prioritisation of interventions in mountain-border regions. Its practical application enabled the identification of bottlenecks in infrastructure placement, route accessibility, institutional coordination and local adaptive capacity, thereby supporting more targeted and environmentally relevant waste management planning in transboundary basin systems (Fig. 1).

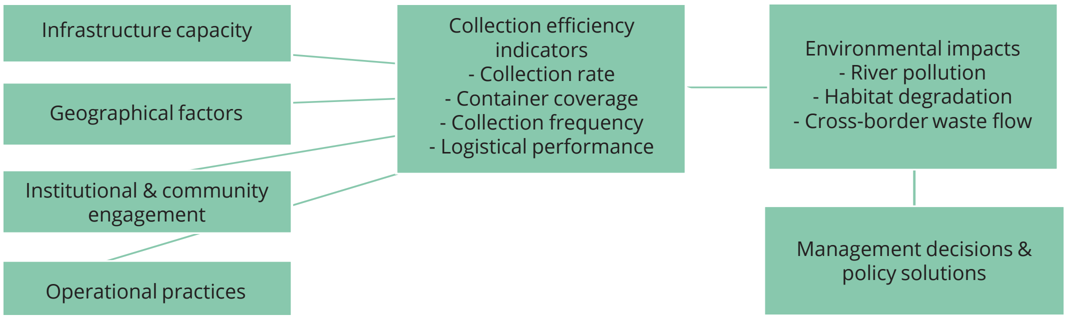

Figure 1. Conceptual model for assessing plastic waste collection efficiency in mountain-border regions

Source: developed by the authors

The conceptual model integrated the main findings of the study into a coherent analytical framework showing that plastic waste collection efficiency in mountain-border regions depended on the interaction of infrastructure availability, spatial accessibility, institutional-logistical capacity and community participation support. It also demonstrated that seasonal pressures and hydrological dynamics amplified pre-existing system weaknesses and increased the environmental significance of local collection deficits. Overall, the model provided a structured basis for interpreting the empirical results and for identifying priority directions for more effective and environmentally oriented waste management planning in transboundary basin regions.

CONCLUSIONS

This study assessed the efficiency of plastic waste collection systems in the Ukrainian section of the Upper Tisza Basin as a mountain-border region characterised by complex terrain, dispersed settlements and transboundary environmental vulnerability. The results confirmed that municipal collection performance was determined by the combined influence of infrastructure availability, spatial accessibility, logistical feasibility and institutional capacity. The most pronounced disparities were identified between lowland and mountainous municipalities. Lower collection rates, weaker infrastructure coverage and reduced service frequency in mountainous communities were associated with terrain-related constraints, limited road accessibility and organisational fragmentation. These factors reduced the practical effectiveness of formal collection systems and increased the risk of unmanaged plastic accumulation in residential and river-adjacent areas. The study confirmed that municipal collection inefficiencies in upstream territories had direct environmental-safety implications. Insufficient plastic waste collection coverage and irregular servicing increased the probability of riverine plastic leakage, particularly during flood periods and heavy rainfall. In the Upper Tisza Basin, such leakage represented not only a local waste-management problem but also a transboundary risk affecting downstream river ecosystems.

Community-driven initiatives demonstrated an important complementary role in improving plastic waste recovery in underserved municipalities. Their practical contribution was greatest in mountainous settlements with infrastructural and logistical deficits; however, the findings also showed that such initiatives were most effective when integrated with stable municipal collection services rather than operating as isolated measures. The proposed assessment framework and conceptual model provided a structured basis for evaluating plastic waste collection efficiency in mountain-border regions exposed to riverine pollution risks. Their practical application may support terrain-sensitive infrastructure planning, seasonal service adaptation, stronger inter-municipal coordination and integration of community-based measures into environmental safety-oriented waste-management strategies.

This study had several limitations. Municipal statistical data were not uniformly available across all communities, which restricted the depth of temporal comparison. Seasonal fluctuations related to tourism intensity and hydrological conditions may also have influenced waste generation patterns and short-term efficiency indicators. In addition, the absence of long-term continuous monitoring data limited the assessment of multi-year performance trends. Nevertheless, the combination of statistical analysis, geospatial modelling and qualitative assessment ensured sufficient analytical consistency for identifying the principal determinants of plastic waste collection efficiency in the study area. The prospects for further research in this area lie in the development and implementation of integrated waste management models that take into account seasonal fluctuations, local infrastructure limitations, and transboundary ecological risks to improve waste collection efficiency in mountain-border regions.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Baltrocchi, A.P.D., Maggi, L., Carnevale Miino, M., Torretta, V., & Rada, E.C. (2024). Comparison of mixed and door-to-door systems for urban waste collection in terms of effectiveness and greenhouse gas emissions: A case study from two mountainous Italian valleys. *Resources*, 13(1), article number 17. doi: 10.3390/resources13010017.

[2] Bhandari, N.L., Bhattarai, S., Bhandari, G., Subedi, S., & Dhakal, K.N. (2021). A review on current practices of plastics waste management and future prospects. *Journal of Institute of Science and Technology*, 26(1), 107-118. doi: 10.3126/jist.v26i1.37837.

[3] Chen, C.C. (2010). Spatial inequality in municipal solid waste disposal across regions in developing countries. *International Journal of Environmental Science and Technology*, 7, 447-456. doi: 10.1007/BF03326154.

[4] Copernicus Data Space Ecosystem. (n.d.). Retrieved from https://dataspace.copernicus.eu/.

[5] Environmental Passport of Zakarpattia Region for 2022. (2023). Retrieved from https://ecozakarpat.gov.ua/wp-content/nd/2022_ecopasport.pdf.

[6] Environmental Passport of Zakarpattia Region for 2023. (2024). Retrieved from https://surl.li/ygrbrx.

[7] Di, J., Xi, K., & Yang, Y. (2025). An enhanced YOLOv8 model for accurate detection of solid floating waste. *Scientific Reports*, 15, article number 25015. doi: 10.1038/s41598-025-10163-2.

[8] Gallitelli, L., & Scalici, M. (2022). Riverine macroplastic gradient along watercourses: A global overview. *Frontiers in Environmental Science*, 10, article number 937944. doi: 10.3389/fenvs.2022.937944.

[9] Interreg Danube Region. (2024). *Policy guide released*. Retrieved from https://interreg-danube.eu/projects/aquatic-plastic/news/policy-guide-released.

[10] Kiss, T., Fórián, S., Szatmári, G., & Sipos, G. (2021). Spatial distribution of microplastics in the fluvial sediments of a transboundary river – a case study of the Tisza River in Central Europe. *Science of the Total Environment*, 785, article number 147306. doi: 10.1016/j.scitotenv.2021.147306.

[11] Kwikima, M.M., & Ngole, F. (2025). GIS-based optimization of solid waste collection points and routes: A case study of Majengo Ward, Sumbawanga Municipality, Tanzania. *Cleaner Waste Systems*, 12, article number 100372. doi: 10.1016/j.clwas.2025.100372.

[12] Leknoi, U., Painmanakul, P., Chawaloesphonsiya, N., Wimolsakcharoen, W., Samritthinanta, C., & Yiengthaisong, A. (2024). Building sustainable community: Insight from successful waste management initiative. *Resources, Conservation & Recycling Advances*, 24, article number 200238. doi: 10.1016/j.rcradv.2024.200238.

[13] Liro, M., Zielonka, A., van Emmerik, T.H.M., Grodzińska-Jurczak, M., Liro, J., Kiss, T., & Mihai, F.C. (2023). Mountains of plastic: Mismanaged plastic waste along the Carpathian watercourses. *Science of the Total Environment*, 888, article number 164058. doi: 10.1016/j.scitotenv.2023.164058.

[14] Mihai, F.-C., *et al*. (2022). Plastic pollution, waste management issues, and circular economy opportunities in rural communities. *Sustainability*, 14(1), article number 20. doi: 10.3390/su14010020.

[15] Nava, V., *et al*. (2024). Plastic pollution affects ecosystem processes including community structure and functional traits in large rivers. *Water Research*, 259, article number 121849. doi: 10.1016/j.watres.2024.121849.

[16] OpenStreetMap. (n.d.). Retrieved from https://surl.li/tcvljj.

[17] Owowenu, E.K., Nnadozie, C.F., Akamagwuna, F., Noundou, X.S., Uku, J.E., & Odume, O.N. (2023). A critical review of environmental factors influencing the transport dynamics of microplastics in riverine systems: Implications for ecological studies. *Aquatic Ecology*, 57, 557-570. doi: 10.1007/s10452-023-10029-7.

[18] Plastic Cup. (2023). *CALL-Action reached 1200 tons*. Retrieved from https://petkupa.hu/eng/?cikkId=call-action-reached-1200-tons.

[19] Popa, C.L., Dontu, S.I., Savastru, D., & Carstea, E.M. (2022). Role of citizen scientists in environmental plastic litter research – a systematic review. *Sustainability*, 14(20), article number 13265. doi: 10.3390/su142013265.

[20] Russell, C.E., Fernández, R., Parsons, D.R., & Gabbott, S.E. (2023). Plastic pollution in riverbeds fundamentally affects natural sand transport processes. *Communications Earth & Environment*, 4, article number 255. doi: 10.1038/s43247-023-00820-7.

[21] Shi, Y., & Zhang, Y. (2026). Marine plastic pollution governance: The role and challenges of international law. *NPJ Emerging Contaminants*, 2, article number 7. doi: 10.1038/s44454-025-00026-w.

[22] Shi, Y., Chen, W., Yang, S., Fan, Y., & Lu, L. (2024). Freshwater microplastics governance and sustainable development: Pollution status, interactions, policies, and prospective studies. *Desalination and Water Treatment*, 320, article number 100704. doi: 10.1016/j.dwt.2024.100704.

[23] State Statistics Service of Ukraine, & Main Department of Statistics in Zakarpattia Region. (2022). *Demographic situation in the region in 2021*. Retrieved from https://www.uz.ukrstat.gov.ua/catalog/2022/bulet_04.pdf.

[24] USGS EarthExplorer. (n.d.). Retrieved from https://earthexplorer.usgs.gov/.

[25] van Emmerik, T.H.M., Frings, R.M., Schreyers, L.J., Hauk, R., de Lange, S.I., & Mellink, Y. A.M. (2023). River plastic transport and deposition amplified by extreme flood. *Nature Water*, 1, 514-522. doi: 10.1038/s44221-023-00092-7.

[26] Wang, S., Truffer, B., & Bai, X. (2025). Pathways towards successful urban community waste management: Insight from 26 experiments in China. *Environmental Innovation and Societal Transitions*, 57, article number 101032. doi: 10.1016/j.eist.2025.101032.

[27] Zakarpattia Regional State Administration. (2021). *Regional waste management plan of Zakarpattia Region until 2030*. Retrieved from https://surl.li/dpulpt.

[28] Zhang, J., Ruan, R., & Bashir, M.J.K. (Eds.). (2023). *Environmental pollution governance and ecological remediation technology*. Cham: Springer. doi: 10.1007/978-3-031-25284-6.

[29] Zhou, K., Zhang, J., Liu, H., & Fan, J. (2024). An integrated framework for assessing the multiscale environmental risks of domestic waste management systems in mountainous regions. *Environmental Impact Assessment Review*, 108, article number 107597. doi: 10.1016/j.eiar.2024.107597.

Оцінка ефективності систем збору пластикових відходів у гірських та прикордонних регіонах: дослідження басейну Верхньої Тиси

Руслан Шварц

Аспірант
ДВНЗ «Ужгородський національний університет»
88000, пл. Народна, 3, м. Ужгород, Україна
https://orcid.org/0009-0003-2560-3975

Олег Глух

Доцент
ДВНЗ «Ужгородський національний університет»
88000, пл. Народна, 3, м. Ужгород, Україна
https://orcid.org/0000-0002-5725-1981

Анотація. Гірські та прикордонні регіони стикаються з вираженими труднощами в організації ефективного збору пластикових відходів через складний рельєф, розсіяні поселення та обмежену інфраструктурну спроможність. Ці труднощі особливо критичні в транскордонних річкових басейнах, де неправильне управління пластиковими відходами створює накопичувальні екологічні ризики вниз за течією. Басейн Верхньої Тиси, що належить Україні та кільком країнам Європейського Союзу, є високоризиковою зоною для транспортування пластику в річках і транскордонного забруднення. Метою цього дослідження була оцінка ефективності регіональних систем збору пластикових відходів у гірських та прикордонних умовах і визначення ключових факторів, які визначають ефективність системи. Дослідження базувалося на змішаному підході, що поєднував статистику муніципальних відходів, аналіз геопросторової доступності, польові спостереження та якісну оцінку на основі муніципальних документів і публічно доступних операційних звітів. Ефективність системи оцінювали за допомогою кількісних показників, включаючи рівень збору пластику на душу населення, покриття контейнерами, частоту збору та логістичну ефективність з урахуванням рельєфу. Результати виявили значні просторові відмінності в ефективності збору. Низовинні муніципалітети досягли рівня збору пластику 9,8-12,3 кг на особу на рік, тоді як гірські муніципалітети збирали лише 3,2-5,7 кг на особу на рік. У гірських районах менше ніж 50 % жителів мали доступ до пунктів збору в межах 300 м, порівняно з понад 80 % у низовинних поселеннях. Логістична неефективність у високогірних муніципалітетах була більш ніж удвічі вищою через поганий доступ до доріг та зміни висоти. Ініціативи, спрямовані на залучення громади, включаючи децентралізовані пункти збору та програми прибирання річок, підвищували локальну ефективність збору до 30 % у недостатньо обслуговуваних районах. Результати показали, що для покращення збору пластикових відходів у гірських та прикордонних регіонах необхідні інтегровані рішення, які поєднують оптимізацію інфраструктури, логістику, чутливу до рельєфу, інституційну координацію та залучення громади. Запропонована система оцінки може бути застосована в інших транскордонних гірських регіонах для підвищення екологічної безпеки та сталого використання ресурсів

Ключові слова: управління пластиковими відходами; просторовий доступ; прикордонні території; річкове забруднення; ефективність збору; управління річковими басейнами; екологічна безпека

Received: 28.11.2025 Revised: 17.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 48-62

UDC 632.7:595.78:582.677.1(477-021)

DOI: 10.31548/biologiya/2.2026.48

Phenological plasticity of *Cydalima perspectalis* and an assessment of the effectiveness of biological control methods in urbanised ecosystems

Lesia Bondareva*

PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-8171-2338

Alla Gnatiuk

PhD in Biological Sciences, Senior Researcher, Senior Scientific Associate
M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine
01014, 1 Sadovo-Botanichna Str., Kyiv, Ukraine
https://orcid.org/0000-0001-5001-971X

Nataliia Kushnir

PhD in Biological Sciences
M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine
01014, 1 Sadovo-Botanichna Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9659-8814

Lyudmila Nemerytska

PhD in Biological Sciences, Associate Professor
Zhytomyr Agrotechnical Vocational College
10031, 96 Pokrovska Str., Zhytomyr, Ukraine
https://orcid.org/0000-0001-7933-0587

Inna Zhuravska

PhD in Agricultural Sciences
Zhytomyr Agrotechnical Vocational College
10031, 96 Pokrovska Str., Zhytomyr, Ukraine
https://orcid.org/0000-0001-9332-1134

Abstract. This study was conducted to develop an effective system for protecting boxwood against the invasive pest *Cydalima perspectalis* in the urban ecosystems of Ukraine. The work was based on field observations and laboratory analyses conducted in 2022-2023 at the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine, the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine, the O.V. Fomin Botanical Garden, as well as in urban green spaces of Kyiv, including the Holosiivskyi National Nature Park and city squares. It was established that under the conditions of Kyiv, the pest forms two complete generations per season and a third generation of caterpillars that enter diapause and overwinter. Pheromone monitoring revealed two peaks of adult flight: in the first ten-day period of July and the first-second ten-day periods of August.

Suggested Citation:
Bondareva, L., Gnatiuk, A., Kushnir, N., Nemerytska, L., & Zhuravska, I. (2026). Phenological plasticity of *Cydalima perspectalis* and an assessment of the effectiveness of biological control methods in urbanised ecosystems. *Biological Systems: Theory and Innovation*, 17(2), 48-62. doi: 10.31548/biologiya/2.2026.48.

*Corresponding author (lnubip69@gmail.com)

A strong statistically significant inverse (negative) relationship ($R^2 = 0.96$) was identified between the daily temperature and the duration of the caterpillar stage. Mild winters ensured the survival of more than 70% of overwintering caterpillars. The evaluation of control methods showed that a biopreparation based on *Bacillus thuringiensis* achieved an efficiency of 96 ± 1.2% against early instar caterpillars (L1-L3). In comparison, the introduction of *Trichogramma ensured* parasitism of 71.5 ± 2.6% of eggs. In contrast, mechanical methods were significantly less effective (45.0 ± 3.9%). Based on the obtained data, a phenologically grounded treatment calendar and differentiated protection schemes for botanical gardens and urban squares were developed, allowing the minimisation of environmental risks and economic costs. The results form the basis for practical recommendations aimed at preserving boxwood plantings and minimising the pest's impact on biodiversity in Ukraine

Keywords: green spaces; *Cydalima perspectalis*; defoliation; pheromone monitoring; *Bacillus thuringiensis*; environmental safety

INTRODUCTION

The box tree moth (*Cydalima perspectalis* Walker, 1859) is an invasive species of the family Crambidae that causes significant damage to boxwood plantings in botanical gardens, park areas, and other urbanised ecosystems. Its high rate of spread, aggressiveness, and the lack of sufficient natural enemies in its secondary range result in considerable ecological and economic impacts. A similar threat to green spaces in Ukraine is posed by other invasive phytophagous insects, in particular the horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimic, 1986) and the emerald ash borer (*Agrilus planipennis* Fairmaire, 1888), which indicates a general trend of the increasing role of invasive insects in the transformation of urbanised ecosystems (Puzrina *et al.*, 2025). Studies on the phenology of *C. perspectalis* in various European regions reveal a high degree of plasticity in its life cycle. In most regions, two complete generations are formed, with the possibility of developing a third facultative generation under favourable temperature conditions. The mechanisms of diapause induction and regulation were studied by L. Poitou *et al.* (2020), while entomological monitoring in urbanised environments has shown that the spatial arrangement of plantings and the microclimate of the urban environment significantly influence the seasonal population dynamics (Marchenko, 2022).

The temperature regime is a key factor determining the rate of development and the number of generations of the species. R. Stan & I. Mitrea (2021) established a significant influence of temperature fluctuations on the duration of individual developmental stages and the overall population dynamics. An increase in mean daily temperatures contributes to a reduction in the duration of the caterpillar stage and an increase in the invasive potential. Thus, climate change may enhance the risks of the pest spreading into new regions. Among modern approaches to regulating the abundance of *C. perspectalis*, priority is given to environmentally safe methods. The effectiveness of biopreparations based on *Bacillus thuringiensis* has been confirmed by a number of studies, including the work of Y.V. Koshelyaeva *et al.* (2024). Biotechnical measures, including pheromone monitoring and trapping, have been considered cost-effective tools for early population suppression in the study by K. Ok *et al.* (2023). In North America, emphasis is placed on the importance of early detection and an integrated approach to managing populations of the box tree moth (Coyle *et al.*, 2022). Most studies confirm the potential of combining biological and other control methods.

Modelling population dynamics and spatial spread is an important tool for predicting invasions. Q. Canelles Trabal (2021) integrated population outbreaks into landscape modelling, which made it possible to assess potential risks to forest ecosystems. Using remote sensing, R. Esmaili *et al.* (2020) carried out mapping of plantings affected by the moth, facilitating a rapid response to the invasion. An assessment of the potential distribution of the species in Europe was conducted by Q. Canelles Trabal *et al.* (2021), confirming the dependence of its range on climatic parameters. Modern molecular genetic studies also expand understanding of the adaptive potential of *C. perspectalis*. An analysis of the mitochondrial genome carried out by Y. Gao *et al.* (2023) has clarified the phylogenetic relationships of the species within the family Crambidae and deepened understanding of its evolutionary history.

Despite the considerable research conducted in Europe, data on phenological dynamics and the effectiveness of control methods against *C. perspectalis* in urbanised ecosystems of Ukraine remain limited. In particular, the influence of urban microclimatic conditions on the development of populations of this pest and the effectiveness of integrated pest management methods are still insufficiently studied. This study aimed to assess the phenological characteristics of the development of *C. perspectalis* under the conditions of the city of Kyiv and to analyse the effectiveness of different pest control methods. The objectives of the study included determining the seasonal dynamics of the development of the box tree moth and examining the influence of climatic factors on the rate of pest development. It also involved comparing the effectiveness of biological control methods, such as the use of *Bacillus thuringiensis* (Berliner, 1915) and entomophages, with mechanical methods at different stages of pest development.

MATERIALS AND METHODS

The study was conducted during 2022-2023 within the city of Kyiv (Ukraine). Observations and experiments were carried out at several representative sites: the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine, the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine, the O.V. Fomin Botanical Garden, as well as in green spaces of the urban environment, including the Holosiivskyi National Nature Park and five urban squares located in four administrative districts of Kyiv: Shevchenkivskyi, Podilskyi (two squares), Pecherskyi, and Desnianskyi. Site selection was based not only on the age of plantings (⩾ 10 years) and the presence of damage, but also taking into account location type (botanical garden/urban square/private plot), insolation (full sun/partial shade), density and varietal composition of the genus *Buxus* (at least 30 shrubs per site with an area of ⩾ 50 m²), maintenance history (pruning, fertilisation, irrigation) and the use of insecticides/biopreparations over the previous 12 months, microclimatic conditions (mean daily temperature, minimum winter temperatures, wind regime), soil and hydrological conditions (pH, drainage), the presence of nearby alternative habitats and sources of infestation (boxwood within a 200 m radius), as well as logistical factors (accessibility for monitoring, the possibility of establishing control subplots, and a minimum distance between sites of ⩾ 200 m to minimise mutual influence).

The objects of the study were populations of *C. perspectalis* and the degree of damage inflicted on plantings of common box (*Buxus sempervirens* L.). For detailed analysis, 50 model plants were selected at each experimental site using a systematic sampling method, totalling 450 plants. The inclusion criterion was the presence of signs of moth damage in the previous season or location within an active infestation focus. The exclusion criteria were as follows: sites were not included in the study if at least one of the following conditions was present – recent treatments (contact insecticides within ⩽ 30 days; systemic insecticides ⩽ 60 days; biopreparations based on *Bacillus thuringiensis* or entomopathogenic fungi ⩽ 10 days; releases of *Trichogramma* spp. ⩽ 21 days); factors distorting damage assessment (intensive sanitary pruning within ⩽ 14 days prior to the start; abiotic damage – frost, drought, or mechanical – with defoliation > 25%; pronounced associated diseases of boxwood, such as box blight (*Calonectria pseudonaviculata*) exceeding level 2 on the damage scale); insufficient representativeness (< 30 *Buxus* shrubs per site, area < 50 m², inability to establish control subplots, distance to another experimental site < 200 m); manageable risks and access limitations (absence of written permission from the managing authority, lack of regular access or security; risk of plant removal or relocation during the study; proximity to sources of chemical drift – active treatments on neighbouring sites within < 20 m); as well as legal or quarantine restrictions preventing field experiments or conflicting with the study design.

To study the phenology and seasonal dynamics of the pest, visual assessment and pheromone monitoring methods were used. At each experimental site, five Delta pheromone traps (manufacturer Biosystems, Spain) equipped with *C. perspectalis* dispensers were installed. The traps were inspected, and the number of captured adults was recorded at 7-day intervals. During each inspection, the trap was temporarily removed, captured insects were counted and identified by species and sex using tweezers and a magnifying lens, and the results were recorded in a field log. After counting, the captured specimens were removed, adhesive inserts were replaced with new ones, and dispensers were renewed according to the manufacturer's instructions to maintain monitoring effectiveness. In parallel, regular visual inspections of plantings were conducted to record the developmental stages of the pest (eggs, caterpillars of different instars, pupae, and adults). To assess the intensity of defoliation, a five-point scale was developed and applied: 0 – no damage; 1 – up to 20% leaf damage; 2 – 21-40%; 3 – 41-60%; 4 – 61-100%. To evaluate the influence of climatic factors, air temperature and relative humidity were recorded daily using portable digital thermo-hygrometers TFA Dostmann (Germany) installed at each site. To assess the relationship between daily temperature and the duration of the caterpillar stage, correlation and regression analysis were applied. A scatter plot was constructed to verify the linearity of the relationship, and model assumptions were tested using residual normality tests and checks for homoscedasticity. For the linear model, regression coefficients (β), the coefficient of determination (R^2), and p-values were reported. Model performance was evaluated using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), allowing selection of the most appropriate model.

The effectiveness of control methods was assessed under field experimental conditions with four replicates (n = 4). A biological preparation based on *Bacillus thuringiensis* var. *kurstaki* (Lepidocide, BTU-Centre Ltd., Ukraine) was applied at a concentration of 0.7% using fine-droplet spraying with a Gloria backpack sprayer (Germany). The introduction of the entomophagous agent *Trichogramma* spp. (manufacturer Biozakhyst, Ukraine) was carried out during the period of mass egg-laying by the moth, at a rate of 3,000 individuals per 100 m².

The effectiveness of the methods was calculated after 7, 14, and 21 days using Abbott's formula (1):

$$E = \frac{n2 - n1}{n2} \times 100\%, \quad (1)$$

where n_1 is the number of live caterpillars in the treated plot, and n_2 is the number of live caterpillars in the control plot.

The economic assessment was carried out from the perspective of the managing authority of the green space, with a time horizon of one season and a base price year of 2023 (euro exchange rate in 2023: 1 EUR = 42.175 UAH (MinFin, 2023), corresponding to the study period); accounting units were UAH per 100 shrubs per season. Direct costs were estimated based on expenditures for biopreparations (Bt), biological material (*Trichogramma* spp.), labour (standard hours for treatment/monitoring), consumables, and pheromone monitoring (traps and dispensers). Economic benefits were assessed in terms of avoided costs for shrub replacement and restoration works. To compare the effectiveness of control strategies, independent experimental plots were established at each of the five study locations. Each experimental strategy and control at each location was represented by four replicates (n = 4). The size of one elementary plot was 25 m² (5 × 5 m) and included at least five boxwood shrubs with moderate or severe damage.

The experimental groups included: Strategy 1 – treatment of phytophagous caterpillars with a biopreparation based on *Bacillus thuringiensis* (Bt). Strategy 2 (mechanical) – exclusive use of weekly mechanical inspections with removal of caterpillars. Strategy 3 – two releases of the entomophagous agent *Trichogramma* spp. followed by regular mechanical inspections. Control – plots without any intervention, only monitoring. Interventions were carried out synchronously across all locations at the recommended phenological periods. Effectiveness was assessed by visually counting live caterpillars on five fixed boxwood shrubs within each plot before the start of the experiment (baseline population) and after 7, 14, and 21 days. Data from individual replicates within each strategy at a given location were averaged for further analysis. Effectiveness at each assessment time was calculated using Abbott's formula (1) by comparison with the control group at the corresponding location.

To determine the sensitivity of different caterpillar instar groups to the *Bacillus thuringiensis*-based biopreparation, a separate experiment was conducted. *C. perspectalis* caterpillars were collected from natural populations and classified into instars based on morphological characteristics (head capsule width, body length) according to a standard scale: L1-L3 – early instars (1st, 2nd, 3rd instars; body length < 10 mm); L4-L5 – late instars (4^{th}, 5^{th} instars; body length 10-20 mm). For each instar group (L1-L3 and L4-L5), four groups (replicates) of 20 individuals each were formed. Caterpillars were maintained on cut boxwood shoots in rearing cages under controlled conditions ($t = 23 \pm 2°C$). Treatment was performed by spraying the plant surface with the recommended working concentration of the biopreparation. In control groups, shoots were sprayed with clean water. Caterpillar mortality was recorded 3, 5, and 7 days after treatment. Effectiveness for each instar group was calculated using Abbott's formula (1) on day 7 and expressed as the mean value of four replicates.

The economic analysis of control methods was carried out by comparing the costs of each method and assessing the total costs for each strategy per unit area. The calculations included expenses for biopreparations (Bt), *Trichogramma*, tools for mechanical collection, and labour costs. All costs were calculated for an area of 100 shrubs (50 m^2). In addition, savings associated with avoiding the replacement of damaged plants and maintenance of green spaces were estimated, depending on the effectiveness of each control method. To assess the robustness of the economic conclusions, a sensitivity analysis was conducted with respect to fluctuations in the price of biopreparations (±20%) and changes in temperature conditions (±2°C). Based on these calculations, total costs for each strategy were determined, allowing comparison with potential benefits for the managing authorities of green spaces.

Statistical data processing was performed using the software package Statistica 10.0 (StatSoft, USA). Linear regression analysis was used to establish the relationship between temperature and the duration of the caterpillar stage. The significance of differences between the effectiveness indicators of different control methods was assessed using Student's t-test at a significance level of $p < 0.05$. All studies were conducted in accordance with the principles of ethical botanical research.

The research was carried out in compliance with the principles of the Convention on Biological Diversity (1992). All methods of controlling *C. perspectalis* populations were coordinated with the administrations of the botanical gardens and complied with the requirements of the Law of Ukraine No. 180-XIV "On Plant Protection" (1998). The application of biological preparations and entomophagous agents was conducted in accordance with the principles of integrated pest management and minimisation of impacts on non-target insect species. No additional permits were required for researching invertebrates, as the box tree moth is an invasive pest, and the research methods were non-invasive or involved only the use of registered plant protection products.

RESULTS AND DISCUSSION

As a result of the conducted studies, it was established that during the growing season, *C. perspectalis* forms two complete generations. At the end of the season, a third generation of caterpillars develops, which enters diapause for the winter period. Overwintering occurs in dense white silken cocoons located between boxwood leaves deep within the shrub, protecting from unfavourable abiotic factors. The age structure of the overwintering population is represented predominantly by third-fourth instar individuals. The lower temperature threshold for caterpillar development is approximately +10°C, which determines the onset of post-winter activity. In spring, overwintered caterpillars resume feeding. It was found that the timing of caterpillar hatching and mass adult flight varies depending on the climatic conditions of the given year. Figure 1 illustrates the phenological calendar of pest development for each generation based on observations in the city of Kyiv.

Despite nearly two decades of invasion in Central Europe, the biology of this species remains a subject of active scientific research. Earlier, S. Nacambo *et al.* (2014) reported that in Europe, the box tree moth may develop one or two generations per year. However, in connection with climate change, recent observations indicate the possibility of forming three to four complete generations per year, depending on local climatic conditions. The results of their own studies on the development of *C. perspectalis* in the city of Kyiv are consistent with the data presented in the work of O.Yu. Andrieieva *et al.* (2024) for the city of Zhytomyr. According to P.K. Bereś *et al.* (2021), who studied the invasion of the box tree moth in Poland, this pest forms two complete generations and one facultative generation. In Ukraine, as in Poland, the number of generations and their timing may vary depending on the temperature and humidity conditions of a given year, which confirms the ecological plasticity of the species in response to climate change. To assess the relationship between daily temperature and the duration of the caterpillar stage, regression analysis was applied. The results of the analysis are presented in Table 1, which includes the regression coefficient for temperature, the coefficient of determination (R^2), and the p-value for testing statistical significance.

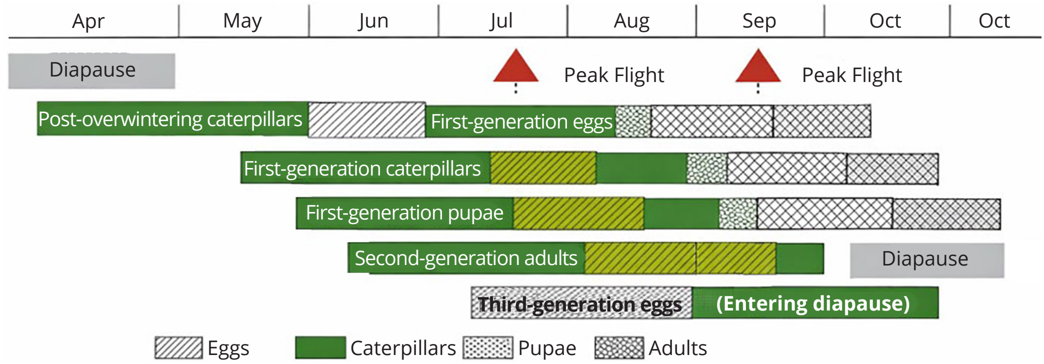

Figure 1. Phenological calendar of the development of *C. perspectalis* in the city of Kyiv
Source: developed by the authors based on their own research

Table 1. Results of correlation and regression analysis between temperature and the duration of the caterpillar stage of *C. perspectalis*

Parameter	Value	95% CI	p-value
β (temperature)	-0.28	[-0.35, -0.21]	<0.001
R^2	0.96		

Source: compiled by the authors based on their own research

The study revealed statistically significant differences in the rate of development of the box tree moth in sites with varying levels of isolation and wind protection. In urban squares with better solar exposure and higher mean daily temperatures, the development of the first generation of the pest occurred 5-7 days faster compared with more shaded and sheltered park areas, including the Holosiivskyi National Nature Park. This was reflected in earlier hatching of *C. perspectalis* caterpillars and a reduced duration of transition to subsequent developmental stages in open, sunlit sites. In June-July, air temperatures in such areas were on average 2-3°C higher, creating favourable conditions for accelerated ontogeny and increased reproductive activity of the pest. Figure 2 shows the relationship between daily temperature and the duration of the caterpillar stage.

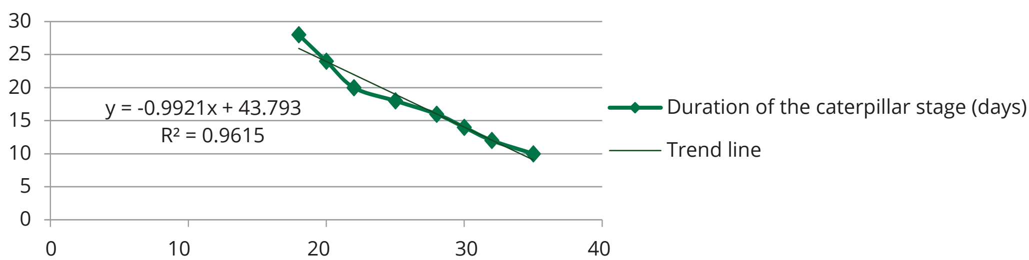

Figure 2. Scatter plot of temperature and the duration of the caterpillar stage of *C. perspectalis*
Source: compiled by the authors based on their own research

With an increase in mean daily temperature, the duration of the caterpillar stage decreases. The regression line clearly demonstrates an inverse correlation between temperature and stage duration, confirming that higher temperatures accelerate caterpillar development. This relationship shows some variation, particularly at extreme temperature values, which may indicate the influence of other factors such as humidity or specific microclimatic conditions. Comparison of information criteria showed that the linear model including only temperature (AIC = 145.2, BIC = 150.1) had an advantage over the quadratic model (AIC = 147.8, BIC = 154.5) and the model including humidity as a covariate (AIC = 146.5, BIC = 153.2), confirming its adequacy and sufficiency for describing the data.

The analysis demonstrated an "inverse (negative)" relationship between daily temperature and the duration of the caterpillar stage. Regression analysis revealed a correlation ($r < -0.80$), confirming a significant influence of temperature on developmental duration. For example, at a mean daily temperature of +25°C, the duration of the caterpillar stage decreased by 20% compared with conditions at +18°C. This indicates that increasing temperature accelerates the development process of caterpillars, reducing the time spent at each developmental stage. Table 2 presents the results of the comparison of caterpillar stage duration at different temperatures.

The study showed that mild winters with minimum temperatures not lower than -15°C ensure high survival of overwintering *C. perspectalis* caterpillars. Under such conditions, their survival rate exceeded 70%, creating favourable conditions for the formation of high population densities in spring. In contrast, in years with more severe winters, when minimum temperatures dropped to -20°C, the survival of overwintering stages decreased significantly, resulting in lower numbers of caterpillars after the resumption of feeding. The obtained results confirm that temperature is a key factor determining the seasonal development of the box tree moth. Similar conclusions were reported by A. Wiesner *et al*. (2021), who found that temperature fluctuations significantly affect the phenology and population dynamics of this pest. Comparable patterns were also recorded in studies conducted in Kyiv. Furthermore, research by D. Schmera & B. Baur (2025) in Switzerland showed that increasing temperature shortens the duration of individual life cycle phases, thereby accelerating species development. Thus, the temperature regime determines not only winter survival but also the rate of generational development and the potential population size, which in turn influences the invasive potential of the species. Modelling of the potential distribution of *C. perspectalis*, carried out by M. Seehausen *et al*. (2024), confirms that climate change may contribute to the expansion of the pest's range. The consistency of local results with international studies indicates general patterns in the influence of climatic factors on the population dynamics of this invasive species.

Table 2. Duration of the *C. perspectalis* caterpillar stage at different temperatures

Temperature (°C)	Duration of the caterpillar stage (days)
18	28
20	24
22	20
25	18

Source: compiled by the authors based on their own research

Increased humidity during rainy periods promoted the development of fungal diseases among early instar caterpillars. Under conditions of cooling and high humidity, a significant spread of epizootics was observed, which reduced population density. These diseases had the greatest impact on early instars (L1-L3), which were generally the most susceptible to such infections. During periods of elevated humidity and low temperatures, a reduction in population size of 15-20% was observed, significantly decreasing the overall impact of the moth on plants (Table 3).

Table 3. Effect of humidity and temperature on mycoses of *C. perspectalis*

Temperature (°C)	Humidity (%)	Reduction in population density (%)
18	85	18
20	75	15

Table 3. Continued

Temperature (°C)	Humidity (%)	Reduction in population density (%)
22	70	10
25	60	5

Source: compiled by the authors based on their own research

Thus, temperature conditions have a significant influence on the rate of development of box tree moth caterpillars, which in turn affects population dynamics throughout the season. Mild winters promote high survival of the overwintering stage, ensuring substantial population development during the growing season. In addition, increased humidity and prolonged rainy periods can significantly reduce population density due to the spread of diseases among caterpillars. These factors are important for predicting population development of the box tree moth and for managing its abundance in ecosystems.

The application of a bioinsecticide based on *Bacillus thuringiensis* var. *kurstaki* demonstrated high effectiveness against early instar caterpillars (L1-L3), with a mortality rate of 96 ± 1.2%. This indicates a high sensitivity of early larval stages to the preparation. At the same time, against later instars (L4-L5), effectiveness decreased to 65.3 ± 2.5%, indicating their lower susceptibility to the bioinsecticide. The mechanism of action of the bioinsecticide lies in the formation of crystalline protein inclusions during bacterial sporulation. In the alkaline environment of the caterpillar gut, these inclusions dissolve, releasing insecticidal toxins (Malinowski, 2000). The obtained results confirm the advisability of early application of the preparation, as treatment at the initial stages of development ensures a substantial reduction in pest population density (Table 4).

Table 4. Effectiveness of a biopreparation based on *Bacillus thuringiensis* var. *kurstaki* against different instar groups of *C. perspectalis* caterpillars (M ± SD; n = 4)

Caterpillar instar group	Effectiveness (%)
L1-L3	96 ± 1.2
L4-L5	65.3 ± 2.5

Note: effectiveness for each age group was calculated using formula (1) on day 7 and expressed as the mean of four replicates
Source: compiled by the authors based on their own research

The introduction of parasitoid wasps of the genus *Trichogramma* during the egg-laying period of the box tree moth in the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine resulted in the parasitism of 71.5 ± 2.6% of moth eggs, which contributed to a significant reduction in the density of leaf-feeding caterpillars. This indicates the high effectiveness of *Trichogramma* as a biological control agent under botanical garden conditions. The results showed that the introduction of *Trichogramma* spp. is an effective method for controlling the abundance of the box tree moth, as it significantly reduces population size at the egg stage, thereby limiting subsequent caterpillar development. A comparative analysis of different control methods for the box tree moth revealed substantial differences in their effectiveness (Table 5). The highest effectiveness was demonstrated by the application of a biopreparation based on *Bacillus thuringiensis* var. *kurstaki* – 95.0 ± 1.4%. This high efficacy is due to the specific mode of action of the preparation, which targets larval stages of the pest and does not harm non-target organisms, thereby ensuring its environmental safety. The introduction of parasitic wasps of the genus *Trichogramma* achieved an effectiveness of 71.5 ± 2.6%. This method targets the destruction of pest eggs and allows population control before caterpillars emerge; however, its effectiveness depends on the timing of release of the entomophagous agent and the population density of the phytophagous pest. The least effective method was mechanical control (45.0 ± 3.9%), which involves shaking and manual collection of caterpillars. The limited effectiveness of this approach is explained by the ability of caterpillars to hide within dense foliage and silken shelters, as well as the rapid recovery of the population due to the presence of multiple generations during the season.

Table 5. Effectiveness of population control methods for *C. perspectalis* (M ± SD; n = 4)

Control method	Effectiveness (%)
Biopreparation (*Bacillus thuringiensis* var. *kurstaki*)	95.0 ± 1.4
Introduction of *Trichogramma* spp.	71.5 ± 2.6
Mechanical destruction of caterpillars	45.0 ± 3.9

Note: efficiency was calculated using the formula (1)
Source: compiled by the authors based on their own research

Thus, the findings of this study are consistent with the results of N.V. Makarenko *et al*. (2019) regarding the effectiveness of a biopreparation based on *Bacillus thuringiensis* var. *kurstaki*, particularly at the early stages of caterpillar development. The effectiveness of biological control is also confirmed by data from other authors. In particular, L.M. Bondareva *et al.* (2021) reported high efficiency of phytocomplexes against early instar caterpillars under the conditions of the O.V. Fomin Botanical Garden. The promise of biological methods is also confirmed in the work of R. Murillo *et al*. (2025), dedicated to the use of insect pathogens. M. Budziszewska & P.K. Bereś (2024) emphasise the importance of biological control methods for the box tree moth in Europe, particularly the use of introduced entomophagous agents such as *Trichogramma* spp. Their findings fully correspond with the results obtained in the city of Kyiv, where the introduction of *Trichogramma* spp. ensured a significant reduction in the population size of the box tree moth. M. Usta *et al.* (2025) investigated the pathogenic effect of *Penicillium glabrum* on the box tree moth. This study opens new perspectives for the use of entomopathogens in the biological control of this phytophagous species, which may also be considered in future research in Ukraine to ensure sustainable management of box tree moth populations. In addition, a positive effect of pheromone monitoring on the accuracy of predicting periods of intensive adult flight has been observed in Ukraine, which is important for the timely application of control measures.

To assess boxwood damage in areas infested by *C. perspectalis*, a five-point scale of defoliation intensity was applied. According to the observations, during the peak population of the second generation, 80% of the studied sites exhibited defoliation at levels 3-4, indicating severe to complete loss of foliage in these areas. According to the scale, level 3 corresponded to partial defoliation (41-60% of leaves), while level 4 corresponded to complete defoliation (61-100% of leaves) (Table 6).

Table 6. Results of the assessment of boxwood damage across all experimental sites

Defoliation level	Description of damage	% of sites with this level of damage
0	No damage	0
1	Light damage (up to 20%)	5
2	Moderate damage (20-40%)	10
3	Severe damage (41-60%)	30
4	Complete defoliation (61-100%)	55

Source: compiled by the authors based on their own research

The least affected sites were observed in urban squares, where only 5% of areas showed light damage (level 1). This may be due to lower exposure to direct sunlight and more favourable conditions for boxwood growth, which reduce pest activity. The most severely affected sites were located in well-lit areas of botanical gardens, where defoliation reached 61-100% on 55% of the sites. This is associated with higher levels of isolation, which created optimal conditions for the development of multiple generations of the box tree moth during the season. The maximum level of boxwood defoliation was observed in July-August, during the development of the second generation of the box tree moth, when caterpillar abundance reached peak levels. This led to significant damage to green spaces and deterioration of their phytosanitary condition. Similar conclusions were reported by A.M. Hnatiuk & M.B. Haponenko (2016), who identified the box tree moth as one of the main threats to green spaces in Kyiv. The obtained results also indicate a negative impact of the pest on the biodiversity of the studied areas, which justifies the need for a comprehensive approach to regulating its population. A similar trend of rapid population growth and active spread of the species was recorded by A. O'Hanlon (2024) in Ireland, confirming the pan-European nature of the invasion and the relevance of timely control measures.

Based on the obtained phenological data, a treatment calendar was developed to optimise the timing of interventions aimed at reducing the abundance of *C. perspectalis* at different stages of its development. The first treatment is recommended in late April to early May, when overwintered caterpillars begin to hatch. This allows control of the pest at a stage when it is most susceptible to biological preparations such as *Bacillus thuringiensis*. The second treatment is scheduled for mid-July, when the peak abundance of L1-L3 caterpillars of the first generation is observed. The third treatment in mid-August significantly reduces population size before the onset of autumn, when the life cycle of most individuals is completed (Table 7).

Table 7. Timing of treatments and corresponding developmental stages of *C. perspectalis*

Generation	Approximate treatment period	Dominant caterpillar instar stages	Biological justification for the application	Purpose of treatment
Overwintered generation	Late April – early May	L3-L4	Resumption of feeding after emergence from diapause	Reduction of the overwintered population
First generation	Mid-July	L1-L3	Mass hatching of caterpillars and the onset of active feeding	Prevention of peak defoliation
Second generation	Mid-August	L1-L3	Onset of development of the second population wave	Limitation of population size before entering diapause

Note: dates may vary depending on the weather conditions in a given year
Source: compiled by the authors based on their own research

To ensure effective control of the box tree moth under different types of plantings, differentiated approaches were proposed. For botanical gardens, where the preservation of biodiversity and ecological balance is essential, priority is given to biological control methods, such as the use of entomophagous agents (e.g., *Trichogramma* spp.) and pheromone monitoring. This allows precise determination of periods of the most intensive adult flight and the timely implementation of population control measures without the use of chemical treatments. In urban squares, where a higher level of urbanisation is often observed, and fewer opportunities exist for the implementation of biological methods, a combined approach is more appropriate. In this case, biopreparations based on *Bacillus thuringiensis* were used to control early instar caterpillars (L1-L3), together with mechanical control methods such as shaking caterpillars from foliage during peak population periods. These methods made it possible to reduce population density while minimising environmental impact (Table 8).

Table 8. Approaches to the protection of boxwood from *C. perspectalis* depending on the type of plantings

Type of plantings	Main control methods	Additional measures
Botanical gardens	Biological methods (*Trichogramma* spp.), pheromone monitoring	Forecasting the timing of treatments
Urban squares	Biopreparations (*Bacillus thuringiensis*), mechanical control	Shaking off caterpillars, timely treatments

Source: compiled by the authors based on their own research

The proposed approaches to protecting plantings from *C. perspectalis* are based on the results of their own phenological observations and take into account the specific characteristics of different types of green spaces. Differentiation of methods depending on the level of urbanisation and the ecological value of plantings makes it possible to optimise control effectiveness and minimise negative environmental impacts. The appropriateness of applying integrated and biologically oriented approaches is also confirmed by the results of international studies. In particular, A.R. Armanda & M. Bjeliš (2025), in their review of the box tree moth invasion in Europe, emphasise the need to combine biological and mechanical population management methods. Similar conclusions have been drawn in Ukrainian studies, where the combination of biopreparations with mechanical control increases overall effectiveness while reducing environmental pressure. Further research indicates that in Europe, *C. perspectalis* lacks sufficiently effective natural enemies capable of significantly limiting its abundance (Budziszewska & Bereś, 2024), which necessitates the systematic application of integrated methods throughout the growing season, especially given the overlap of generations. In addition, modern approaches to pest population management are increasingly based on predictive distribution models. M. Kenis (2022) and Ş. Yaman & M. Yaman (2024) demonstrated the effectiveness of using spatial distribution models to assess invasion risks. The obtained forecasts can be adapted to the conditions of Ukraine, taking into account climate change, which contributes to the expansion of the species' range.

The economic justification of a comprehensive approach to controlling *C. perspectalis* populations, taking into account the market cost of labour (100 UAH/hour; EUR 1 = UAH 42.175), confirmed the feasibility of combining biological and mechanical methods, while also demonstrating a significant increase in the share of labour costs within the overall cost structure. The use of biopreparations at early stages of pest development contributes to a substantial reduction in population size, potentially decreasing the need for intensive mechanical measures and chemical treatments. The economic efficiency of control methods was assessed by comparing direct costs for each measure and determining total costs per unit area. Direct costs included the price of biopreparations, labour, and expenses for materials and tools used in mechanical control. The cost of the biopreparation *Bacillus thuringiensis* var. *kurstaki* was UAH 500 (EUR 11.86) per litre, sufficient to treat 1,000 m^2. For an area of 100 shrubs (approximately 50 m^2), the cost of the preparation amounted to UAH 25 (EUR 0.59), while labour costs for a 2-hour treatment were UAH 200 (EUR 4.74). The total cost of applying the biopreparation was therefore UAH 225 (EUR 5.33) per 100 shrubs. The introduction of *Trichogramma* spp. cost UAH 150 (EUR 3.56) per 1,000 individuals (per 100 m^2); for 50 m^2, the cost of biological material was UAH 75 (EUR 1.78). Including labour costs (approximately 1.33 hours, or UAH 133 – EUR 3.15), the total amounted to UAH 208 (EUR 4.93) per 100 shrubs. Mechanical control through shaking and manual collection of caterpillars required approximately 2.67 hours of work, resulting in labour costs of UAH 267 (EUR 6.33) per 100 shrubs. The total cost of the combined approach (biopreparation + *Trichogramma* spp. + mechanical control) was UAH 700 (EUR 16.60) per 100 shrubs. The results indicate that under current economic conditions, labour costs are the determining factor in the overall cost structure, with mechanical methods representing the most expensive component of the control system.

To assess the impact of changes in control method costs, a sensitivity analysis was conducted regarding fluctuations in the price of biopreparations and changes in temperature conditions. The modelling results showed that a 20% increase in the cost of *Bacillus thuringiensis* leads to an increase in total costs by UAH 6-7 (EUR 0.14-0.16) per 100 shrubs. Temperature changes of 2°C affect caterpillar development and, consequently, the effectiveness of biopreparations, potentially altering costs by 5-10% depending on the number of required treatments. Combined control methods for the box tree moth, including biopreparations and the introduction of *Trichogramma* spp., minimise environmental risks and plant losses, making these strategies optimal for long-term pest population management. Thus, the practical recommendations proposed in this study are consistent with contemporary international approaches to regulating *C. perspectalis* populations and take into account both local phenological characteristics and broader European trends in the spread of this pest.

CONCLUSIONS

As a result of the conducted study under the conditions of the city of Kyiv, comprehensive data on the bioecology of *C. perspectalis* were obtained, and elements of a system for protecting boxwood from this invasive pest were developed. It was established that in Kyiv, the pest forms two complete generations. At the end of the season, a third generation of caterpillars develops, which enters diapause for the winter period. The periods of mass hatching of early instar caterpillars are critical for population control. A statistically significant relationship between air temperature and the duration of caterpillar development was identified. An increase in mean daily temperature from 18°C to 25°C reduces the duration of the caterpillar stage by 20%, which significantly affects population dynamics. It was demonstrated that mild winters with minimum temperatures not lower than -15°C ensure the survival of more than 70% of overwintering stages, leading to intensified reproduction in the following year.

The evaluation of the effectiveness of different control methods showed the advantage of biological approaches. A biopreparation based on *Bacillus thuringiensis* demonstrated an effectiveness of 96 ± 1.2% against early instar caterpillars (L1-L3), whereas against later instars (L4-L5) this rate decreased to 65.3 ± 2.5%. The introduction of *Trichogramma* spp. ensured parasitism of 71.5 ± 2.6% of eggs, which is a high level for biological control. Mechanical methods proved to be the least effective (45.0 ± 3.9%), but may be used as a supplementary element of an integrated system. A differentiated approach to plant protection is proposed: for botanical gardens, priority is given to biological methods and pheromone monitoring, whereas for urban squares, a combination of biopreparations and mechanical control is recommended. The developed phenologically grounded treatment calendar allows optimisation of intervention timing and improvement of its effectiveness.

The limitations of the study include its regional scope and the two-year observation period, which did not allow for a full assessment of the impact of extreme weather conditions on pest populations. In addition, the research was conducted primarily in urbanised ecosystems, and therefore, the obtained results may differ from those under forest conditions. Prospects for further research include the study of the effectiveness of new biopreparations, monitoring of the duration of pest resistance, and investigation of the impact of the box tree moth on different varieties and forms of boxwood, which is of particular importance for the conservation of biodiversity in urban ecosystems of Ukraine.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Andrieieva, O.Yu., Martynchuk, I.V., Ivaniuk, T.M., Matkovska, S.I., & Marchuk, D.O. (2024). First data on the seasonal development of the box tree moth in urban green plantings of Zhytomyr. *Forestry and Forest Melioration*, 144, 110-118. doi: 10.33220/1026-3365.144.2024.110.

[2] Armanda, A.R., & Bjeliš, M. (2025). Overview of invasion and control management options of *Cydalima perspectalis* (Lepidoptera, Crambidae). *Šumarski List*, 149(1-2), 67-76. doi: 10.31298/sl.149.1-2.6.

[3] Bereś, P.K., Ziętara, P., Nakonieczny, M., Kontowski, Ł., Grzbiela, M., & Augustyniak, M. (2021). *Cydalima perspectalis* in Poland – 8 years of invasion against the background of three other invasive species. *Diversity*, 14(1), article number 22. doi: 10.3390/d14010022.

[4] Bondareva, L.M., Chumak, P.Ya., & Zaichenko, E.M. (2021). Control of the number of *Cydalima perspectalis* (Lepidoptera: Crambidae) using phytocomplexons in the Botanical Garden named after Academician A.V. Fomin. In O.A. Shust *et al.* (Eds.), *Study and preservation of the biodiversity of biocenoses of Ukraine: Proceedings of the all-Ukrainian scientific and practical conference of higher education applicants and young scientists* (pp. 56-57). Bila Tserkva: Bila Tserkva National Agrarian University.

[5] Budziszewska, M., & Bereś, P.K. (2024). The box tree moth *Cydalima perspectalis*: A review of biology, invasiveness, management practices and future perspectives of control strategy in Europe. *Journal of Plant Protection Research*, 64(4), 275-286. doi: 10.24425/jppr. 2024.152886.
[6] Canelles Trabal, Q. (2021). *Incorporating insect pest outbreaks into landscape modelling: The case of Cydalima perspectalis invasion in Europe*. (Doctoral dissertation, Autonomous University of Barcelona, Barcelona, Spain).
[7] Canelles Trabal, Q., Bassols, E., Vayreda, J., & Brotons, L. (2021). Predicting the potential distribution and forest impact of the invasive species *Cydalima perspectalis* in Europe. *Ecology and Evolution*, 11(10), 5713-5727. doi: 10.1002/ece3.7476.
[8] Convention on Biological Diversity. (1992, June). Retrieved from https://www.cbd.int/.
[9] Coyle, D.R., Adams, J., Bullas-Appleton, E., Llewellyn, J., Rimmer, A., Skvarla, M.J., Smith, S.M., & Chong, J.-H. (2022). Identification and management of *Cydalima perspectalis* (Lepidoptera: Crambidae) in North America. *Journal of Integrated Pest Management*, 13(1), article number 24. doi: 10.1093/jipm/pmac020.
[10] Esmaili, R., Jouibary, S.S., Soosani, J., & Naghavi, H. (2020). Mapping of understory infested boxwood trees using high-resolution imagery. *Remote Sensing Applications: Society and Environment*, 18, article number 100289. doi: 10.1016/j.rsase.2020.100289.
[11] Gao, Y., Zhang, J., Wang, Q., Liu, Q., & Tang, B. (2023). The complete mitochondrial genome of box tree moth *Cydalima perspectalis* and insights into phylogenetics in Pyraloidea. *Animals*, 13(6), article number 1045. doi: 10.3390/ani13061045.
[12] Gnatiuk, A.M, & Gaponenko, M.B. (2016). A new invasive pest, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) in Kyiv (Ukraine). In S.I. Galkin (Ed.), *Current trends in the preservation, restoration and enrichment of phytodiversity of botanical gardens and arboretums: Proceedings of the international scientific conference dedicated to the 70th anniversary of the Dendrological Park "Olexandria" as a scientific institution of the NAS of Ukraine* (pp. 99-101). Bila Tserkva: Bila Tserkva National Agrarian University.
[13] Kenis, M. (2022). *Cydalima perspectalis* (box tree moth). *CABI Compendium*. doi: 10.1079/cabicompendium.118433.
[14] Koshelyaeva, Y.V., Ivanov, S.O., Hryhorov, S.Yu., & Zinchenko, O.V. (2024). Box tree moth (*Cydalima perspectalis* (Walker, 1859)) in urban plantings of Kharkiv. In A.O. Rozhkov & V.L. Meshkova (Eds.), Plant protection and quarantine in the 21st century: Challenges and prospects. Proceedings of the 3rd international scientific and practical conference dedicated to the anniversaries of prominent phytopathologists, professors V.F. Peresypkin and F.M. Mariutin (pp. 83-86). Zhytomyr: Ruta Publishing.
[15] Law of Ukraine No. 180-XIV "On Plant Protection". (1998, October). Retrieved from https://zakon.rada.gov.ua/laws/show/180-14#Text.
[16] Makarenko, N.V., Gnatiuk, A.M., Pylypchuk, V.F., & Shevchenko, Y.S. (2019). Effectiveness of biological agents against the boxwood firefly *Cydalima perspectalis* (Walker, 1859). *Introduction to Plants*, 4, 97-106. doi: 10.5281/zenodo.3566638.
[17] Malinowski, H. (2000). The use of *Bacillus thuringiensis* in plant protection: Prospects and limitations. *Biotechnologia*, 3(50), 81-92.
[18] Marchenko, A.B. (2022). Ecological aspects of manifestations of the invasive species Cydalima perspectalis (Walker, 1859) in urbanized ecosystems. *Agrobiology*, 2, 153-160.
[19] MinFin. (2023). *Exchange rate archive. Results of the day 12/31/2023*. Retrieved from https://index.minfin.com.ua/ua/exchange/archive/2023-12-31/.
[20] Murillo, R., Bengoa, P., Santesteban, X., Molina, C., & de Escudero, I.R. (2025). Exploring indigenous entomopathogens for sustainable management of the invasive box tree moth (*Cydalima perspectalis*). *IOBC-WPRS Bulletin*, 176, 110-113.
[21] Nacambo, S., Leuthardt, F.L.G., Wan, H., Li, H., Haye, T., Baur, B., Weiss, R.M., & Kenis, M. (2014). Development characteristics of the box-tree moth *Cydalima perspectalis* and its potential distribution in Europe. *Journal of Applied Entomology*, 138, 14-26. doi: 10.1111/jen.12078.

[22] O'Hanlon, A. (2024). Notes on the box-tree moth (*Cydalima perspectalis*) (Lepidoptera: Crambidae) in Ireland, with observations on breeding and rapid distribution increase. *The Irish Naturalists' Journal*, 40, 144-145.

[23] Ok, K., Ünal, S., & Kaya, G. (2023). Economic analysis of a biotechnical control method against the box tree moth (*Cydalima perspectalis*) in Türkiye. *Bosque*, 44(1), 55-64. doi: 10.4067/S0717-92002023000100055.

[24] Poitou, L., Bras, A., Pineau, P., Lorme, P., Roques, A., Rousselet, J., Auger-Rozenberg, M.-A., & Laparie, M. (2020). Diapause regulation in newly invaded environments: Termination timing allows matching novel climatic constraints in the box tree moth, *Cydalima perspectalis* (Lepidoptera: Crambidae). *Insects*, 11(9), article number 629. doi: 10.3390/insects11090629.

[25] Puzrina, N., Bala, O., Boyko, H., Sovakov, O., & Nosenko, Yu. (2025). Infestation of ash emerald ash borer *Agrilus planipennis* Fairmaire, (Coleoptera: Buprestidae) on the territory of National University of Life and Environmental Sciences (NULES) of Ukraine. *Ukrainian Journal of Forest and Wood Science*, 16(1), 8-22. doi: 10.31548/forest/1.2025.08.

[26] Schmera, D., & Baur, B. (2025). Climate warming and the dynamics of the invasive box-tree moth *Cydalima perspectalis* in the Suburbs of Basel (Switzerland) and in the nearby natural box-tree forest: A 15-year study. *Journal of Applied Entomology*, 149(2), 256-265. doi: 10.1111/jen.13381.

[27] Seehausen, M.L., Rimmer, A., Wiesner, A., Kenis, M., Scott-Dupree, C., & Smith, S.M. (2024). Modelling potential distribution of the invasive box tree moth across Asia, Europe, and North America. *PLOS One*, 19(4), article number e0302259. doi: 10.1371/journal.pone.0302259.

[28] Stan, R., & Mitrea, I. (2021). Evolutionary cycle of *Cydalima perspectalis* Walk. under the influence of climate conditions in Craiova area. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 21(3), 739-744.

[29] Usta, M., Biryol, S., Soydinç, A., & Işık, S. (2025). A novel entomopathogenic fungus from *Cydalima perspectalis*: Pathogenic effects of *Penicillium glabrum* under laboratory conditions. *Journal of Plant Diseases and Protection*, 132, article number 164. doi: 10.1007/s41348-025-01155-5.

[30] Wiesner, A., Llewellyn, J., Smith, S.M., & Scott-Dupree, C. (2021). Biology and distribution of box tree moth (*Cydalima perspectalis*) (Walker, 1859) in southern Ontario. In *Proceedings of the 1st International electronic conference on entomology*. Basel: MDPI. doi: 10.3390/IECE-10514.

[31] Yaman, Ş., & Yaman, M. (2024). Predictive determination of *Cydalima perspectalis* spread areas by using Maxent model. *Journal of the Entomological Research Society*, 26(3), 337-346. doi: 10.51963/jers.v26i3.2604.

Фенологічна пластичність *Cydalima perspectalis* та оцінка ефективності біологічних методів контролю в умовах урбанізованих екосистем

Леся Бондарева

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-8171-2338

Алла Гнатюк

Кандидат біологічних наук, старший дослідник, старший науковий співробітник
Національний ботанічний сад імені М. М. Гришка НАН України
01014, вул. Садово-Ботанічна, 1, м. Київ, Україна
https://orcid.org/0000-0001-5001-971X

Наталія Кушнір

Кандидат біологічних наук, науковий співробітник
Національний ботанічний сад імені М. М. Гришка НАН України
01014, вул. Садово-Ботанічна, 1, м. Київ, Україна
https://orcid.org/0000-0002-9659-8814

Людмила Немерицька

Кандидат біологічних наук, доцент
Житомирський агротехнічний фаховий коледж
10031, вул. Покровська, 96, м. Житомир, Україна
https://orcid.org/0000-0001-7933-0587

Інна Журавська

Кандидат сільськогосподарських наук
Житомирський агротехнічний фаховий коледж
10031, вул. Покровська, 96, м. Житомир, Україна
https://orcid.org/0000-0001-9332-1134

Анотація. Дослідження проведено з метою розробки ефективної системи захисту самшиту від інвазійного шкідника *Cydalima perspectalis* в умовах урбанізованих екосистем України. Робота ґрунтувалася на польових спостереженнях та лабораторному аналізі, проведених у 2022-2023 роках на території Ботанічного саду Національного університету біоресурсів і природокористування України, Національного ботанічного саду імені М. М. Гришка Національної академії наук України, Ботанічного саду імені академіка О. В. Фоміна, а також у зелених насадженнях м. Києва, зокрема в Національному природному парку «Голосіївський» та міських скверах. Встановлено, що в умовах м. Києва шкідник формує дві повні генерації за сезон і третє покоління гусені, що впадає у діапаузу і зимує. Феромонний моніторинг виявив два піки льоту імаго: у I декаді липня і I–II декадах серпня. Виявлено сильний статистично значущий зворотний (негативний) зв'язок (R^2 = 0,96) між середньодобовою температурою повітря та тривалістю стадії гусені. М'які зими забезпечували виживаність понад 70 % зимуючих гусениць. Оцінка ефективності методів контролю показала, що біопрепарат на основі *Bacillus thuringiensis* досягав ефективності 96 ± 1,2 % проти гусеней молодших вікових стадій (L1-L3), а інтродукція трихограми забезпечила паразитування 71,5 ± 2,6 % яєць. Натомість механічні методи були значно менш ефективними (45,0 ± 3,9 %). На підставі отриманих даних розроблено фенологічно обґрунтований календар обробок та диференційовані схеми захисту для ботанічних садів і міських скверів, що дозволяють мінімізувати екологічні ризики та економічні витрати. Результати є основою для практичних рекомендацій щодо збереження самшитових насаджень та мінімізації впливу шкідника на біорізноманіття в Україні

Ключові слова: зелені насадження; *Cydalima perspectalis*; дефоліація; феромонний моніторинг; *Bacillus thuringiensis*; екологіна безпека

Received: 19.12.2025 Revised: 24.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 63-75

UDC 581.143:547.7/.8:631.811.98

DOI: 10.31548/biologiya/2.2026.63

Prospects for the use of growth stimulators based on nitrogen-containing heterocycles in plant propagation technologies

Mykhailo Zavhorodnii

PhD in Biological Sciences, Associate Professor
Khortytsia National Academy
69017, 59 Naukove Mistechko Str., Zaporizhzhia, Ukraine
https://orcid.org/0000-0002-7234-6153

Viktoriia Gencheva

PhD in Biological Sciences, Associate Professor
Zaporizhzhia National University
69011, 66 Universytetska Str., Zaporizhzhia, Ukraine
https://orcid.org/0000-0002-8764-4559

Vasyl Shupeniuk*

PhD in Chemical Sciences, Assistant
Vasyl Stefanyk Precarpathian National University
76018, 57 Shevchenko Str., Ivano-Frankivsk, Ukraine
https://orcid.org/0000-0003-3965-2201

Oleksandr Brazhko

Doctor of Biological Sciences, Professor
Zaporizhzhia National University
69011, 66 Universytetska Str., Zaporizhzhia, Ukraine
https://orcid.org/0000-0002-5212-2689

Valerii Horban

PhD in Biological Sciences, Associate Professor
Zaporizhzhia National University
69011, 66 Universytetska Str., Zaporizhzhia, Ukraine
https://orcid.org/0000-0003-4720-293X

Abstract. Nitrogen-containing heterocycles have attracted the attention of researchers as compounds widely represented in the structures of both natural and synthetic plant growth regulators. The relevance of using natural and synthetic growth regulators in plant propagation is extremely high, as they play a key role in regulating plant growth and development processes, including reproduction. Propagation technologies that employ growth regulators are a vital tool in modern horticulture, agriculture, and biotechnology, enabling the production of high-quality planting material and enhancing production efficiency. The aim of this study was to analyse current approaches to the use of both natural and synthetic growth stimulators based on nitrogen-containing heterocycles in plant propagation, and to further determine the prospects for their application. It has been established

Suggested Citation:
Zavhorodnii, M., Gencheva, V., Shupeniuk, V., Brazhko, O., & Horban, V. (2026). Prospects for the use of growth stimulators based on nitrogen-containing heterocycles in plant propagation technologies. *Biological Systems: Theory and Innovation*, 17(2), 63-75. doi: 10.31548/biologiya/2.2026.63.

*Corresponding author (vasyl.shupeniuk@pnu.edu.ua)

that nitrogen-containing natural and synthetic growth regulators are most effectively used for the rooting of cuttings, microclonal propagation (*in vitro* culture), seed pre-treatment, and the regulation of flowering and fruiting processes. Current strategies for the application of growth stimulators based on nitrogen-containing heterocycles have been systematised, and modern approaches to the development of new, highly efficient compounds using molecular modelling have been outlined. It was demonstrated that the integration of different pharmacophoric fragments within a single molecule can significantly enhance biological activity. Furthermore, the use of newly synthesised compounds, multicomponent formulations, methods for improving their efficiency, and controlled-release systems has shown high effectiveness. The practical significance of this work lays in its potential to guide the development and application of more effective growth stimulators, thereby improving plant propagation efficiency, increasing crop productivity, and optimising biotechnological processes in agriculture and plant science

Keywords: quinoline and succinate derivatives; hybrid molecules; vegetative propagation of plants; microclonal propagation; mixtures of growth stimulators; quinoline derivatives

INTRODUCTION

The modern development of agriculture, biotechnology, and landscape gardening requires the implementation of efficient plant propagation technologies to ensure the production of high-quality planting material and to increase plant productivity and adaptability to changing environmental conditions. In this context, the use of plant growth regulators that specifically influence physiological processes, including rooting, organogenesis, flowering, and fruiting, is particularly important. Among a wide range of biologically active substances, nitrogen-containing heterocyclic compounds occupy a significant place due to their high biological activity, structural diversity, and the ability to modify their properties. Analysis of contemporary scientific studies indicates the active development of this research area. J. Cohen & L. Strader (2024) emphasise that indole compounds' ability to mimic natural phytohormones opens broad prospects for the development of new growth regulators. Auxins are key regulators of plant development, controlling cell division, differentiation, and organ formation processes. The balance between different auxin forms and their transport is critical for the normal functioning of the plant organism. G. Gomes & K. Scortecci (2021) and P. Sun *et al*. (2023) note that indole derivatives not only stimulate growth but also enhance plant resistance to biotic and abiotic stresses by activating protective metabolic pathways. This opens new opportunities for the application of such compounds under stress conditions. At the same time, the effectiveness of growth stimulants depends on both their concentration and the genetic characteristics of plants. An important research direction is the search for an individualised approach to the application of growth regulators for different plant species. Studies confirm the importance of using growth regulators in tissue culture, where they promote the induction of adventitious roots and the synthesis of secondary metabolites. Such approaches are promising for the biotechnological production of valuable compounds.

A number of studies by S.-H. Kim *et al*. (2021), D. Loconsole *et al*. (2023) and F. da Silva *et al*. (2025) demonstrate practical aspects of using growth regulators for biotechnological production and vegetative propagation of ornamental plants. The use of synthetic regulators yields more stable results than natural extracts. To stimulate rooting in difficult-to-root species and to preserve rare and endemic plants, new synthetic growth stimulants based on nitrogen-containing heterocycles are being actively investigated. Considerable interest is focused on studies of nanomaterials and novel biologically active compounds, particularly quinoline and pyrimidine derivatives, that enhance the efficiency of plant growth regulation and provide more controlled effects on physiological processes. At the same time, the development of environmentally safe preparations with high efficiency and low toxicity remains relevant.

The application of 6-benzylaminopurine (6-BAP) in *in vitro* culture stimulates shoot formation, increasing the efficiency of plant propagation. At the same time, different concentrations of this growth regulator affect the accumulation of photosynthetic pigments (chlorophylls and carotenoids) in plants after acclimatisation. Optimal doses of 6-BAP promote both intensive shoot formation and improved plant physiological condition, as reflected in increased pigment content. The study by J. Seka *et al*. (2025) demonstrated that the addition of cytokinins – kinetin or thidiazuron (TDZ) – to the culture medium significantly improves shoot regeneration and overall *in vitro* culture efficiency. TDZ proved to be particularly effective, stimulating more intensive shoot formation in most of the studied varieties. These results indicate the potential to overcome cassava recalcitrance and are of great importance for the mass propagation of valuable agronomic varieties and for the development of agricultural biotechnology. Research by V. Tsygankova *et al*. (2024) and M. Zavhorodnii *et al*. (2024) demonstrated the perspective of the direction modification of the heterocycle and the residue of S-heterocyclic acids. In particular, residues of acetic and succinic acids influenced plant metabolic processes, stimulating growth and propagation.

Thus, the analysis of modern literature indicates significant scientific interest in the use of nitrogen-containing heterocyclic growth regulators in plant propagation technologies. At the same time, further research is needed to clarify their mechanisms of action, optimal concentrations, and the potential to develop new, effective compounds with targeted properties. The aim of this study was to conduct a systematic analysis of modern approaches to the use of natural and synthetic nitrogen-containing heterocycle-based growth stimulators in plant propagation technologies, and to identify prospects for their further application.

MATERIALS AND METHODS

This study was conducted as a narrative review aimed at systematising and summarising current scientific data on the use of nitrogen-containing heterocycle-based natural and synthetic plant growth regulators in plant propagation technologies. The literature search was carried out during the period 2020-2025 using leading international scientific databases, including Scopus, Web of Science, PubMed, Google Scholar, as well as electronic libraries of specialised scientific journals. The search employed keywords in English and Ukrainian, including: plant growth regulators, nitrogen-containing heterocycles, auxins, micropropagation, plant propagation technologies, indole derivatives, quinoline derivatives, rhizogenesis, and their Ukrainian equivalents. The inclusion criteria were: publications in peer-reviewed scientific journals; relevance to the research topic (plant growth regulators, biologically active heterocyclic compounds, plant propagation technologies); timeliness of sources (preference given to studies with emphasis on 2023-2025); availability of experimental or review data on mechanisms of action, efficiency, or practical application of growth regulators. The exclusion criteria included: non-peer-reviewed publications; studies lacking sufficient information on mechanisms of action or application results; duplication of results or secondary sources without new data. In total, 35 scientific sources were analysed, including original studies, review articles, and experimental works on the synthesis, properties, and applications of growth regulators based on nitrogen-containing heterocycles. The literature analysis was performed using comparative, systemic, and structural-functional approaches. Particular attention was paid to studies examining the mechanisms of action of indole, quinoline, and pyrimidine derivatives, as well as their effects on root formation, organogenesis, seed germination, and plant adaptation to stress conditions.

To generalise the obtained information, qualitative analysis methods were used, including: systematisation of data by types of growth regulators; classification of compounds according to their chemical nature and mechanisms of action; comparison of the effectiveness of natural and synthetic stimulators; analysis of modern approaches to the development of new biologically active substances, including molecular modelling methods and the combination of pharmacophoric groups. Technological aspects of the application of growth regulators in plant production and biotechnology were also considered, including micropropagation (*in vitro*), cutting rooting, seed treatment, and the use of prolonged-release formulations. The analysis covers the current stage of scientific development (2020-2025), enabling identification of trends in the development and application of plant growth regulators. The main emphasis was on recent studies (2023-2025) demonstrating the potential of nitrogen-containing heterocyclic compounds to enhance the efficiency of plant propagation technologies.

RESULTS AND DISCUSSION

The modern development of plant science and biotechnology necessitates the search for effective means of regulating plant growth and development. Particular attention has been paid to compounds based on nitrogen-containing heterocycles, which are characterised by high biological activity and the ability to exert targeted effects on plant physiological processes. In this context, it is important to analyse current approaches to the development and application of such growth stimulators, as well as to determine their role in enhancing the efficiency of plant propagation. O. Nedukha (2015) and V. Tsygankova *et al*. (2024) believe that natural and synthetic growth stimulators based on nitrogen-containing heterocycles have attracted researchers' attention due to their high efficiency and potential for targeted influence on plant physiological processes. The development of modern growth regulators is proceeding in the following directions: creating safe, environmentally friendly growth stimulators; employing nanomaterials to enhance the delivery of active substances; and combining multiple active compounds to achieve synergistic effects. J. Cohen & L. Strader (2024) believe that indole compounds, with their unique capacity to mimic peptide structures and reversibly bind to enzymes, are of great importance in regulating plant growth. They stimulate the formation of roots and fruits while activating the plant's immune system against harmful biotic and abiotic factors. Analysing target recognition, receptor recognition, key activation sites, and the mechanism by which indoles activate processes in plants to improve crop growth or disease resistance is a crucial step in further designing such compounds as plant growth regulators and immunity inducers. Understanding the mechanisms of action of indoles is crucial in contemporary strategies for applying growth regulators to control plant growth and enhance their tolerance to biotic and abiotic stresses (Tsygankova *et al*., 2024).

The paper by M. Faizan & S. Hayat (2024) presents the features of synthesis, potential applications, mechanisms of stress resistance formation, and aspects of stability for plant growth regulators. Strigolactones, karrikins, and heme-mediated regulation of plant biology are considered. Plant growth regulators act as biostimulants, enhancing plant tolerance to adverse conditions. Particular attention is paid to the fact that the application of these substances in low concentrations ensures significant improvement in plant vitality and contributes to increased crop yields. The publication by O. Nedukha (2015) indicates that among synthetic nitrogen-containing auxins, the most commonly used are 4-amino-3,5,6-trichloropyridine-2-carboxylic acid (picloram) (Fig. 1a) and 5,6-dichloroindole-3-acetic acid (Fig. 1b).

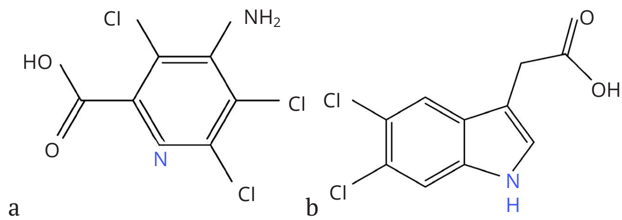

Figure 1. 4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid (a), 2-(5,6-dichloro-1H-indol-3-yl) acetic acid (b)
Source: developed by the authors based on O. Nedukha (2015)

Auxin is a key phytohormone regulating plant growth and development under various conditions. Even at low levels, it influences gene expression via specific transcription factors and signalling proteins (Sun *et al*., 2023). Auxin is synthesised in actively dividing tissues and transported by specialised carrier proteins. Precise control of auxin levels, particularly of its primary form – indole-3-acetic acid (IAA), is crucial for organ formation and is regulated by converting IAA from conjugated forms and precursors (Gomes & Scortecci, 2021; Sosnowski *et al*., 2023). One such precursor is indole-3-butyric acid (IBA), which is converted to IAA via β-oxidation in peroxisomes. Disruptions in this conversion result in developmental defects, underscoring IBA's importance for maintaining auxin homeostasis (Roth *et al*., 2024). Like IAA, IBA can also be transported within plants, though many of its transporters remain unidentified. Auxin biosynthesis occurs through both tryptophan-dependent and tryptophan-independent pathways, involving indole intermediates and key enzymes such as YUC and TAA (Gomes & Scortecci, 2021). Crucially, indole compounds play a significant role in regulating plant growth and influencing resistance to biotic and abiotic stresses. They can enhance plant tolerance to stresses directly or indirectly through the salicylic acid, jasmonic acid, and malate dehydrogenase pathways, and by increasing the activity of related defence enzymes (Roth *et al*., 2024).

Studies by H. Li *et al*. (2024) have shown that triazole plant growth regulators are highly effective at preventing plant lodging and increasing crop yield. However, their prolonged persistence in soil may negatively affect subsequent crop development. Compounds of cyclohexanecarboxylic acid are characterised by moderate effectiveness and rapid soil degradation; however, prohexadione calcium requires caution due to the risk of soil toxicity. The use of B9 in succinic acid derivatives is limited by its elevated toxicity. Y. Zaytseva (2024) developed an effective system for regeneration of *Rhododendron yedoense* var. *poukhanense* (H. Lév.) Nakai from seedlings using TDZ as an inducer of meristem proliferation. Seedlings germinated *in vitro* were used as explants. The highest average number of shoots per explant was obtained after elongation under conditions of direct cultivation on AM medium supplemented with 1.0 μM TDZ, and after a pulse treatment with 30.0 μM TDZ (9.32 and 10.32, respectively). Rooting of plants (50%) was recorded after a 4-hour pulse treatment with indole-3-butyric acid with *in vitro* cultivation on hormone-free AM0 medium. M. Khanam *et al*. (2022) showed that growth regulators play a crucial role in ornamental nurseries, ensuring rapid production of well-developed seedlings. Prolonged growth in small pots leads to root deformation, hindering water and nutrient uptake and reducing plant stress tolerance. IBA often combined with 1-naphthaleneacetic acid, is commonly used in nurseries (Tsaktsira *et al*., 2021). A study on rhododendron propagation (*R. ponticum*, *R. luteum*, *R. ungernii*, and *R. caucasicum*) examined the effects of IBA dosage (0, 500, and 1,500 ppm) and propagation season (autumn/spring) on rooting. *R. caucasicum* and *R. ungernii* showed 100% rooting efficiency, while *R. ponticum* and *R. luteum* had lower rates. The highest root dry mass, volume, and diameter were observed in *R. caucasicum* (autumn, 1,500 ppm IBA), while the longest roots were found in *R. ungernii* (spring, 500 ppm IBA) (Altun, 2023).

Vegetative propagation is crucial for reproducing high-yielding trees (Elouaflin *et al*., 2023). In Ivorian cashew orchards, air-layering protocols were developed, testing various substrates, stem diameters, and IBA concentrations. Sawdust, rice husk, and coconut peat yielded rooting percentages of 88.09%, 79.36%, and 61.90%, respectively, while coffee husk yielded no roots. Larger diameter stems (8-12 mm) and 5 mg/L IBA resulted in the highest rooting (85.18%) and survival (89.41%), with over 80% survival after transplanting. Thus, 8-12 mm stems treated with 5 mg/L IBA in sawdust are optimal for cashew air-layering in Côte d'Ivoire. Vegetative propagation is also effective for species with complex seed germination requirements, such as red osier dogwood (*Cornus sericea* L.). Stem cuttings were used with varying IBA concentrations and cutting ages (previous-year shoots/PYS and current-year shoots/CYS). After four months, IBA improved survival, height, and biomass in PYS cuttings but not in CYS. IBA concentration and cutting age significantly affected root morphology; higher IBA increased root surface area and length, particularly in PYS cuttings, potentially enhancing resource uptake and growth (Inoue *et al*., 2023).

Another important avenue for using propagation technologies with growth regulators involves specific adaptations to challenging environmental conditions. Propagating plants for harsh environments requires tailoring them to specific stresses. A study on *Prunus* rootstocks showed that genotypes significantly affected auxin-induced root formation. This suggests that a plant's genetic makeup plays a key role in its ability to develop stress-resistant roots (Justamante *et al*., 2022). S.-H. Kim *et al*. (2021) showed that IBA is a common growth stimulator used in stem cuttings. Research on *V. dahurica* and *V. pusanensis* confirms that IBA is more effective than NAA in promoting root growth. IBA at 1.5 mg/L significantly increased root number in both species and increased root length in *V. dahurica*. These findings highlight IBA's potential for vegetative propagation in these plants. The influence of mother plant age on adventitious root formation and IBA uptake was studied in *Prunus subhirtella* 'Autumnalis' leaf cuttings. IBA and IAA concentrations were measured over 24 hours in cuttings from mature (60 years), rejuvenated (16 years), and juvenile (*in vitro*) mother plants. Rooting success and root quality were then assessed. Semi-mature plants showed significantly higher rooting (95%) than mature plants (68%), with less callus formation. While IBA concentration in cuttings wasn't affected by mother plant age or time after cutting, the study highlights the impact of mother plant physiology on rooting success and root quality.

Traditional peach rootstock propagation relies on seeds because they are readily available and easy to handle. However, stem cuttings are crucial for propagating hybrids and maintaining genetic uniformity (Lesmes-Vesga *et al.*, 2021). A study comparing K-IBA (potassium indole-3-butyric acid) concentrations (0-0.4%) on peach cuttings in aeroponics and rooting trays found that 0.2% K-IBA optimised rooting and survival, while 0.2% and 0.4% promoted the most adventitious roots. Rooting trays supported better root growth, but aeroponics proved equally effective for rooting with improved control and resource management. In floriculture, vegetative propagation is vital for preserving rose varieties. Research evaluating powdered and gel IBA on hybrid tea ('Mr. Lincolin') and floribunda ('Iceberg') roses, using cuttings of varying lengths (20-35 cm) and an anti-desiccant (Vapor Gard), found that 30 cm cuttings treated with Vapor Gard for three weeks yielded the highest rooting percentages (73-83%). Notably, gel IBA significantly outperformed the powdered form for floribunda roses, improving rooting (93%), root length (15.7 cm), root number (21), shoot number (4), shoot length (19.7 cm), leaf number (20), and leaf area (19.6 cm^2). While both forms were comparable in terms of hybrid tea rose leaf area, gel IBA was superior in all other aspects. This suggests that gel IBA with longer cuttings enhances rose rooting and may be applicable to other ornamentals (Mohammed *et al.*, 2024).

This study evaluated the effect of different numbers of leaflets and concentrations of IBA on the rooting ability of leafy stem cuttings of *Hevea brasiliensis*. Rooting of *Hevea brasiliensis* leafy stem cuttings can be an effective tool for cultivation, conservation of genetic diversity, and restoration of this species in Peru, which has been decimated by decades of extractive practices (Gomes & Scortecci, 2021). Rubber tree cuttings (6-7 cm) with 2 or 5 leaflets were treated with 0-5,000 ppm IBA and rooted in sandy substrate under mist irrigation. After 29 days, cuttings with 5 leaflets and 2,000 ppm IBA showed the best results: 100% survival, 79% rooting, 4.1 roots, and 2.9 cm root length. This technique is a simple yet effective tool for conserving and commercialising rubber trees. A study by J. Seka *et al.* (2025) showed that the addition of exogenous plant growth regulators: KIN at concentrations of 0.12 and 0.24 μM and TDZ at concentrations of 5 and 10 nM, contributed to the optimisation of micropropagation *in vitro*. When reducing the concentration of mineral salts Murashige and Skoog (MS) by half, this was also effective, especially in combination with PGR. The authors of M. Seliem *et al.* (2025), investigated that although 1.0 mg L-1 TDZ produced the highest significant number of shoots, it did not promote rooting. The authors recommended using BAP at a concentration of 4.0 mg L-1. The separate effect of a single cytokinin, added to MS medium, on the *in vitro* propagation of A. sisalana was also studied.

One modern approach to the search for effective compounds is molecular modelling based on known natural and synthetic compounds. Research results from recent years by M. Zavhorodnii *et al.* (2024) show that the combination of a nitrogen-containing heterocycle and mercapto-carboxylic acids enhances biological activity or reveals new effects. An analysis of the lipophilicity, toxicity, and rhizogenesis effects of the studied compounds was conducted. The most toxic compounds had acetic and propanoic acid residues in the 4th position. Among the triazoles, the compound containing bromine in the 4th position of the anthraenedione ring was the most toxic. This is associated with increased bioavailability of more lipophilic compounds (Matada *et al.*, 2021).

The studied compounds exhibited strong stimulatory activity for rhizogenesis *in vitro* in explants of *Paulownia* clone 112 and *Rosa damascena* variety 'Lada'. Some of the studied compounds – (2-((7-chloroquinolin-4-yl)thio)acetic acid) and (3-((7-chloroquinolin-4-yl)thio) propanoic acid) – exceeded the reference drug – 2-(naphthalen-5-ol)acetate acid – by 10 to 20% (Shupeniuk *et al*., 2023). The presence of L-cysteine and N-acetyl in the 4th position of the quinoline cycle reduced activity. The introduction of substituents into the anthraenedione ring did not significantly affect the activity of the triazoles. A number of effective compounds were obtained by combining a heterocycle and a succinic acid residue in a single molecule (Shupeniuk *et al*., 2023) (Fig. 2).

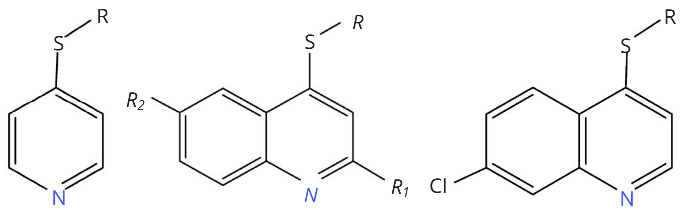

Figure 2. General structure of S-heteryl succinates
Note: R = CH(COOHCH$_2$-COOH); R$_1$ = H, CH$_3$; R$_2$ = H, -OCH$_3$, -OC$_2$H$_5$
Source: developed by the authors based on V. Shupeniuk *et al.* (2023)

The presented research results show that on a nutrient medium supplemented with the compounds: 2-((quinolin-4-yl)thio)succinic acid and 2-((7-chloroquinolin-4-yl)thio)succinic acid, cloned *Paulownia* plants form 4.22 ± 0.31 and 4.50 ± 0.71 roots ($p < 0.001$), respectively, and the rhizogenesis frequency reached 93%. Overall, *Paulownia* clone 112 on a hormone-free nutrient medium initiates the fewest roots and the shortest roots among all tested medium variants. On a nutrient medium containing the compounds with the addition of: 2-((quinolin-4-yl)thio)succinic acid and 2-((7-chloroquinolin-4-yl) thio)succinic acid, rose explants also had a significantly greater number of roots ($p < 0.001$) (Fig. 3).

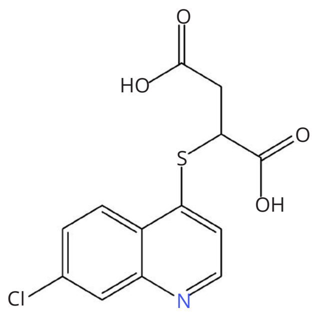

Figure 3. Structure of 2-((7-chloroquinolin-4-yl)thio)succinic acid
Source: developed by the authors based on M. Zavhorodnii *et al*. (2024)

The presented studies show that the compound 2-((7-chloroquinolin-4-yl)thio)succinic acid is a promising plant growth regulator (Zavhorodnii *et al*., 2024). Thus, the creation of new compounds based on succinic acid, an important intermediate in the Krebs cycle and in plant energy metabolism, is a promising direction for the development of effective growth stimulators (Labenska, 2016). It is known that the main derivative of succinic acid used in growth stimulators is gibberellins. In addition to gibberellins, there are other growth stimulators that can be derived from succinic acid or have a similar structure. The triazoles obtained from anthracenedione derivatives showed a moderate stimulatory effect on rhizogenesis *in vitro* in explants of *Paulownia* clone 112 and *Rosa damascena* variety 'Lada'. The presence of a sulphur atom contributes to both antiradical protection (OH) and inhibition of lipid peroxidation processes (ROOH) due to reducing properties with the formation of sulfoxide and six-membered complexes with metals of variable valency. New synthetic pyrimidine derivatives that have a regulatory effect similar to that of plant hormones on root and shoot growth are being actively researched as novel plant growth regulators, pesticides, and fungicides at concentrations of 10^{-5}-10^{-9} (Shupeniuk *et al.*, 2023). A comparative assessment of the restorative activity of synthetic low-molecular-weight nitrogen-containing heterocyclic compounds was carried out, including derivatives of sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur, Kamethur), as well as new thioxopyrimidine derivatives. The most biologically active compounds were identified – Methyur, Kamethur and thioxopyrimidine derivatives (Tsygankova *et al.*, 2025).

Research is exploring the use of natural extracts for plant propagation. A study on *Photinia* 'Red Robin' cuttings compared IBA and seaweed extracts. While seaweed extracts promoted early callus formation, IBA at 1% concentration ultimately yielded the best overall rooting. Seaweed extracts, despite increasing root number, inhibited root length and didn't improve biomass compared to the control, suggesting they aren't suitable for this species' propagation using this method (Loconsole *et al.*, 2023). B. Altun (2023) conducted a study examining the effects of IBA dose and breeding season on rooting rate and several root parameters in rhododendron species: *R. ponticum* L., *R. luteum* Sweet, *R. ungernii* Trautv and *R. caucasicum* Pallas. The author noted that the highest rooting efficiency (100%) was obtained in *R. caucasicum* and *R. ungernii*.

Combining plant growth regulators, particularly auxins and cytokinins, shows promise, enhancing root regeneration, yield, and disease resistance, though some effects on plant chemistry are noted by D. Loconsole *et al.* (2023), J. Sosnowski *et al.* (2023) and V. Tsygankova *et al.* (2024). Direct organogenesis in mature *Pinus massoniana* was optimised using specific disinfection and PGR combinations (6-BA, IBA, NAA), establishing a regeneration system (Wan & Fan, 2024). Propagation of *Ilex aquifolium* benefited significantly from NAA application, with apical cuttings achieving 100% rooting (Tsaktsira *et al.*, 2021). The paper by T. Thakur *et al.* (2025) demonstrates that plant growth retardants are widely used in ornamental horticulture to regulate plant morphology and quality, as well as flowering processes, to enhance ornamental value. Their action is associated with the inhibition of gibberellin biosynthesis. By limiting stem elongation, these substances promote the formation of more compact and bushier plants. It is well known that growth retardants can strengthen stems, increase plant stability, enhance stress tolerance, reduce the risk of lodging, and ensure more uniform flowering.

A. Pourkhaloee (2021) investigated the vegetative propagation of field elm (*Ulmus minor* Mill.) by softwood cuttings that were quickly immersed (8 s) in aqueous solutions of IBA (0, 1,500 and 3,000 mg/L) followed by foliar spraying with a commercial seaweed extract (Citoking – Anoca Chemistry, Spain) at concentrations of 0, 1.5 and 3 ml/L. The highest rooting percentage (78.9) was obtained with 1,500 mg/L IBA, which showed a significant difference compared to the control (20.9). The longest root and shoot lengths (18.0 cm and 55.8 mm, respectively) were observed with 1,500 mg/L IBA + 1.5 ml/L seaweed extract (SWE). The application of SWE had no significant effect on root diameter, leaf bud germination and number of new leaves. In contrast, all these traits improved with 1,500 mg L-1 IBA. Cutting survival during the rooting period was not affected by SWE. Increasing the IBA concentration from 0 to 3,000 mg L-1 decreased survival from 98.6% to 91.7%, respectively. Finally, adventitious root cultures provide a sustainable method for producing metabolites from medicinal plants, thereby addressing limitations in accessing wild plants (Khanam *et al.*, 2022).

Tetrastigma hemsleyanum, a valuable medicinal liana, faces endangerment due to limited wild populations and low seed yield. A study developed protocols for shoot induction and organogenesis from leaves and petioles (Pang *et al.*, 2024). A combination of IBA and thidiazuron induced callus and shoots, with optimal shoot induction on MS medium containing 1.0 mg/L IBA and 0.1 mg/L NAA, achieving a shoot proliferation coefficient (SPC) of 6.73. Increasing light intensity further improved SPC and chlorophyll content. Adventitious shoots readily formed roots (100%) on MS medium with NAA or IBA (0.1-2.0 mg/L). Plants exhibited high survival rates (>98%) after transplanting. These protocols enable mass production of shoots for conservation and potential commercial propagation.

New vegetative propagation methods are being developed for industrially important plants like bamboo (Kumari *et al.*, 2024). A study investigated the effects of citrate- and CTAB-coated gold nanoparticles (AuNPs) on *Bambusa balcooa in vitro*. 400 µM citrate-AuNPs significantly improved shoot proliferation, photosynthetic pigment accumulation, and antioxidant activity. Conversely, 600 µM CTAB-AuNPs decreased growth. Transcriptome analysis identified differentially expressed genes and metabolic pathways associated with nanoelicitation and plant growth, including those involved in oxidative stress response and cell proliferation. This provides insights into the molecular mechanisms by which AuNPs modulate micropropagation.

Methods involving radical activation of plant growth regulators are also under development (Gil *et al.*, 2020). A study explored the effects of light treatment (blue, red, fluorescent) and NAA application on the rooting of single-node stem cuttings. Blue light combined with NAA effectively induced adventitious root formation in otherwise poorly rooting cuttings. Gene expression analysis revealed that cmLBD1 was upregulated by blue light after 5 days, initiating rooting, while other root formation genes were upregulated by fluorescent light after 14 days. This indicates that blue light and NAA are more effective for rapid rooting in single-node cuttings.

New approaches using mixtures of plant growth regulators are crucial for propagating endangered and endemic species. A study focused on *Hieracium lucidum* subsp. *lucidum*, a critically endangered Sicilian endemic (Gianguzzi *et al.*, 2024). Seed germination tests showed high germination rates (70-95%) across various temperatures, indicating no dormancy. Vegetative propagation via stem cuttings was enhanced by IBA treatment, with all treated cuttings rooting within two months compared to 50% rooting in untreated cuttings over a longer period. An efficient *in vitro* propagation protocol using leaf explants was also developed. The combination of meta-topolin (mT, 2 mg/L) and 2,4-D (1 mg/L) in MS medium maximised callus induction and shoot regeneration. Root regeneration was optimal on hormone-free MS and MS with IBA (1 mg/L). This research demonstrates that *H. lucidum* can be successfully propagated using various methods, depending on the available material.

CONCLUSIONS

The use of natural and synthetic plant growth regulators based on nitrogen-containing heterocycles is a promising direction in modern plant propagation technologies. It has been shown that nitrogen-containing heterocyclic plant growth regulators are effective across various modern plant propagation technologies. A review of current research demonstrating the effectiveness of using different groups of growth stimulators for the propagation of plants with extremely complex propagation due to long stratification requirements and low seed germination, for the reproduction of potentially high-yielding plants, under conditions of specific plant adaptations to harsh environmental conditions, is presented. Plant propagation technologies involving plant growth regulators encompass a wide range of methods that stimulate and regulate propagation processes using natural and synthetic substances that affect plant growth and development. It has been shown that plant growth regulators based on nitrogen-containing heterocycles, both natural and synthetic, are effectively used in the main plant propagation technologies – rooting of cuttings, microclonal propagation (*in vitro* tissue culture), seed treatment, and regulation of flowering and fruiting. The review article analyses approaches to the design of new and effective compounds using molecular modelling. It has been shown that combining known pharmacophores into a single molecule increases the effect. Thus, the combination of a nitrogen-containing heterocycle and mercaptocarboxylic acids leads to an enhancement of biological activity or the appearance of new effects. The use of new synthetic preparations, mixtures of growth stimulators, various methods to enhance their effects, and slow-release strategies for growth stimulators has demonstrated their effectiveness. However, it is important to consider possible environmental risks and conduct further research to create safe and effective preparations. Future research should focus on elucidating the mechanisms of action, optimising molecular structure, and evaluating environmental safety and field-scale efficacy of growth stimulators based on nitrogen-containing heterocycles across diverse plant propagation systems.

ACKNOWLEDGEMENTS

None.

FUNDING

Funded by the National Research Fund of Ukraine (NRFU). Project registration number: 2025.05/0026 Development of biologically active nitrogen-containing anthracenedione derivatives.

CONFLICT OF INTEREST

The authors declare no competing interests relevant to this article.

REFERENCES

[1] Altun, B. (2023). Effects of seasons and indole-3-buteric acid doses on the propagation of some native rhododendron species by air layering technique in their natural habitats. *BioResources*, 18(3), 5209-5221. doi: 10.15376/biores.18.3.5209-5221.

[2] Cohen, J.D., & Strader, L.C. (2024). An auxin research odyssey: 1989-2023. *The Plant Cell*, 36(5), 1410-1428. doi: 10.1093/plcell/koae054.

[3] da Silva, F.R., Stefanello, C.A., & Fraga, H.P.F. (2025). 6-benzylaminopurine promotes the shoots formation during plantlets *in vitro* culture and affects the photosynthetic pigments accumulation in acclimatized plants of *Maxillaria picta* (Orchidaceae). *Plant Cell, Tissue and Organ Culture*, 160, article number 58. doi: 10.1007/s11240-025-03002-9.

[4] Elouaflin, A.Y.A., Kouakou, K.L., Kouakou, C., Dao, J.P., & Zoro, B.I.A. (2023). Branch diameters, substrates, and indole-3-butyric acid effect on cashew (*Anacardium occidentale* L.) tree propagation by air layering. *Forestist*, 73(3), 278-284. doi: 10.5152/forestist.2023.22044.

[5] Faizan, M., & Hayat, S. (2024). *Plant growth regulators: Resilience for sustainable agriculture*. Singapore: Springer. doi: 10.1007/978-981-97-2918-0.

[6] Gianguzzi, V., Di Gristina, E., Barone, G., Sottile, F., & Domina, G. (2024). Seed germination and vegetative and *in vitro* propagation of *Hieracium lucidum* subsp. *lucidum* (*Asteraceae*), a critically endangered endemic taxon of the Sicilian flora. *PeerJ*, 12, article number e16839. doi: 10.7717/peerj.16839.

[7] Gil, C.S., Jung, H.Y., Lee, C., & Eom, S.H. (2020). Blue light and NAA treatment significantly improve rooting in single-leaf-bud cuttings of chrysanthemum by upregulating rooting-related genes. *Scientia Horticulturae*, 274, article number 109650. doi: 10.1016/j.scienta.2020.109650.

[8] Gomes, G.L.B., & Scortecci, K.C. (2021). Auxin and its role in plant development: Structure, signaling, regulation and response mechanisms. *Plant Biology*, 23(6), 894-904. doi: 10.1111/plb.13303.

[9] Inoue, S., Ilogu, C., & Sobze, J.-M. (2023). Effects of indole-3-butyric acid and age of stem cuttings on root morphology, growth, and survival of *Cornus sericea*. *Journal of Forestry Research*, 34, 433-440. doi: 10.1007/s11676-022-01490-5.

[10] Justamante, M.S., Mhimdi, M., Molina-Pérez, M., Albacete, A., Moreno, M.A., Mataix, I., & Pérez-Pérez, J.M. (2022). Effects of auxin (indole-3-butyric acid) on adventitious root formation in peach-based *Prunus* rootstocks. *Plants*, 11(7), article number 913. doi: 10.3390/plants11070913.

[11] Khanam, M.N., Anis, M., Javed, S.B., Mottaghipisheh, J., & Csupor, D. (2022). Adventitious root culture – an alternative strategy for secondary metabolite production: A review. *Agronomy*, 12(5), article number 1178. doi: 10.3390/agronomy12051178.

[12] Kim, S.-H., Kim, J.-H., Oh, H.-J., Kim, S.-Y., & Suh, G.-U. (2021). Vegetative propagation of *Veronica dahurica* and *Veronica pusanensis* by stem cuttings with auxins. *Rhizosphere*, 17, article number 100315. doi: 10.1016/j.rhisph.2021.100315.

[13] Kumari, A., Joshi, S., Dar, A.I., & Joshi, R. (2024). Physiological responses and transcriptomic profiles unveil pivotal genes and pathways implicated in nano-elicited *in vitro* shoot proliferation of *Bambusa balcooa*. *Plant Cell, Tissue and Organ Culture*, 158, article number 9. doi: 10.1007/s11240-024-02812-7.

[14] Labenska, I.B. (2016). Succinic acid as a potential pharmacophore in modelling new bioregulators based on nitrogen-containing heterocycles. *Pharmacology and Drug Toxicology*, 2(48), 3-13.

[15] Lesmes-Vesga, R.A., Chaparro, J.X., Sarkhosh, A., Ritenour, M.A., Cano, L.M., & Rossi, L. (2021). Effect of propagation systems and indole-3-butyric acid potassium salt (K-IBA) concentrations on the propagation of peach rootstocks by stem cuttings. *Plants*, 10(6), article number 1151. doi: 10.3390/plants10061151.

[16] Li, H., Cui, G., Li, G., Lu, H., Wei, H., Zhang, H., & Zhang, H. (2024). Assessing the efficacy and residual impact of plant growth retardants on crop lodging and overgrowth: A review. *European Journal of Agronomy*, 159, article number 127276. doi: 10.1016/j.eja.2024.127276.

[17] Loconsole, D., Sdao, A.E., Cristiano, G., & De Lucia, B. (2023). Different responses to adventitious rhizogenesis under indole-3-butyric acid and seaweed extracts in ornamental cuttings: First results in *Photinia x fraseri* 'Red Robin'. *Agriculture*, 13(3), article number 513. doi: 10.3390/agriculture13030513.

[18] Matada, B.S., Pattanashettar, R., & Yernale, N.G. (2021). A comprehensive review on the biological interest of quinoline and its derivatives. *Bioorganic Medicinal Chemistry*, 32, article number 115973. doi: 10.1016/j.bmc.2020.115973.

[19] Mohammed, I., Karrar, H., Al-Taey, D.K.A., Li, G., Yonglin, R., & Alsaffar, M.F. (2024). Response of rose stem cuttings to indole-3-butyric acid for root formation and growth traits. *Sabrao Journal of Breeding and Genetics*, 56(3), 1187-1198. doi: 10.54910/sabrao2024.56.3.25.

[20] Nedukha, O. (2015). *Cell wall of plants and environment*. Kyiv: Alterpress.

[21] Pang, J., *et al*. (2024). Shoot organogenesis from *Tetrastigma hemsleyanum* leaf and petiole explants, and subsequent plant regeneration and acclimatisation. *Journal of Plant Growth Regulation*, 43, 4782-4795. doi: 10.1007/s00344-024-11433-5.

[22] Pourkhaloee, A. (2021). Indole-3-butyric acid and a seaweed-based biostimulant improve vegetative propagation of field elm (*Ulmus minor* Mill.) by softwood cuttings. *Propagation of Ornamental Plants*, 21(4), 107-115.

[23] Roth, O., *et al*. (2024). Slow release of synthetic auxin induces formation of adventitious roots in recalcitrant woody plants. *Nature Biotechnology*, 42, 1705-1716. doi: 10.1038/s41587-023-02065-3.

[24] Seka, J.S.S., Kouassi, M.K., Yéo, E.F., Saki, F.M., Otron, D.H., Tiendrébéogo, F., Eni, A., Kouassi, N.K., & Pita, J.S. (2025). Removing recalcitrance to the micropropagation of five farmer-preferred cassava varieties in Côte d'Ivoire by supplementing culture medium with kinetin or thidiazuron. *Frontiers in Plant Science*, 16, article number 1538799. doi: 10.3389/fpls.2025.1538799.

[25] Seliem, M.K., Abdalla, N., & El-Mahrouk, M.E. (2025). Cytokinin potentials on *in vitro* shoot proliferation and subsequent rooting of *Agave sisalana* Perr. Syn. *Horticulturae*, 11(8), article number 929. doi: 10.3390/horticulturae11080929.

[26] Shupeniuk, V.I., Zavhorodnii, M.P., Derevyanko, N.P., Taras, T.N., Shkopynska, T.Ye., Brazhko, O.A., & Matkivskyi, M.P. (2023). Search of regulators among (7-chloroquinoline-4-ylthio) carbonic acids and triazoles of anthracenedione for microclonal propagation of plants. *Journal of Chemistry and Technologies*, 30(4), 568-576. doi: 10.15421/jchemtech.v31i1.271400.

[27] Sosnowski, J., Truba, M., & Vasileva, V. (2023). The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture*, 13(3), article number 724. doi: 10.3390/agriculture13030724.

[28] Sun, P., Huang, Y., Yang, X., Liao, A., & Wu, J. (2023). The role of indole derivative in the growth of plants: A review. *Frontiers in Plant Science*, 13, article number 1120613. doi: 10.3389/fpls.2022.1120613.

[29] Thakur, T., Garg, A., & Kaur, P. (2025). Growth retardants: Efficient tool for regulating plant architecture and flowering in ornamental crops. *South African Journal of Botany*, 184, 911-922. doi: 10.1016/j.sajb.2025.06.047.

[30] Tsaktsira, M., *et al*. (2021). Vegetative propagation and ISSR-based genetic identification of genotypes of *Ilex aquifolium* 'Agrifoglio Commune'. *Sustainability*, 13(18), article number 10345. doi: 10.3390/su131810345.

[31] Tsygankova, V., Andrusevich, Ya., Kopich, V., Vasylenko, N., Solomyannyi, R., Popilnichenko, S., Kachaeva, M., Kozachenko, O., Pilyo, S., & Brovarets, V. (2024). Wheat growth in the vegetative phase under the regulatory effect of furopyrimidine derivatives. *The Scientific Heritage*, 140, 3-12. doi: 10.5281/zenodo.12720608.

[32] Tsygankova, V.A., Vasylenko, N.M., Andrusevich, Ya.V., Kopich, V.M., Solomyannyi, R.M., Kachaeva, M.V., Bondarenko, O.M., Pilyo, S.G., Popilnichenko, S.V., & Brovarets, V.S. (2025). Screening of synthetic auxin-like and cytokinin-like compounds, derivatives of thioxopyrimidine as new plant growth regulators. *Significances of Bioengineering & Biosciences*, 7(2), 779-789. doi: 10.31031/SBB.2025.07.000657.

[33] Wan, Y., & Fan, F. (2024). Direct organ regeneration from apical shoot buds of adult *Pinus massoniana* Lamb. *In Vitro Cellular & Developmental Biology – Plant*, 60, 202-213. doi: 10.1007/s11627-024-10415-2.
[34] Zavhorodnii, M.P., Derevianko, N.P., Shkopynska, T.E., Brazhko, O.A., Kornet, M.M., Gencheva, V.I., Luchkevych, E.R., & Shupenyuk, V.I. (2024). Study of the effect of S-heterilsuccinates on microclonal propagation of ornament.al plants. *Pharmaceutical Review*, 3, 21-34. doi: 10.11603/2312-0967.2024.3.14838.
[35] Zaytseva, Y.G. (2024). *In vitro* conservation and thidiazuron-induced micropropagation of *Rhododendron yedoense* var. *poukhanense* (H. Lév.) Nakai. *Contemporary Problems of Ecology*, 17, 928-935. doi: 10.1134/S1995425524700677.

Перспективи використання стимуляторів росту на основі азотовмісних гетероциклів у технологіях розмноження рослин

Михайло Завгородній
Кандидат біологічних наук, доцент
Хортицька національна академія
69017, вул. Наукове Містечко, 59, м. Запоріжжя, Україна
https://orcid.org/0000-0002-7234-6153

Вікторія Генчева
Кандидат біологічних наук, доцент
Запорізький національний університет
69011, вул. Університетська, 66, м. Запоріжжя, Україна
https://orcid.org/0000-0002-8764-4559

Василь Шупенюк
Кандидат хімічних наук, асистент
Карпатський національний університет імені Василя Стефаника
76018, вул. Шевченка, 57, м. Івано-Франківськ, Україна
https://orcid.org/0000-0003-3965-2201

Олександр Бражко
Доктор біологічних наук, професор
Запорізький національний університет
69011, вул. Університетська, 66, м. Запоріжжя, Україна
https://orcid.org/0000-0002-5212-2689

Валерій Горбань
Кандидат біологічних наук, доцент
Запорізький національний університет
69011, вул. Університетська, 66, м. Запоріжжя, Україна
https://orcid.org/0000-0003-4720-293X

Анотація. Азотовмісні гетероциклічні сполуки привертають увагу дослідників як речовини, широко представлені у структурах як природних, так і синтетичних регуляторів росту рослин. Актуальність використання природних і синтетичних регуляторів росту в розмноженні рослин є надзвичайно високою, оскільки вони відіграють ключову роль у регуляції процесів росту і розвитку рослин, включаючи розмноження. Технології розмноження із застосуванням регуляторів росту є важливим інструментом сучасного садівництва, сільського господарства та біотехнології, забезпечуючи отримання високоякісного посадкового матеріалу та підвищення ефективності виробництва. Метою цього дослідження було проаналізувати сучасні підходи до використання як природних, так і синтетичних стимуляторів росту на основі азотовмісних гетероциклів, а також визначити перспективи їх подальшого застосування. Встановлено, що азотовмісні природні та синтетичні регулятори росту найефективніше застосовуються для укорінення живців, мікроклонального розмноження (*in vitro* культура), передпосівної обробки насіння, а також регулювання процесів цвітіння і плодоношення. Систематизовано сучасні стратегії застосування стимуляторів росту на основі азотовмісних гетероциклів та окреслено сучасні підходи до розробки нових високоефективних сполук із використанням молекулярного моделювання. Показано, що інтеграція різних фармакофорних фрагментів в одній молекулі може суттєво підвищувати біологічну активність. Крім того, використання новосинтезованих сполук, багатокомпонентних препаратів, методів підвищення їх ефективності та систем контрольованого вивільнення продемонструвало високу результативність. Практичне значення роботи полягає у можливості використання її результатів для розробки та впровадження більш ефективних стимуляторів росту, що сприятиме підвищенню ефективності розмноження рослин, зростанню врожайності та оптимізації біотехнологічних процесів у сільському господарстві та рослинництві

Ключові слова: похідні хіноліну та сукцинату; гібридні молекули; вегетативне розмноження рослин; мікроклональне розмноження; суміші стимуляторів росту; похідні хіноліну

Received: 07.01.2026 Revised: 25.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 76-91

UDC 577.21:612.017.1

DOI: 10.31548/biologiya/2.2026.76

Theoretical aspects of the formation of key elements of the macrophage activation dichotomy concept: Literature review

Anhelina Andriushchenko*

Postgraduate Student
Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine
02000, 9 Leontovych Str., Kyiv, Ukraine
https://orcid.org/0000-0003-4174-2740

Abstract. The aim of this study was to analyse the theoretical aspects of the formation of the macrophage activation dichotomy concept (M1/M2), which, despite the complexity of immune response regulation, reflects the existence of a highly efficient multilevel system controlling macrophage functional states and ensuring the proper course of inflammatory processes. Disruption of this system is associated with the development of pathological conditions, including chronic inflammation and tumour progression. The materials of the study consisted of contemporary publications presenting data from fundamental and experimental research on the mechanisms of macrophage activation and polarisation. The methods used include systematisation, comparative analysis, and generalisation of modern scientific approaches, with emphasis on molecular mechanisms and recent technological advances. The classical model of macrophage polarisation distinguishes between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes; however, this classification is increasingly considered oversimplified and does not fully reflect macrophage heterogeneity *in vivo*. It was shown that cytokines such as interferon-gamma (IFN-γ), interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor-beta (TGF-β) play a key role in shaping macrophage phenotypes and regulating their involvement in inflammation, tissue repair, and tumour microenvironment processes. Furthermore, macrophages are capable of simultaneously expressing features of different activation programmes, forming mixed or hybrid phenotypes. Based on the analysis of current data, it was concluded that the M1/M2 dichotomy has limited explanatory capacity and should be replaced by a model that considers macrophage activation as a dynamic continuum dependent on microenvironmental conditions. The results of this study may be useful for researchers and clinicians in immunology and oncology for improving approaches to immunomodulatory therapy and the development of targeted treatment strategies

Keywords: macrophage polarisation; M1/M2; cytokines; immune regulation; inflammation; plasticity; tumour microenvironment

INTRODUCTION

Macrophages are highly specialised cells of the innate immune system that play a key role in maintaining tissue homeostasis, regulating inflammation, and coordinating tissue repair. Due to their pronounced functional plasticity, macrophages can rapidly respond to signals from the microenvironment and acquire different activation states under physiological and pathological conditions. Interest in macrophage polarisation has significantly increased in recent years because dysregulation of macrophage activation is associated with chronic inflammatory diseases, fibrosis,

Suggested Citation:
Andriushchenko, A. (2026). Theoretical aspects of the formation of key elements of the macrophage activation dichotomy concept: Literature review. *Biological Systems: Theory and Innovation*, 17(2), 76-91. doi: 10.31548/biologiya/2.2026.76.

*Corresponding author (02222angel@gmail.com)

autoimmune disorders, cardiovascular pathology, and cancer progression. Therefore, understanding the mechanisms of macrophage activation is important for the development of modern immunotherapeutic approaches and targeted immune modulation strategies.

Recent studies have substantially expanded current understanding of macrophage heterogeneity and functional specialisation. Authors S. Pérez & S. Rius-Pérez (2022), investigating macrophage polarisation during acute inflammation, demonstrated that redox signalling pathways critically regulate macrophage reprogramming. According to their findings, oxidative stress promotes the pro-inflammatory M1 phenotype, whereas antioxidant mechanisms support M2-associated tissue repair responses. The authors also reported that M1 and M2 macrophages possess distinct metabolic profiles regulated by redox-dependent mechanisms. J. Cornice *et al*. (2024) analysed the role of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling in tumour-associated macrophages and showed that NF-κB transcription factors function as major regulators of macrophage activation in cancer. Their study demonstrated that activation of the NF-κB pathway contributes to polarisation toward an M2-like immunosuppressive phenotype, thereby promoting tumour progression and suppressing anti-tumour immunity. Additional insight into metabolic regulation of macrophage polarisation was provided by H. Guo *et al*. (2025), who investigated the relationship between lactylation and macrophage functional reprogramming. The authors demonstrated that lactylation acts as an epigenetic mechanism regulating gene expression associated with macrophage polarisation. Increased lactylation was shown to promote M2 polarisation and contribute to tumour progression and inflammatory dysregulation.

The role of macrophage polarisation in fibrotic diseases was investigated by L. Xie *et al*. (2023). The authors demonstrated that activation of G protein-coupled oestrogen receptor 1 (GPER1) inhibited both classically activated macrophages (M1) and alternatively activated macrophages (M2) polarisation and reduced inflammatory and fibrotic lesions in kidney tissues. They additionally reported that suppression of mitogen-activated protein kinase signalling pathways contributed to decreased macrophage-mediated tissue injury. Macrophage polarisation has also been actively studied in autoimmune diseases. N. Zerrouk *et al*. (2024), using computational modelling in rheumatoid arthritis research, reported that the balance between classically activated macrophages (M1) and alternatively activated macrophages (M2) plays a crucial role in chronic inflammation. The authors identified nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Janus kinase 1/Janus kinase 2, and extracellular signal-regulated kinase 1/Notch homolog 1 signalling pathways as potential therapeutic targets capable of selectively suppressing pro-inflammatory macrophage activity.

The dynamic nature of macrophage activation was emphasised by A. Marrufo & A. Flores-Mireles (2024). The authors described macrophages as highly plastic immune cells capable of polarising along a continuum between M1-like and M2-like phenotypes depending on tissue microenvironmental signals. According to their analysis, dysregulated macrophage activation may contribute to persistent inflammatory responses and unfavourable clinical outcomes. An important contribution to understanding macrophage behaviour in the tumour microenvironment was made by M. Bruch-Oms *et al*. (2023). Investigating interactions between cancer-associated fibroblasts and macrophages, the authors demonstrated that activated fibroblasts promote macrophage polarisation toward immunosuppressive phenotypes. Their findings showed that stromal components of the tumour microenvironment significantly influence macrophage functional programming and tumour immune evasion. Similar observations were reported by S. Pisano *et al*. (2025), who analysed interactions between ovarian cancer cells and macrophage populations using organotypic spheroid models. The authors demonstrated that anti-inflammatory macrophages promoted epithelial-to-mesenchymal transition and contributed to metastatic progression through paracrine signalling mechanisms. Collectively, contemporary studies indicate that the traditional M1/M2 dichotomy does not fully reflect the diversity of macrophage phenotypes observed in vivo. Current evidence suggests that macrophage activation is regulated through complex interactions between cytokines, stromal cells, metabolic pathways, and tissue-specific environmental signals. Consequently, macrophage activation is increasingly interpreted as a dynamic continuum of functional states rather than a strict binary classification.

The aim of this study was to analyse current theoretical approaches to macrophage activation and evaluate the evolution of the macrophage polarisation concept from the classical M1/M2 dichotomy toward a continuum-based model of macrophage functional states. The literature review for this study was conducted using the PubMed and Google Scholar databases. Publications published between 2020 and 2026 were analysed to identify recent advances related to macrophage activation, polarisation, tissue heterogeneity, fibrosis, autoimmune disorders, and tumour-associated macrophages. The search strategy included the keywords "macrophage polarisation", "M1 macrophages", "M2 macrophages", "macrophage plasticity", "tumour microenvironment", and "macrophage heterogeneity". Priority was given to peer-reviewed English-language articles indexed in international scientific databases. Both original experimental studies and review articles were included if they addressed theoretical or functional aspects of macrophage activation. Studies published before 2020 or papers lacking relevance to macrophage polarisation mechanisms were excluded from the analysis. In this study, the author systematised and critically analysed current data on the concept of macrophage activation dichotomy.

Origin, ontogeny, and tissue heterogeneity of macrophages

In mammalian organisms, macrophages develop from bone marrow stem cells. In humans, the bone marrow contains resident macrophages, as well as their progenitor cells: monocytes, promonocytes, and monoblasts, which represent successive stages of mononuclear phagocyte differentiation. E. Kvedaraite *et al*. (2024) demonstrated that monocytes and neutrophils share a common myeloid precursor, highlighting the close developmental relationship between these innate immune cell lineages. The monoblast represents the least differentiated stage of the mononuclear phagocyte system. Through mitotic division, monoblasts give rise to promonocytes, which subsequently differentiate into circulating monocytes. B. Corleis *et al*. (2023) showed that monocytes exit the bone marrow and enter peripheral blood, where they circulate for a limited period before migrating into tissues under the influence of chemotactic signals.

Historically, it was assumed that tissue-resident macrophages are predominantly derived from circulating monocytes. However, E. Mass *et al*. (2023) provided evidence that a substantial proportion of tissue macrophages originate from embryonic precursors and can be maintained locally with minimal contribution from monocyte recruitment. This finding revised the traditional concept of macrophage ontogeny and emphasised their dual origin. Nevertheless, tissue macrophages represent a highly dynamic and adaptable cell population. Their differentiation from monocytes into fully functional macrophages is not a terminal state but rather a continuous process of functional adaptation shaped by cytokines and microenvironmental signals. This plasticity is essential for their dual role in initiating inflammatory responses and promoting tissue repair. The ultrastructural characteristics of macrophages under physiological and infectious conditions are summarised in Figure 1.

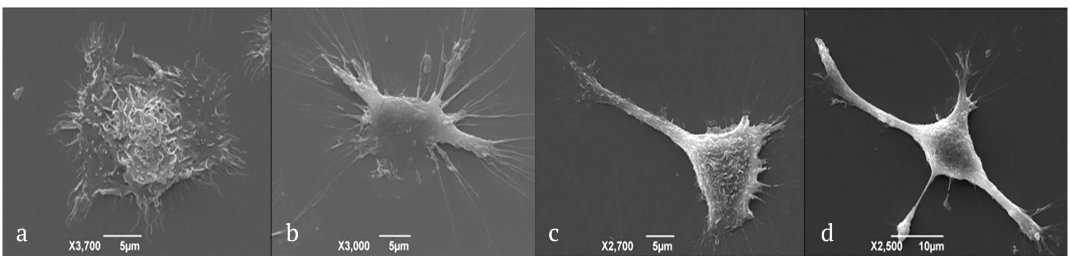

Figure 1. Morphological differences between non-infected and *Trypanosoma cruzi*-infected macrophages

Note: infected macrophages exhibit a more elongated and stellate morphology compared to non-infected cells. Scanning electron microscopy images show peritoneal macrophages from BALB/c mice cultured on polystyrene surfaces, 2D biofilms, and 3D matrices. Non-infected cells are presented in panels (A, C), while infected cells are shown in panels (B, D). Infection was performed using metacyclic trypomastigotes (clone Dm28c) at a 1:1 parasite-to-cell ratio, and images were obtained 48 hours post-infection

Source: J. Logullo *et al.* (2023)

The fate of senescent macrophages remains incompletely understood. D. Guan *et al*. (2025) reported that macrophages are capable of migrating from peripheral tissues to lymphoid organs, where they undergo apoptosis or participate in antigen presentation. This supports the concept of dynamic turnover and systemic integration of macrophage populations. The distribution and specialisation of mononuclear phagocytes across different tissues are summarised in Table 1.

Table 1. Mononuclear phagocytes in the human body

Location	Organ/tissue	Mononuclear phagocyte
Bone marrow		**Monoblasts, promonocytes, monocytes, macrophages**
Peripheral blood		**Monocytes**
Organs	Liver Lugs Spleen Lymph nodes Thymus Bones Synovium Gastrointestinal tract Urogenital tract Endocrine system Central nervous system Skin	Kupffer cells Alveolar macrophages Macrophages of the red pulp Macrophages Macrophages Osteoclasts Type a cells Macrophages Macrophages Macrophages Microglia Langerhans cells
Serous cavities		Pleural macrophages, peritoneal macrophages
Sites of inflammation		Epithelioid cells, exudate macrophages, multinucleated giant cells

Source: developed by the author

During acute inflammatory responses, circulating monocytes are actively recruited to affected tissues, where they differentiate into inflammatory macrophages. R. Britt *et al*. (2023) demonstrated that these recruited cells actively contribute to immune cell infiltration and the production of pro-inflammatory cytokines, thereby amplifying local inflammatory responses.

Metabolic reprogramming and plasticity of macrophages

Macrophage metabolism represents a highly dynamic and tightly regulated system that undergoes profound changes during the transition from circulating monocytes to differentiated tissue macrophages. This process is accompanied by extensive metabolic reprogramming that enables macrophages to adapt to diverse microenvironmental conditions and functional demands. As demonstrated by O. Kolliniati *et al*. (2022), macrophage activation is closely linked to shifts in core metabolic pathways, including glycolysis, oxidative phosphorylation, and fatty acid metabolism. Similarly, S. Wculek *et al*. (2022) emphasised that tissue-resident macrophages display remarkable metabolic heterogeneity, which reflects both their developmental origin and local environmental cues. Recent findings by A. Wong *et al*. (2025) further highlight that macrophage energy metabolism plays a critical role in maintaining cellular function under both physiological and pathological conditions, particularly in cardiometabolic diseases. During differentiation, macrophages exhibit an increase in mitochondrial biogenesis and enhanced oxidative capacity, which supports their functional specialisation. At the same time, glycolytic pathways remain essential, particularly under inflammatory conditions. L. Wang *et al*. (2023) demonstrated that immunometabolic reprogramming is a central mechanism regulating macrophage polarisation, especially in acute inflammatory states such as lung injury. In addition, X. Chen *et al*. (2025) showed that metabolic intermediates themselves can act as signalling molecules, modulating inflammatory responses and contributing to the phenomenon of meta-inflammation observed in obesity and diabetes. These findings indicate that metabolism is not merely a source of energy but also a key regulatory system controlling macrophage behaviour.

Adenosine triphosphate generated through metabolic pathways is essential for supporting energy-intensive processes such as phagocytosis, migration, and cytokine secretion. According to B. Wang *et al*. (2021), lipid metabolism pathways, including arachidonic acid metabolism, play a crucial role in shaping inflammatory responses and determining macrophage functional states. Furthermore, it was demonstrated that interactions with mesenchymal stem cells can induce metabolic reprogramming in macrophages, thereby altering their phenotype and functional activity. These observations underscore the importance of metabolic plasticity as a fundamental property of macrophages. Macrophages are capable of maintaining functional activity under a wide range of environmental conditions, including hypoxia. This adaptability is particularly important in pathological contexts such as tumours, chronic inflammation, and ischemic tissues. As shown by N. Tran & E. Mills (2024), redox regulation is a key component of macrophage metabolism, linking cellular metabolic state with reactive oxygen species production and signalling pathways. The generation of reactive oxygen intermediates plays a critical role in antimicrobial defence but must be tightly controlled to prevent tissue damage. In this context, T. Liu *et al*. (2025) highlighted the importance of metabolic enzymes in regulating inflammatory and pathological processes, particularly in liver diseases and cancer.

In addition to classical metabolic pathways, recent studies have identified novel regulatory mechanisms involving transcriptional and post-transcriptional control. For example, J. Fernandes *et al*. (2024) demonstrated that microRNA-mediated regulation of polyamine metabolism significantly influences macrophage activation and pathogen response. Similarly, J. Galley *et al*. (2021) reported that systemic metabolic changes, including alterations in tryptophan metabolism, can indirectly affect macrophage function through host-microbiome interactions. These findings suggest that macrophage metabolism is integrated into broader systemic metabolic networks. Importantly, macrophage metabolic states are closely linked to their functional phenotypes. Pro-inflammatory macrophages are typically associated with increased glycolysis and disrupted mitochondrial respiration, whereas anti-inflammatory or tissue-repair phenotypes rely more on oxidative metabolism and lipid utilisation. M. Klichinsky *et al*. (2020) demonstrated that targeted metabolic reprogramming can enhance macrophage anti-tumour activity, opening new perspectives for immunotherapy. Moreover, F. Guan *et al*. (2025) emphasised that macrophage heterogeneity and metabolic diversity are critical determinants of disease progression and therapeutic response. Thus, macrophage metabolism should be considered not only as a supportive system for cellular energy supply but as a central regulatory hub integrating environmental signals, intracellular pathways, and functional outcomes. The ability of macrophages to dynamically reprogram their metabolism in response to changing conditions represents a key mechanism underlying their plasticity and versatility in both health and disease.

Macrophage receptors: Structure, diversity, and functional roles in activation and signalling

To perform their diverse functions in the body, macrophages possess a wide array of receptors responsible for regulating processes such as growth, differentiation, activation, ligand recognition, endocytosis, phagocytosis, migration, and secretion. As emphasised by M. Wang *et al*. (2021), macrophage populations exhibit substantial heterogeneity in receptor expression depending on tissue localisation and developmental origin, which directly affects their functional specialisation. The complexity of macrophage receptor systems reflects their central role in both innate and adaptive immunity, as well as in maintaining tissue homeostasis. Among the first described macrophage receptors are Fc receptors, which are receptors for the Fc fragment of Immunoglobulin G (IgG) and receptors for components of the complement system. According to H. Bauer-Smith *et al*. (2023), Fc receptor-mediated signalling plays a crucial role in determining the threshold of immune activation and coordinating downstream effector responses. In addition, A. Petty *et al*. (2022) demonstrated that alterations in Fc receptor expression are associated with pro-inflammatory states, further highlighting their regulatory importance. Ligand binding to Fc receptors activates a wide variety of processes in macrophages, ranging from endocytosis and cytoskeletal rearrangement to phagocytosis and the secretion of molecules necessary for the antibacterial response. The Fc receptor family includes the high-affinity receptor cluster of differentiation 64 (CD64), as well as the low-affinity receptors CD32 and CD16. Stimulation of macrophages with IL-4 activates the expression of another low-affinity Fc receptor, CD23, a low-affinity Fc receptor that binds Immunoglobulin E (IgE), linking Fc receptor signalling to alternative activation pathways, as also supported by N. Kern *et al*. (2021). In parallel with Fc receptors, macrophages express receptors for components of the complement system, including complement receptors CR1, CR3, and CR4. These receptors are responsible for the recognition, binding, and internalisation of opsonised particles. A. Lokki *et al*. (2021) demonstrated that functional alterations in complement receptors, particularly CR3 and CR4, can significantly affect immune responses and are associated with pathological conditions such as pre-eclampsia. The coordinated action of Fc and complement receptors ensures efficient pathogen clearance and modulation of inflammatory responses.

Macrophages also express a large number of receptors for cytokines and growth factors. Cytokine receptors are subdivided into families depending on homology to one of the following receptors: IL-2R (interleukin-2 receptor), IL-3R (interleukin-3 receptor), IL-4R (interleukin-4 receptor), GM-CSFR (granulocyte-macrophage colony-stimulating factor receptor), immunoglobulin-like receptor, TNFR (tumour necrosis factor receptor) and TGF-βR (transforming growth factor-β receptor). The signalling systems activated by these receptors often use kinase activity as the first stage of signal transduction. This is followed by the activation of various transcription factors, among which it is worth noting NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), activated in response to TNF-α (tumour necrosis factor alpha) and IFN-γ (interferon-gamma), transcription regulators of the STAT proteins (signal transducers and activators of transcription), and Smad family members (proteins homologous to Caenorhabditis elegans Sma and Drosophila Mad). As demonstrated by C. Li *et al*. (2024), interferon-gamma (IFN-γ) signalling is a key driver of macrophage-mediated inflammatory responses, particularly in respiratory pathologies. Exposure to specific cytokines or their combinations can profoundly alter macrophage function. For example, IL-4 (interleukin-4) and IL-13 (interleukin-13) promote alternative activation and increase expression of the mannose receptor, whereas IFN-γ enhances oxidative and bactericidal activity. In addition, H. Jo *et al*. (2026) showed that extracellular signals, such as platelet-derived factors, can reprogram macrophage responses through receptor-mediated mechanisms, further emphasising the plasticity of these cells. Given the diversity of ligands and signalling pathways involved, macrophage receptor systems can be broadly categorised according to their functional roles. A structured overview of the major receptor classes expressed by monocytes and macrophages, along with their corresponding ligands, is presented in Table 2.

Table 2. Monocyte and macrophage receptors

Receptor	Ligands
Fc receptors	Immunoglobulin (Ig)G, A, E
Complement receptors	Complement component (C3)b, d, anaphylatoxin, complement component 1q, Inactivated complement component 3b (iC3b)
Cytokine receptors	MIF[1], MAF[2], LIF[3], CF[4], MFF[5], Interleukin (IL)-1, -2, -3, -4, -6, -7, -10, -13, -17, Interferon (IFN)-α, β, γ, GM-CSF[6], CSF1[7]
Chemokine receptors	MIP-1α, -1β[8], RANTES[9], MCP-1, -2, -3, -4[10], IL-8[11], SDF-1[12]
Hormone receptors	Insulin, glucocorticosteroids, angiotensin
Transferrin & lactoferrin receptors	Transferrin, lactoferrin
Lipoprotein & lipid receptors	Low-density lipoproteins (LDL), prostaglandin E_2, leukotriene B_4, leukotriene D_4, apolipoproteins B and E
Peptide & small molecule receptors	1,25-dihydroxyvitamin D_3, endorphins, substance P, vasopressin
Coagulation & anticoagulation factor receptors	Fibrinogen/fibrin, coagulation factor VII, heparin
Fibronectin receptors	Fibronectin
AGE receptors	Advanced glycation end-products (AGEs)

Table 2. Continued

Receptor	Ligands
Lectin-like receptors	Fucose, mannose, galactose, sialic acid
Toll-like receptors (TLRs)	Pathogen-specific molecular patterns (lipopolysaccharide, unmethylated cytosine-phosphate-guanine DNA motifs (CpG oligonucleotides)

Note: MIF – macrophage migration inhibitory factor; MAF – macrophage activating factor; LIF – leukaemia inhibitory factor; CF – chemotactic factor; MFF – macrophage fusion factor; GM-CSF – granulocyte-macrophage colony-stimulating factor; CSF-1 – colony-stimulating factor 1 (macrophage CSF, M-CSF); MIP-1α / MIP-1β – macrophage inflammatory protein-1 alpha/beta; RANTES – regulated upon activation, normal T cell expressed and secreted (CCL5); MCP-1, -2, -3, -4 – monocyte chemoattractant proteins (CCL2, CCL8, CCL7, CCL13); IL-8 – interleukin-8 (CXCL8); SDF-1 – stromal cell-derived factor 1 (CXCL12)
Source: developed by the author

In addition to a wide spectrum of cytokine receptors, a large number of various chemokine receptors, macrophages express a large number of various chemokine receptors that regulate their migration and positioning within tissues. As shown by N. Zhang *et al*. (2022), receptors such as CXCR4 (C-X-C chemokine receptor type 4) play a critical role in macrophage-mediated intercellular communication and tissue remodelling, particularly in cardiovascular diseases. Representatives of the CC (chemokine ligand family with two adjacent cysteines), CXC (chemokine ligand family with one amino acid separating two cysteines), and CX3C (chemokine ligand family with three amino acids between two cysteines) receptor families, including CCR1 (CC chemokine receptor 1), CCR2 (CC chemokine receptor 2), CCR5 (CC chemokine receptor 5), CXCR1 (CXC chemokine receptor 1), CXCR2 (CXC chemokine receptor 2), CXCR4 (CXC chemokine receptor 4), and CX3CR1 (CX3C chemokine receptor 1), enable macrophages to respond dynamically to chemotactic gradients and adapt to changing physiological conditions. Furthermore, H. Piao *et al*. (2022) demonstrated that chemokine receptor signalling contributes to the interaction between tumour-associated macrophages and cancer cells, promoting disease progression. Morphological differences between macrophage phenotypes under various culture conditions are illustrated in Figure 2. A special place among macrophage receptors is occupied by lipoprotein receptors. These receptors enable macrophages to bind, internalise, and break down cholesterol-containing lipoproteins and low-density lipoproteins (LDL). According to Z. You *et al*. (2023), dysregulation of lipid receptor pathways can lead to pathological processes such as ferroptosis and contribute to the development of atherosclerosis. This is further supported by Y. Liu *et al*. (2026), who highlighted the role of macrophage receptor-mediated lipid uptake in disease progression and therapeutic targeting. Scavenger receptors, in particular, play a dual role: under physiological conditions they contribute to the clearance of modified lipoproteins, while under pathological conditions they promote intracellular lipid accumulation and foam cell formation.

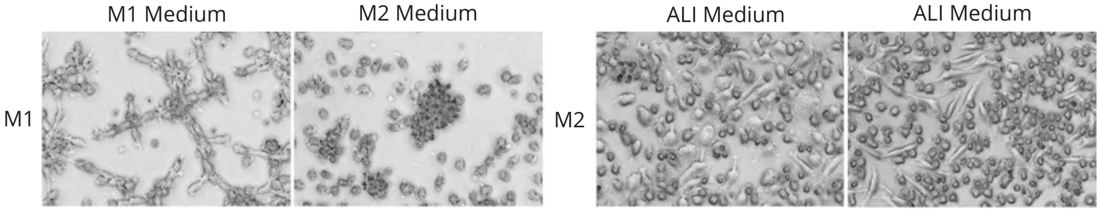

Figure 2. Morphology of M1, and M2 macrophages after 72 hours of culture
Note: macrophages of different activation states (M0, M1, and M2) were cultured either in their respective differentiation media or in air-liquid interface (ALI) medium, under monoculture conditions or in co-culture with human bronchial epithelial cells (HBECs); morphological characteristics were assessed using white light microscopy after 72 hours of culture
Source: N. Ronaghan *et al.* (2022)

Lectin-like receptors represent another important class of macrophage receptors that recognise carbohydrate structures on the pathogen surfaces. These receptors, including the mannose receptor, bind oligosaccharides containing terminal residues of mannose, fucose, galactose, and sialic acid. Their activation allows macrophages to recognise and internalise non-opsonised pathogens. This mechanism is essential for early innate immune responses and can function independently of Fc or complement receptor pathways. In addition to lectin-like receptors, macrophages express Toll-like receptors (TLRs), which are key pattern recognition receptors involved in detecting pathogen-associated molecular patterns. Among them, TLR2, TLR4, and TLR9 play a particularly important role. A. Mansouri *et al*. (2025) demonstrated that activation of TLR2/1 and TLR4 induces the production of pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), highlighting their central role in innate immune activation. The broad ligand specificity and high sensitivity of TLRs enable macrophages to detect a wide range of pathogens and mount an appropriate immune response without the need for prior sensitisation.

Effector functions of macrophages in host defence and tissue homeostasis

Mononuclear phagocytes provide the first line of defence against invading pathogens such as bacteria, viruses, fungi, and protozoa. Macrophages are capable of actively migrating to the site of pathogen invasion by orienting themselves along the gradient of molecules produced by the pathogen. During recognition, several receptor systems can participate simultaneously, including complement receptors, Fc receptors, lectin-like receptors, and scavenger receptors, ensuring efficient detection and response to pathogens; disruption of this process significantly impairs macrophage infiltration and function (Ikebukuro *et al*., 2023; Mvubu, 2025).

The processes of pathogen recognition and internalisation typically initiate the mechanism for its destruction. Following the formation of the phagosome, it fuses with lysosomes, releasing lysosomal enzymes necessary for destroying the pathogen. Furthermore, upon activation of macrophages induces the generation of reactive oxygen species, contributing to microbial killing but also influencing intracellular signalling pathways (Mangiaterra *et al*., 2024). Despite the high efficacy of macrophages in destroying pathogens, some microorganisms have developed protective mechanisms that allow them not only to avoid destruction but also to replicate inside macrophages. The most prominent example of a bacterium utilising this survival strategy is *Mycobacterium tuberculosis*, which produces sulpholipids that prevent phagosome-lysosome fusion, thereby avoiding degradation (Saha *et al*., 2021; Bo *et al*., 2023). The capture and destruction of a pathogen provide macrophages with the opportunity to activate the acquired immune system for more effective protection of the organism. After destroying the pathogen in the phagosome, pathogen-specific antigens are processed inside the cell, forming peptides that are presented on the macrophage surface in complex with major histocompatibility complex class II (MHC class II) molecules, allowing interaction with T lymphocytes and initiation of antigen-specific immune responses. However, the efficiency of macrophages in activating T cells remains context-dependent and may vary depending on the microenvironment. In addition to their antimicrobial functions, macrophages play a central role in maintaining immune homeostasis through the secretion of a wide range of bioactive molecules. These include enzymes, cytokines, chemokines, soluble receptors, coagulation factors, complement components, and extracellular matrix proteins. Protein secretion in macrophages involves synthesis in the rough endoplasmic reticulum, processing in the Golgi apparatus, and transport via vesicles to the plasma membrane. Some mediators are released through classical exocytosis, while others require more complex regulatory mechanisms. For example, TNF is initially synthesised as a transmembrane precursor, and its release depends on cleavage by the metalloproteinase ADAM17 (a disintegrin and metalloproteinase 17) (Gnosa *et al*., 2022; Aljohmani *et al*., 2026). Similarly, interleukin-1β (IL-1β) is produced as an inactive precursor in the cytoplasm and requires proteolytic activation by caspases, particularly caspase-1, as well as non-canonical pathways involving caspase-4/5/11 (Chaintreuil *et al*., 2022; Pollock *et al*., 2023). The mechanisms regulating the extracellular release of the mature cytokine remain an active area of research.

The secretory products of macrophages regulate numerous physiological processes, including immune cell recruitment, inflammation, tissue remodelling, and angiogenesis. Dysregulation of these processes may lead to pathological conditions such as chronic inflammation, autoimmune diseases, fibrosis, and impaired immune responses. Given the diversity of macrophage functions and their central role in maintaining physiological balance, dysfunction of these cells can lead to a wide range of diseases. These pathological alterations may affect different stages of macrophage activity, including pathogen recognition, phagocytosis, cytokine production, antigen presentation, and tissue remodelling. To summarise these relationships, the key mechanisms of macrophage dysfunction and their associated pathological conditions are presented in Table 3.

Table 3. Examples of macrophage dysfunction and associated diseases

Dysfunction	Mechanism	Disease
Avoidance of phagolysosome fusion	Sulpholipids produced by Mycobacterium tuberculosis	Tuberculosis
Excessive proinflammatory cytokine secretion	Dysregulation of TNF, interleukin (IL)-1, -6 synthesis	Rheumatoid arthritis, psoriasis, atherosclerosis
Impaired antigen presentation	Reduced MHC class II[1] (human leukocyte antigen (HLA)-DR, -DP, -DQ isotypes) expression	Chronic infections, immunodeficiencies
Tumour immune evasion	Suppression of macrophage activity in the tumour microenvironment	Cancer progression, metastasis
Defective pathogen recognition / phagocytosis	Mutations in scavenger receptors, Fc receptors, or complement system	Recurrent bacterial infections, autoimmunelike disorders
Aberrant tissue remodelling / fibrosis	Overproduction of growth factors (TGF-β[2], PDGF) and matrix enzymes	Organ fibrosis (pulmonary, hepatic), sarcoidosis

Note: MHC class II – major histocompatibility complex class II; TGF-β – transforming growth factor; PDGF – platelet-derived growth factor; TNF – tumour necrosis factor
Source: developed by the author

In addition to maintaining homeostasis, timely detection, and destruction of bacteria, the functions of macrophages in the body include controlling the emergence of tumour cells. Macrophages can directly lyse tumour cells through cytotoxic mechanisms and contribute to the activation of anti-tumour immune responses involving cytotoxic lymphocytes (Yu *et al.*, 2026). Experimental studies have demonstrated that activation of macrophages enhances their ability to eliminate tumour cells. This effect is mediated both by soluble factors and by direct cell-to-cell interactions. Among soluble mediators, cytokines such as TNF and interleukins play a critical role in suppressing tumour cell proliferation and modulating the tumour microenvironment (Mortezaee & Majidpoor, 2022). In addition to cytokine-mediated effects, macrophages exhibit cytotoxic activity upon direct contact with tumour cells. Antibody-dependent cellular cytotoxicity represents another important mechanism, in which macrophages recognise antibody-coated tumour cells and induce their destruction (Wilhelm *et al.*, 2025). However, under conditions of immunosuppression, tumour cells can evade macrophage-mediated immune responses. In such cases, tumours not only escape immune surveillance but may also exploit macrophages to promote tumour progression, invasion, and metastasis.

Concept of macrophage activation dichotomy

As mentioned earlier, during differentiation, macrophages acquire a phenotype characteristic of the physiological state of the tissue. This process is accompanied by profound metabolic and functional reprogramming that defines their role in immune responses (Yan *et al.*, 2024). For a long time, it was believed that in a healthy organism, macrophages reside in a state awaiting activation by exogenous or endogenous factors. The main factors activating macrophages were considered to be bacterial products and interferon gamma (IFN-γ), which induced strong antimicrobial and pro-inflammatory responses. However, subsequent studies demonstrated that macrophage activation is not limited to this pathway. Under the influence of interleukin-4 (IL-4), macrophages not only lose part of their bactericidal activity but also acquire distinct functional properties associated with tissue repair and immunoregulation. These findings led to the introduction of the concept of alternative macrophage activation and significantly expanded the understanding of macrophage functional diversity (Wei *et al.*, 2021). The development of this concept was closely linked to the established type 1 T helper (Th1) and type 2 T helper (Th2) paradigm of T helper cell differentiation, which provided a theoretical framework for classifying macrophage activation states. To illustrate this analogy and the functional relationship between T helper cell subsets and macrophage activation pathways, Figure 3 presents the polarisation of T helper cells and their associated immune responses.

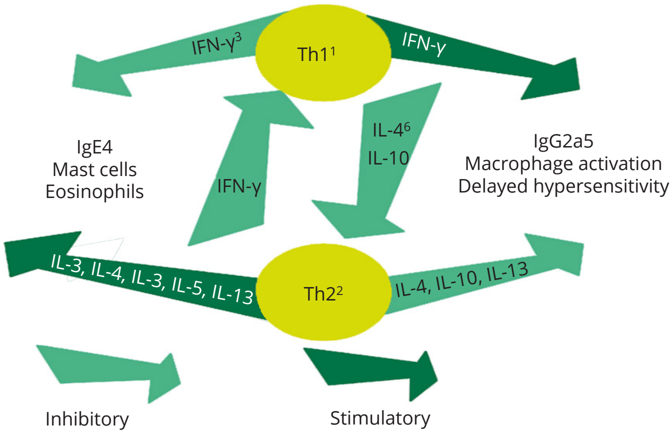

Figure 3. Functional polarisation of T helper cells in immune responses

Note: Th1 – T helper 1 cells; Th2 – T helper 2 cells; IFN-γ – interferon-gamma; IgE – immunoglobulin E; IgG2a – immunoglobulin G2a; IL – interleukin

Source: developed by the author

According to this framework, classical macrophage activation corresponds to stimulation by Th1-associated cytokines, primarily interferon-gamma (IFN-γ), whereas alternative activation is driven by Th2-associated cytokines such as interleukin 4 and interleukin 13. This analogy led to the introduction of the M1/M2 terminology, which became widely used to describe macrophage activation states (Shoeran & Anand, 2025). By the late 1990s, the dichotomous model of macrophage activation had taken its final form. M1 macrophages were defined as pro-inflammatory cells induced by IFN-γ or bacterial components such as lipopolysaccharide, whereas M2 macrophages were associated with anti-inflammatory responses and tissue repair, being induced by cytokines such as interleukin 4, interleukin 10, and transforming growth factor beta (TGF-β). Despite its conceptual usefulness, this model has significant limitations. Increasing evidence indicates that macrophage activation is highly context-dependent and cannot be fully explained by a binary classification.

In response to the limitations of the dichotomy, the scientific community is proposing new, more flexible classification systems. For example, it has been proposed to consider macrophages through the prism of their main functions in a specific context: inflammation, clearance (efferocytosis), tissue repair, or immunoregulation (Kumar Jha *et al.*, 2024). Another approach is to define disease-specific signatures. Thus, the study of tumour-associated macrophages has identified dozens of unique subpopulations that are not M1 or M2 analogues but have prognostic significance for cancer progression and response to immunotherapy (Ferrarini *et al.*, 2023; Pavlov *et al.*, 2026). Thus, the current emphasis is shifting from labelling to understanding the causal relationships between the microenvironment, transcriptomic profile, and functional outcome. Furthermore, studies in cancer biology demonstrate that macrophage behaviour is strongly influenced by the tumour microenvironment, including hypoxia and stromal interactions, which shape their immunosuppressive or pro-tumorigenic functions (Nazon *et al.*, 2022; Carlson *et al.*, 2025). Thus, modern research by R. Li *et al.* (2024) increasingly shifts the focus from rigid classification toward understanding the dynamic relationships between the microenvironment, transcriptional programmes, and functional outcomes of macrophage activation. To systematise the classical view of macrophage activation and highlight the key molecular and functional differences between M1 and M2 phenotypes, these characteristics are summarised in Table 4.

Table 4. Molecular and functional features of type 1 and type 2 activated macrophages

Type 1 activation (M1)	Type 2 activation (M2)
Tumour necrosis factor (TNF) Interleukin 1 (IL-1) Interleukin 6 (IL-6) Interleukin 12 (IL-12)	Interleukin 10 (IL-10) Interleukin 4 (IL-4) Interleukin-1 receptor antagonist (IL-lra) Alternative macrophage activation-associated CC-chemokine-1 (AMAC-1) C-C motif chemokine ligand 18 (CCL18)
Fc gamma receptor I (FcγRI) Fc gamma receptor II (FcγRII) Fc gamma receptor III (FcγRIII) Oxidative burst Bactericidal activity	Mannose receptor (CD206) β-glucan receptor (Dectin-1) Scavenger receptors class A (SR-A/CD204) Haemoglobin scavenger receptor (CD163) Endocytosis of degradation products

Source: developed by the author

Type 1 activated macrophages (M1) are relatively well characterised and are defined by the production of pro-inflammatory cytokines, including TNF (tumour necrosis factor), IL-1 (interleukin-1), IL-6 (interleukin-6), and IL-12 (interleukin-12), as well as high bactericidal activity and the capacity to generate an oxidative burst. These properties make them critical for host defence against intracellular pathogens. In contrast, type 2 activated macrophages (M2) exhibit a broader and more heterogeneous range of functions. They are primarily associated with anti-inflammatory responses, tissue remodelling, and repair processes. M2 macrophages produce cytokines such as IL-10 (interleukin-10) and express receptors involved in endocytosis and clearance of cellular debris, including lectin-like and scavenger receptors (Bongartz *et al.*, 2024; D. Guan *et al.*, 2025). At the same time, M2 macrophages demonstrate reduced bactericidal activity compared to M1 macrophages and are often involved in resolving inflammation and restoring tissue homeostasis. Recent advances in high-throughput technologies, including single-cell transcriptomics and multi-omics analysis, have further challenged the dichotomous model. These approaches reveal that macrophages frequently express gene signatures characteristic of both M1 and M2 phenotypes simultaneously, forming hybrid activation states (Yan *et al.*, 2024). Such hybrid phenotypes are highly dynamic and can rapidly change in response to local environmental signals. This plasticity highlights the limitations of the M1/M2 classification and supports the concept of macrophage activation as a continuum rather than discrete states (Kumar Jha *et al.*, 2024).

In summary, the understanding of macrophage activation has evolved from a simplified dichotomous M1/M2 framework toward a more dynamic and context-dependent model. Although the M1/M2 classification remains useful for describing general pro-inflammatory and anti-inflammatory tendencies, accumulating evidence demonstrates that macrophages exhibit substantial phenotypic plasticity and often adopt hybrid activation states shaped by local microenvironmental cues. Therefore, contemporary research increasingly focuses not on rigid categorisation but on identifying the molecular mechanisms and functional programmes that determine macrophage behaviour in specific physiological and pathological contexts.

CONCLUSIONS

When describing M2 macrophages, emphasis is often placed on the shared functional outcomes induced by different stimuli, while the underlying molecular differences receive less attention. Although cytokines such as interleukin 4 and interleukin 13 produce similar effects due to activation of a common receptor complex and downstream signalling pathway, other mediators, including interleukin 10, operate through distinct signalling mechanisms. As a result, macrophages activated by these factors exhibit significant molecular and functional differences despite being grouped within the same category. Comparable variability is observed when contrasting cytokine-induced activation with hormone-mediated effects. For instance, macrophages stimulated by interleukin 4 differ substantially from those activated by glucocorticoids in terms of receptor expression and functional capabilities. These differences highlight that grouping such activation states under a single classification does not adequately reflect their biological diversity. Taken together, these observations indicate that the classical dichotomous model of macrophage activation has limited explanatory power. It does not fully account for the diversity of phenotypes that arise in response to specific cytokines, hormones, and microenvironmental signals. A more refined framework is therefore required, one that considers the distinct activation programmes and their regulatory mechanisms.

The modern understanding of macrophage biology increasingly shifts from a binary classification toward a continuum-based and context-dependent model. This conceptual transition has important therapeutic implications. Rather than attempting to broadly shift macrophages from one generalised state to another, current strategies focus on the precise modulation of specific functional programmes within defined tissue environments. In particular, enhancing targeted processes such as the clearance of apoptotic cells represents a promising direction for therapeutic intervention. Clinical observations further underscore the importance of this approach. In the context of implant integration, excessive accumulation of macrophages and elevated levels of pro-inflammatory mediators are associated with adverse outcomes, including implant failure. At the same time, successful tissue remodelling depends on a coordinated sequence of macrophage activation states, where early pro-inflammatory responses are followed by reparative processes that support regeneration. These findings suggest that effective biomaterial design should prioritise immunomodulation rather than simple suppression of immune responses. Materials capable of directing macrophage activation toward beneficial functional programmes may improve tissue integration while minimising chronic inflammation and foreign body reactions. Similarly, controlling macrophage-driven fibrotic responses may offer therapeutic benefits in chronic inflammatory conditions. Future research integrating single-cell analysis, spatial transcriptomics, and functional studies will provide a more detailed understanding of macrophage heterogeneity in pathological settings. This will enable the development of targeted strategies for precise regulation of macrophage activity in clinical practice.

ACKNOWLEDGEMENTS

None.

FUNDING

This study was conducted without financial support.

CONFLICT OF INTEREST

The author declares no conflict of interest related to this study, including financial, personal, authorship-related, or any other interests that could have influenced the research or the results presented in this article.

REFERENCES

[1] Aljohmani, A., *et al*. (2026). Leukocytic ADAM10 and ADAM17 modulate disease severity and systemic outcome in bacterial and viral pneumonia. *European Respiratory Journal*, 67(2), article number 2402060. doi: 10.1183/13993003.02060-2024.

[2] Bauer-Smith, H., Sudol, A.S.L., Beers, S.A., & Crispin, M. (2023). Serum immunoglobulin and the threshold of Fc receptor-mediated immune activation. *Biochimica et Biophysica Acta – General Subjects*, 1867(11), article number 130448. doi: 10.1016/j.bbagen.2023.130448.

[3] Bo, H., Moure, U.A.E., Yang, Y., Pan, J., Li, L., Wang, M., Ke, X., & Cui, H. (2023). *Mycobacterium tuberculosis*-macrophage interaction: Molecular updates. *Frontiers in Cellular and Infection Microbiology*, 13, article number 1062963. doi: 10.3389/fcimb.2023.1062963.

[4] Bongartz, H., Bradfield, C., Gross, J., Fraser, I.D.C., Nita-Lazar, A., & Meier-Schellersheim, M. (2024). IL-10 dependent adaptation allows macrophages to adjust inflammatory responses to TLR4 stimulation history. *Biorxiv*. doi: 10.1101/2024.03.28.587272.

[5] Britt, R.D., Ruwanpathirana, A., Ford, M.L., & Lewis, B.W. (2023). Macrophages orchestrate airway inflammation, remodeling, and resolution in asthma. *International Journal of Molecular Sciences*, 24(13), article number 10451. doi: 10.3390/ijms241310451.

[6] Bruch-Oms, M., Olivera-Salguero, R., Mazzolini, R., del Valle-Pérez, B., Mayo-González, P., Beteta, Á., Peña, R., & García de Herreros, A. (2023). Analyzing the role of cancer-associated fibroblast activation on macrophage polarization. *Molecular Oncology*, 17(8), 1492-1513. doi: 10.1002/1878-0261.13454.

[7] Carlson, E.G., Lopez, J.C., Yamaguchi, Y., Gibson, J., Priceman, S.J., & LaBarge, M.A. (2025). $CD105^+$ fibroblasts support an immunosuppressive niche in women at high risk of breast cancer initiation. *Breast Cancer Research*, 27, article number 81. doi: 10.1186/s13058-025-02040-7.

[8] Chaintreuil, P., *et al*. (2022). Reprogramming monocyte-derived macrophages through caspase inhibition. *Oncoimmunology*, 11(1), article number 2015859. doi: 10.1080/2162402X.2021.2015859.

[9] Chen, X., *et al*. (2025). FFAR4-mediated IL-6 release from islet macrophages promotes insulin secretion and is compromised in type-2 diabetes. *Nature Communications*, 16, article number 3422. doi: 10.1038/s41467-025-58706-5.

[10] Corleis, B., *et al*. (2023). Tobacco smoke exposure recruits inflammatory airspace monocytes that establish permissive lung niches for *Mycobacterium tuberculosis*. *Science Translational Medicine*, 15(725), article number eadg3451. doi: 10.1126/scitranslmed.adg3451.

[11] Cornice, J., Verzella, D., Arboretto, P., Vecchiotti, D., Capece, D., Zazzeroni, F., & Franzoso, G. (2024). NF-κB: Governing macrophages in cancer. *Genes*, 15(2), article number 197. doi: 10.3390/genes15020197.

[12] Fernandes, J.C.R., Muxel, S.M., López-Gonzálvez, M.A., Barbas, C., & Floeter-Winter, L.M. (2024). Early *Leishmania* infectivity depends on miR-372/373/520d family-mediated reprogramming of polyamines metabolism in THP-1-derived macrophages. *Scientific Reports*, 14, article number 996. doi: 10.1038/s41598-024-51511-y.

[13] Ferrarini, I., Bernardelli, A., Lovato, E., Schena, A., Krampera, M., & Visco, C. (2023). An updated portrait of monocyte-macrophages in classical Hodgkin lymphoma. *Frontiers in Oncology*, 13, article number 1149616. doi: 10.3389/fonc.2023.1149616.

[14] Galley, J.D., Chen, H.J., Antonson, A.M., & Gur, T.L. (2021). Prenatal stress-induced disruptions in microbial and host tryptophan metabolism and transport. *Behavioural Brain Research*, 414, article number 113471. doi: 10.1016/j.bbr.2021.113471.

[15] Gnosa, S.P., Blasco, L.P., Piotrowski, K.B., Freiberg, M.L., Savickas, S., Madsen, D.H., auf dem Keller, U., Kronqvist, P., & Kveiborg, M. (2022). ADAM17-mediated EGFR ligand shedding directs macrophage-promoted cancer cell invasion. *JCI Insight*, 7(18), article number e155296. doi: 10.1172/jci.insight.155296.

[16] Guan, D., *et al*. (2025). Deficiency of myeloid NPC1 exacerbates liver injury and fibrosis by impairing macrophage efferocytosis. *Journal of Advanced Research*, 72, 213-227. doi: 10.1016/j.jare.2024.11.020.

[17] Guan, F., Wang, R., Yi, Z., Luo, P., Liu, W., Xie, Y., Liu, Z., Xia, Z., Zhang, H., & Cheng, Q. (2025). Tissue macrophages: Origin, heterogeneity, biological functions, diseases and therapeutic targets. *Signal Transduction and Targeted Therapy*, 10, article number 93. doi: 10.1038/s41392-025-02124-y.

[18] Guo, H., Luan, N., Gao, J., Pang, X., Bi, J., & Zhu, L. (2025). Exploring the mechanisms of mutual influence between lactylation and macrophage polarization in the context of disease. *Clinical and Translational Medicine*, 15(11), article number e70499. doi: 10.1002/ctm2.70499.

[19] Ikebukuro, T., Arima, T., Kasamatsu, M., Nakano, Y., Tobita, Y., Uchiyama, M., Terashima, Y., Toda, E., Shimizu, A., & Takahashi, H. (2023). Disulfiram ophthalmic solution inhibited macrophage infiltration by suppressing macrophage pseudopodia formation in a rat corneal alkali burn model. *International Journal of Molecular Sciences*, 24(1), article number 735. doi: 10.3390/ijms24010735.

[20] Jo, H.Y., Kim, M.K., Kim, K.T., Choi, C., & Rhee, W.J. (2026). Extracellular vesicles for macrophage reprogramming: An emerging paradigm in immunomodulatory therapeutics. *Journal of Biological Engineering*, 20, article number 13. doi: 10.1186/s13036-025-00594-w.

[21] Kern, N., Dong, R., Douglas, S.M., Vale, R.D., & Morrissey, M.A. (2021). Tight nanoscale clustering of Fcγ receptors using DNA origami promotes phagocytosis. *eLife*, 10, article number e68311. doi: 10.7554/eLife.68311.
[22] Klichinsky, M., *et al*. (2020). Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nature Biotechnology*, 38, 947-953. doi: 10.1038/s41587-020-0462-y.
[23] Kolliniati, O., Ieronymaki, E., Vergadi, E., & Tsatsanis, C. (2022). Metabolic regulation of macrophage activation. *Journal of Innate Immunity*, 14(1), 51-68. doi: 10.1159/000516780.
[24] Kumar Jha, P., Aikawa, M., & Aikawa, E. (2024). Macrophage heterogeneity and efferocytosis: Beyond the M1/M2 dichotomy. *Circulation Research*, 134(2), 186-188. doi: 10.1161/CIRCRESAHA.123.324011.
[25] Kvedaraite, E., *et al*. (2024). Intestinal stroma guides monocyte differentiation to macrophages through GM-CSF. *Nature Communications*, 15, article number 1752. doi: 10.1038/s41467-024-46076-3.
[26] Li, C., *et al*. (2024). Comparative single-cell analysis reveals IFN-γ as a driver of respiratory sequelae after acute COVID-19. *Science Translational Medicine*, 16(756), article number eadn0136. doi: 10.1126/scitranslmed.adn0136.
[27] Li, R., Huang, J., Wei, Y., Wang, Y., Lu, C., Liu, J., & Ma, X. (2024). Nanotherapeutics for macrophage network modulation in tumor microenvironments: Targets and tools. *International Journal of Nanomedicine*, 19, 13615-13651. doi: 10.2147/IJN.S491573.
[28] Liu, T., Zhang, F.Y., Feng, Y., Han, P.S., & Gao, Y.H. (2025). Alcohol-metabolizing enzymes, liver diseases and cancer. *Seminars in Liver Disease*, 45(1), 99-113. doi: 10.1055/a-2551-3320.
[29] Liu, Y., *et al*. (2026). Recent advances in nanodelivery systems based on extracellular and intracellular reprogramming strategies for enhanced therapy of atherosclerosis. *International Journal of Nanomedicine*, 21, article number 541504. doi: 10.2147/IJN.S541504.
[30] Logullo, J., *et al*. (2023). Increased *Trypanosoma cruzi* growth during infection of macrophages cultured on collagen I matrix. *Life*, 13(4), article number 1063. doi: 10.3390/life13041063.
[31] Lokki, A., *et al*. (2021). Dysfunction of complement receptors CR3 (CD11b/18) and CR4 (CD11c/18) in pre-eclampsia: A genetic and functional study. *BJOG: An International Journal of Obstetrics and Gynaecology*, 128(8), 1282-1291. doi: 10.1111/1471-0528.16660.
[32] Mangiaterra, S., Gavazza, A., Biagini, L., & Rossi, G. (2024). Study of macrophage activity in cats with FIP and naturally FCoV-shedding healthy cats. *Pathogens*, 13(6), article number 437. doi: 10.3390/pathogens13060437.
[33] Mansouri, A., Akthar, I., & Miyamoto, A. (2025). TLR2 and TLR4 bridge physiological and pathological inflammation in the reproductive system. *Communications Biology*, 8, article number 1008. doi: 10.1038/s42003-025-08424-x.
[34] Marrufo, A.M., & Flores-Mireles, A.L. (2024). Macrophage fate: To kill or not to kill? *Infection and Immunity*, 92(9), article number e00476-23. doi: 10.1128/iai.00476-23.
[35] Mass, E., Nimmerjahn, F., Kierdorf, K., & Schlitzer, A. (2023). Tissue-specific macrophages: How they develop and choreograph tissue biology. *Nature Reviews Immunology*, 23, 563-579. doi: 10.1038/s41577-023-00848-y.
[36] Mortezaee, K., & Majidpoor, J. (2022). Roles for macrophage-polarizing interleukins in cancer immunity and immunotherapy. *Cellular Oncology*, 45, 333-353. doi: 10.1007/s13402-022-00667-8.
[37] Mvubu, N.E. (2025). Innate immune recognition of *Mycobacterium tuberculosis*: Receptor engagement and inflammatory outcomes at the site of infection. *The Cell Surface*, 14, article number 100150. doi: 10.1016/j.tcsw.2025.100150.
[38] Nazon, C., *et al*. (2022). Together intra-tumor hypoxia and macrophagic immunity are driven worst outcome in pediatric high-grade osteosarcomas. *Cancers*, 14(6), article number 1482. doi: 10.3390/cancers14061482.
[39] Pavlov, S., Ali, E., Ambrozkiewicz, F., Ye, W., Rajtmajerová, M., Liška, V., Hemminki, K., & Trailin, A. (2026). Macrophages in colorectal cancer: From normal mucosa to distant metastasis: Beyond the M1/M2 paradigm. *Journal of Cancer*, 17(1), 157-176. doi: 10.7150/jca.126772.
[40] Pérez, S., & Rius-Pérez, S. (2022). Macrophage polarization and reprogramming in acute inflammation: A redox perspective. *Antioxidants*, 11(7), article number 1394. doi: 10.3390/antiox11071394.

[41] Petty, A., Glass, L.J., Rothmond, D.A., Purves-Tyson, T., Sweeney, A., Kondo, Y., Kubo, S., Matsumoto, M., & Weickert, C.S. (2022). Increased levels of a pro-inflammatory IgG receptor in the midbrain of people with schizophrenia. *Journal of Neuroinflammation*, 19, article number 188. doi: 10.1186/s12974-022-02541-8.

[42] Piao, H., Fu, L., Wang, Y., Liu, Y., Wang, Y., Meng, X., Yang, D., Xiao, X., & Zhang, J. (2022). A positive feedback loop between gastric cancer cells and tumor-associated macrophage induces malignancy progression. *Journal of Experimental & Clinical Cancer Research*, 41, article number 174. doi: 10.1186/s13046-022-02366-6.

[43] Pisano, S., Jimenez, Y.S., Rees, P., Xiao, J., Gonzalez, D., Conlan, R.S., & Corradetti, B. (2025). Insights into spheroid formation: Interaction of ovarian cancer cells with macrophage populations in the tumor microenvironment. *Journal of Translational Medicine*, 23, article number 1192. doi: 10.1186/s12967-025-07162-2.

[44] Pollock, T.Y., Vázquez Marrero, V.R., Brodsky, I.E., & Shin, S. (2023). TNF licenses macrophages to undergo rapid caspase-1, -11, and -8-mediated cell death that restricts *Legionella pneumophila* infection. *Plos Pathogens*, 19(6), article number e1010767. doi: 10.1371/journal.ppat.1010767.

[45] Ronaghan, N.J., Soo, M., Pena, U., Tellis, M., Duan, W., Tabatabaei-Zavareh, N., Kramer, P., Hou, J., & Moraes, T.J. (2022). M1-like, but not M0- or M2-like, macrophages, reduce RSV infection of primary bronchial epithelial cells in a media-dependent fashion. *Plos One*, 17(10), article number e0276013. doi: 10.1371/journal.pone.0276013.

[46] Saha, S., Hazra, A., Ghatak, D., Singh, A.V., Roy, S., & Dasgupta, S.B. (2021). A bumpy ride of mycobacterial phagosome maturation: Roleplay of coronin1 through cofilin1 and cAMP. *Frontiers in Immunology*, 12, article number 687044. doi: 10.3389/fimmu.2021.687044.

[47] Shoeran, G., & Anand, N. (2025). Interplay of autophagy and Th1/Th2-mediated macrophage polarization in host-pathogen dynamics. *Frontiers in Cellular and Infection Microbiology*, 15, article number 1679514. doi: 10.3389/fcimb.2025.1679514.

[48] Tran, N., & Mills, E.L. (2024). Redox regulation of macrophages. *Redox Biology*, 72, article number 103123. doi: 10.1016/j.redox.2024.103123.

[49] Wang, B., Wu, L., Chen, J., Dong, L., Chen, C., Wen, Z., Hu, J., Fleming, I., & Wang, D.W. (2021). Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets. *Signal Transduction and Targeted Therapy*, 6, article number 94. doi: 10.1038/s41392-020-00443-w.

[50] Wang, L., Wang, D., Zhang, T., Ma, Y., Tong, X., & Fan, H. (2023). The role of immunometabolism in macrophage polarization and its impact on acute lung injury/acute respiratory distress syndrome. *Frontiers in Immunology*, 14, article number 1117548. doi: 10.3389/fimmu.2023.1117548.

[51] Wang, M., *et al*. (2021). Two populations of self-maintaining monocyte-independent macrophages exist in adult epididymis and testis. *Proceedings of the National Academy of Sciences of the United States of America*, 118(1), article number e2013686117. doi: 10.1073/pnas.2013686117.

[52] Wculek, S.K., Dunphy, G., Heras-Murillo, I., Mastrangelo, A., & Sancho, D. (2022). Metabolism of tissue macrophages in homeostasis and pathology. *Cellular & Molecular Immunology*, 19, 384-408. doi: 10.1038/s41423-021-00791-9.

[53] Wei, Y., Wang, M., Ma, Y., Que, Z., & Yao, D. (2021). Classical dichotomy of macrophages and alternative activation models proposed with technological progress. *BioMed Research International*, 2021, article number 9910596. doi: 10.1155/2021/9910596.

[54] Wilhelm, O., *et al*. (2025). Afucosylated broadly neutralizing antibodies targeting the HIV envelope elicit enhanced NK-cell-mediated cytotoxicity against HIV-infected $CD4^+$ T-cell and macrophage targets. *Journal of Leukocyte Biology*, 117(5), article number qiaf033. doi: 10.1093/jleuko/qiaf033.

[55] Wong, A., Sun, Q., Latif, I.I., & Karwi, Q.G. (2025). Macrophage energy metabolism in cardiometabolic disease. *Molecular and Cellular Biochemistry*, 480, 1763-1783. doi: 10.1007/s11010-024-05099-6.

[56] Xie, L., *et al*. (2023). Activation of GPER1 in macrophages ameliorates UUO-induced renal fibrosis. *Cell Death & Disease*, 14, article number 818. doi: 10.1038/s41419-023-06338-2.

[57] Yan, L., Wang, J., Cai, X., Liou, Y.-C., Shen, H.-M., Hao, J., Huang, C., Luo, G., & He, W. (2024). Macrophage plasticity: Signaling pathways, tissue repair, and regeneration. *MedComm*, 5(8), article number e658. doi: 10.1002/mco2.658.

[58] You, Z., Ye, X., Jiang, M., Gu, N., & Liang, C. (2023). lnc-MRGPRF-6:1 promotes ox-LDL-induced macrophage ferroptosis via suppressing GPX4. *Mediators of Inflammation*, article number 5513245. doi: 10.1155/2023/5513245.

[59] Yu, L., *et al*. (2026). Reinforced immunotherapy of M1 macrophage-derived exosomes with CEL on TNBC via regulating macrophage functions. *Journal of Advanced Research*, 82, 1163-1178. doi: 10.1016/j.jare.2025.07.024.

[60] Zerrouk, N., Alcraft, R., Hall, B.A., Augé, F., & Niarakis, A. (2024). Large-scale computational modelling of the M1 and M2 synovial macrophages in rheumatoid arthritis. *NPJ Systems Biology and Applications*, 10, article number 10. doi: 10.1038/s41540-024-00337-5.

[61] Zhang, N., Ma, Q., You, Y., Xia, X., Xie, C., Huang, Y., Wang, Z., Ye, F., Yu, Z., & Xie, X. (2022). CXCR4-dependent macrophage-to-fibroblast signaling contributes to cardiac diastolic dysfunction in heart failure with preserved ejection fraction. *International Journal of Biological Sciences*, 18(3), 1271-1287. doi: 10.7150/ijbs.65802.

Теоретичні аспекти формування ключових елементів концепції дихотомії активації макрофагів: огляд літератури

Ангеліна Андрющенко

Аспірант
Інститут біохімії ім. О. В. Палладіна Національної академії наук України
02000, вул. Леонтовича, 9, м. Київ, Україна
https://orcid.org/0000-0003-4174-2740

Анотація. Метою цього дослідження був аналіз теоретичних аспектів формування концепції дихотомії активації макрофагів (M1/M2), яка, незважаючи на складність регуляції імунної відповіді, відображає існування високоефективної багаторівневої системи контролю функціональних станів макрофагів і забезпечує належний перебіг запальних процесів. Порушення цієї системи пов'язане з розвитком патологічних станів, зокрема хронічного запалення та прогресії пухлин. Матеріали дослідження охопили сучасні публікації, що містять дані фундаментальних та експериментальних досліджень механізмів активації та поляризації макрофагів. Використані методи включали систематизацію, порівняльний аналіз і узагальнення сучасних наукових підходів з акцентом на молекулярні механізми та новітні технологічні досягнення. Класична модель поляризації макрофагів розрізняє прозапальні (M1) та протизапальні (M2) фенотипи; однак така класифікація дедалі частіше розглядається як спрощена і не повністю відображає гетерогенність макрофагів in vivo. Показано, що такі цитокіни, як інтерферон-γ (IFN-γ), інтерлейкін-4 (IL-4), інтерлейкін-10 (IL-10) та трансформувальний фактор росту-β (TGF-β), відіграють ключову роль у формуванні фенотипів макрофагів і регуляції їх участі в запаленні, відновленні тканин і процесах пухлинного мікрооточення. Крім того, макрофаги здатні одночасно проявляти ознаки різних програм активації, формуючи змішані або гібридні фенотипи. На основі аналізу сучасних даних зроблено висновок, що дихотомія M1/M2 має обмежену пояснювальну здатність і повинна бути замінена моделлю, яка розглядає активацію макрофагів як динамічний континуум, залежний від умов мікрооточення. Результати дослідження можуть бути корисними для науковців і клініцистів у галузях імунології та онкології з метою вдосконалення підходів до імуномодулювальної терапії та розроблення таргетних стратегій лікування

Ключові слова: поляризація макрофагів; M1/M2; цитокіни; імунна регуляція; запалення; пластичність; пухлинне мікрооточення

Received: 21.12.2025 Revised: 19.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 92-105

UDC 633.34:631.53.01:631.67(477.7)

DOI: 10.31548/biologiya/2.2026.92

Enhancing soybean adaptive potential and productivity with endophyte-rhizobial inoculation

Liudmyla Tytova

PhD in Biological Sciences, Senior Researcher
D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine
03143, 154 Akademik Zabolotny Str., Kyiv, Ukraine
https://orcid.org/0000-0003-3131-4355

Olena Dubynska

PhD in Biological Sciences, Senior Researcher
Institute of Climate-Smart Agriculture of the National Academy of Agricultural Sciences of Ukraine
67667, 24 Maiatska Doroga Str., Khlibodarske, Ukraine
https://orcid.org/0000-0002-5572-0094

Nadiia Shevchuk*

PhD Student
D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine
03143, 154 Akademik Zabolotny Str., Kyiv, Ukraine
https://orcid.org/0000-0001-5574-4961

Galyna Iutynska

Doctor of Biological Sciences, Professor, Corresponding Member of the National Academy of Sciences of Ukraine
D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine
03143, 154 Akademik Zabolotny Str., Kyiv, Ukraine
https://orcid.org/0000-0001-6692-2946

Abstract. The research aim was to investigate the effect of pre-sowing complex inoculation with endophytic and nodule bacteria on the rhizosphere microbiocenosis, soil macronutrient content, adaptive potential and productivity of soybean under the influence of stressful weather and climatic factors. A split-plots design was used to conduct a two-factor field experiment with soybean varieties of different maturity groups: the ultra-early maturing 'Diona' and the medium-early maturing 'Sviatohor'. Microbiological methods were used to obtain inoculants based on nodule and endophytic bacteria (*Bradyrhizobium japonicum* as part of the complex bioformulation Ryzobin^{K} and their mixtures with endophytic bacteria *Bacillus velezensis* IMV B-8134, B-8135, *Pseudomonas* sp. 6), as well as to determine the abundance of microorganisms from various ecological-functional groups by inoculating soil suspensions onto selective agar media. Agrochemical methods were used to determine soil macronutrients, and statistical methods were applied for data analysis. The stimulatory and stress-protective effects of co-inoculation of seeds with nodule and endophytic bacteria on microbial communities in the rhizosphere soil, as well as on the development and yield

Suggested Citation:
Tytova, L., Dubynska, O., Shevchuk, N., & Iutynska, G. (2026). Enhancing soybean adaptive potential and productivity with endophyte-rhizobial inoculation. *Biological Systems: Theory and Innovation*, 17(2), 92-105. doi: 10.31548/biologiya/2.2026.92.

*Corresponding author (nadia.shevchuk.48@ukr.net)

of various soybean varieties on irrigated lands in southern Ukraine, have been demonstrated. An increase in the number of microorganisms belonging to the main ecological- functional groups and in the content of available forms of nitrogen, phosphorus and potassium in the rhizosphere soil was established. The most effective treatment in terms of synergistic action on soybean was found to be the treatment of seeds with Ryzobin^K in combination with *Bacillus velezensis* IMB B-8134. The average yield for the 'Diona' variety was 2.69 t/ha and for the 'Sviatohor' variety – 2.95 t/ha, which exceeded the control values by 38-40%. Practical value lies in effective pre-sowing endophyte-rhizobial co-inoculation of soybean seeds, improving productivity and stability of agrophytocenoses under climate change

Keywords: *Glycine max* (L.) Merr.; symbiotic bacteria; stressful climate factors; irrigation; rhizosphere microbiota; macronutrients; effectiveness

INTRODUCTION

In the context of global climate change, the design of agricultural systems with enhanced resistance to stress factors has become one of the priority areas of scientific research and innovation. The use of biological preparations based on active strains of symbiotic microorganisms capable of forming mutualistic relationships with the host plant contributes to plant adaptation to extreme environmental conditions and increases crop productivity. The use of complex multi-strain formulations with a multi-vector stress-protective effect is particularly promising. As noted by L. Shangguan (2023), global warming alters the duration of the growing season and the geographical distribution of agricultural crops, resulting in some traditional cultivation regions potentially losing suitability for certain crops, while the demand for water resources increases. At the same time, W. Zhu *et al*. (2024) emphasised that changes in precipitation patterns negatively affect plant growth and productivity, which may lead to yield reductions and create risks to food security. According to M. Yanagi (2024), climate change affects the distribution and availability of water resources, potentially causing water shortages and droughts and, consequently, significantly complicating irrigated agriculture. Soybean (*Glycine max* (L.) Merr.) is an important global agricultural crop that supplies protein and oil for the food industry and feed production. However, soybean is highly vulnerable to various abiotic stresses, particularly drought and heat during the flowering stage, which may significantly reduce yield (Xia *et al*., 2026). Soybean grows best at temperatures between 20°C and 25°C, but prolonged exposure to temperatures above 29°C results in a reduction in bean number and seed weight. Temperatures above 35°C severely disrupt flowering, bean setting and grain filling (Thenveettil *et al*., 2025). With ongoing global climate change, the simultaneous occurrence of heat and drought stress is becoming increasingly frequent, especially in semi-arid and drought-prone regions.

Modern agricultural practices, as highlighted by H. Tariq *et al.* (2025), depend heavily on synthetic fertilisers and pesticides, which are major contributors to greenhouse gas emissions, groundwater contamination and disruption of agroecosystem dynamics. These issues underline the urgent need for sustainable alternatives that maintain crop productivity while minimising environmental impact. In the study by I.S. Marquez *et al.* (2025), it was shown that despite the significant influence of abiotic factors – temperature, precipitation regime and nutrient availability – on plant resilience and productivity, interactions with microorganisms constitute an important mechanism for improving physiological status and yield. According to D.M. Zeffa *et al.* (2020), co-inoculation of soybean with *Bradyrhizobium* strains and plant growth-promoting rhizobacteria provides a synergistic effect manifested in enhanced root system development, increased nodule formation, improved efficiency of symbiotic nitrogen fixation and, consequently, increased productivity. Bacterial endophytes, both rhizobial and non-rhizobial, are considered critically important for the growth and resilience of legume host plants, as they participate in atmospheric nitrogen fixation, phytohormone synthesis, nutrient mobilisation and regulation of metabolic processes, while also increasing plant tolerance to abiotic and biotic stresses, including drought, temperature fluctuations and pathogen infection (Mayhood & Mirza, 2021). According to N. Shevchuk *et al.* (2025) and Y. Zhakypbek *et al.* (2026), due to their localisation within internal plant compartments, endophytes are able to interact closely with plant tissues and influence processes such as regulation of water balance and stomatal activity, nutrient distribution and stress signalling pathways, thereby contributing to improved tolerance to water deficit. The development and implementation of new environmentally safe biological preparations based on rhizobial and non-rhizobial endophytic bacteria therefore remain highly relevant. Accordingly, the aim of the study was to determine the effect of pre-sowing complex inoculation with endophytic and nodule-forming bacteria on the rhizosphere microbiocenosis, soil macronutrient content, adaptive potential and productivity of soybean varieties differing in maturity group under the influence of stressful weather and climatic factors.

MATERIALS AND METHODS

Field experiments were conducted during 2024-2025 on irrigated land located in the urban-type settlement of Naddniprianske, Kherson Region, on a specially equipped irrigated field site, following generally accepted agronomic methods and methodological guidelines. The geographical coordinates of the site were 46°44'53.6" N latitude and 32°42'55.7" E longitude, at an altitude of 50 m above sea level. The soil was dark chestnut, medium loamy, slightly solonetzic, formed on carbonate loess. The humus content (according to Tyurin) in the arable layer was 2.2%, total nitrogen 0.17%, available phosphorus (according to Machigin) 30.0 mg/kg, and exchangeable potassium 300 mg/kg of soil. The lowest moisture capacity of the 0-100 cm soil layer was 21.3%, and the wilting point was 9.5% relative to the mass of absolutely dry soil. Bulk density was 1.41 t/m³, and the pH of the aqueous soil extract in the arable layer ranged from 6.8 to 7.2. A two-factor field experiment was established using a split-plot design. The sowing plot area was 15 m² and the accounting plot area was 10 m², with four replications. In the experimental design, the main plots (first-order plots, factor A) consisted of soybean varieties: the ultra-early maturing 'Diona' and the medium-early maturing 'Sviatohor'. The subplots (second-order plots, factor B) included microbial inoculants: Control 1 (without water spraying); Control 2 (water spraying); Ryzobin^{K} (an association of three strains of *Bradyrhizobium japonicum*: *B. japonicum* UCM B-6018, UCM B-6023, UCM B-6035); Ryzobin^{K} + *Bacillus velezensis* IMV B-8134; Ryzobin^{K} + *B. velezensis* IMV B-8135; and Ryzobin^{K} + *Pseudomonas* sp. 6. For the complex endophyte-rhizobial inoculation of soybean seeds of different maturity groups, strains of microorganisms from the culture collection of the Department of General and Soil Microbiology of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine were used. Seed inoculation was performed according to the methods described by H. Iutynska *et al.* (2022). The titre of symbiotic bacteria in the inoculant was at least 3-5 × 10^9 cells/ml. Treatment was carried out by spraying the seeds, with an inoculant application rate of 1.0 l per 1 t of seed.

Weather and climatic factors influencing production processes during the yield formation period of soybean varieties differing in maturity group were determined by assessing average temperature, relative air humidity and atmospheric precipitation during interphase periods, i.e. the main indicators significantly affecting evaporation rate, moisture deficit and the moisture coefficient (Romanchuk, 2020). These indicators, taking weather conditions into account, were determined according to generally accepted methods described by V.O. Ushkarenko *et al.* (2019). The overall characterisation of the main weather parameters during the interphase periods of both soybean varieties was based on observations from the meteorological station in the settlement of Naddniprianske. Soybean varieties were sown during the first ten days of May with a row spacing of 45 cm and a sowing depth of 2-3 cm. The sowing rate for the 'Diona' variety was 800,000 viable seeds per hectare and for 'Sviatohor' 600,000 viable seeds per hectare. During the flowering to early bean formation stage, the abundance of microorganisms belonging to the main ecological-functional groups in the soybean rhizosphere was determined. Enumeration was performed using the surface plating method of soil suspensions with appropriate tenfold dilutions onto selective agar media. In particular, phosphate-mobilising microorganisms were cultured on mineral medium with sodium phenolphthalein phosphate, pedotrophic microorganisms on soil agar, oligotrophic microorganisms on starvation (water) agar, and oligonitrotrophic and nitrogen-fixing microorganisms on Ashby medium. Incubation was carried out at 28°C for 5-7 days. The number of microorganisms was expressed as colony-forming units per 1 g of absolutely dry soil, taking soil moisture into account (CFU g^{-1} soil), which was determined gravimetrically. At the bean maturity stage, yield assessment was conducted with adjustment to standard seed moisture content. Harvesting was carried out using a Sampo-130 combine harvester (Sampo Rosenlew Ltd, Finland). Soybean yield was evaluated according to the generally accepted methods of field experimentation described by V.O. Ushkarenko *et al.* (2019) and expressed in t/ha. The results of all laboratory and field experiments with four replications were analysed using Microsoft Excel 2010 software. Multifactor analysis of variance (ANOVA) was applied for statistical analysis. Differences were considered statistically significant at $p < 0.05$. Field studies were conducted in accordance with the principles of biodiversity conservation under the provisions of the Convention on Biological Diversity (1992), as well as bioethical principles and environmental safety standards. Sampling was performed with minimal impact on plants and their habitats.

RESULTS AND DISCUSSION

The agrometeorological conditions of the growing season in the southern part of the Steppe zone (Kherson Region) were characterised by a moderately hot and extremely arid climate. Evaporation and moisture deficit during the growing season varied considerably and depended on average daily temperature, relative air humidity and the amount of productive atmospheric precipitation. The total duration of the interphase period "emergence – beginning of branching" during the cultivation of both soybean varieties in 2024-2025 did not exceed 18 days, during which 21.1 mm of precipitation fell, accounting for 12.3-20.9% of the total precipitation received throughout the crop growing season. The duration of the interphase period "beginning of branching – beginning of flowering" for the soybean variety 'Diona' was 22 days, during which 24.6 mm of atmospheric precipitation fell, representing 24.5% of the total precipitation amount (Table 1).

Table 1. Hydrothermal conditions during the interphase periods of soybean varieties differing in maturity group, average for 2024-2025

Calendar dates	Average air temperature, °C	Precipitation, mm	Relative air humidity, %	Evaporation, mm	Moisture deficit, mm	Moisture coefficient (MC)
'Diona' variety						
Emergence – beginning of branching (18 days)						
18.05-04.06	19.3	21.1	57.0	151.9	130.8	0.14
Beginning of branching – beginning of flowering (22 days)						
05.06-26.06	22.9	24.6	46.0	223.0	198.4	0.11
Beginning of flowering – beginning of bean formation (15 days)						
27.06-11.07	26.5	19.0	40.0	286.4	267.4	0.07
Beginning of bean formation – beginning of seed filling (16 days)						
12.07-27.07	28.3	9.3	41.0	301.7	292.4	0.03
Beginning of seed filling – beginning of seed ripening (16 days)						
28.07-12.08	25.8	6.4	44.0	260.1	253.7	0.02
Beginning of seed ripening – full seed maturity (14 days)						
13.08-26.08	24.2	19.9	40.0	261.4	241.5	0.08
Total						
101 days	24.5	100.3	45.0	1,484.5	1,384.2	0.07
'Sviatohor' variety						
Emergence – beginning of branching (18 days)						
19.05-05.06	19.8	21.1	56.0	158.9	137.8	0.13
Beginning of branching – beginning of flowering (26 days)						
06.06-01.07	23.2	24.6	46.0	225.8	201.2	0.11

Table 1. Continued

Calendar dates	Average air temperature, °C	Precipitation, mm	Relative air humidity, %	Evaporation, mm	Moisture deficit, mm	Moisture coefficient (MC)
Beginning of flowering – beginning of bean formation (20 days)						
02.07-21.07	28.1	28.3	40.0	304.5	276.2	0.09
Beginning of bean formation – beginning of seed filling (22 days)						
22.07-12.08	26.3	6.4	43.0	270.0	263.6	0.02
Beginning of seed filling – beginning of seed ripening (22 days)						
13.08 -03.09	24.2	28.6	41.0	257.1	228.5	0.11
Beginning of seed ripening – full seed maturity (16 days)						
04.09-19.09	20.7	61.5	49.0	191.7	130.2	0.32
Total						
124 days	24.1	171.0	46.0	1,408.5	1,237.5	0.12

Source: compiled by the authors

The duration of this interphase period for the variety 'Sviatohor' was 26 days, during which 24.6 mm of precipitation fell, accounting for 14.4% of the total precipitation during the crop growing season. At an average daily temperature of 22.9°C and relative air humidity of 46.0%, evaporation during this interphase period under cultivation of the variety 'Diona' reached 223.0 mm, while the moisture deficit amounted to 198.4 mm. During cultivation of the variety 'Sviatohor' in the interphase period "beginning of branching – beginning of flowering", 24.6 mm of atmospheric precipitation fell and evaporation amounted to 225.8 mm, resulting in a moisture deficit of 201.2 mm. The moisture coefficient during cultivation of the variety 'Diona' reached 0.11 and, correspondingly, 0.11 for the variety 'Sviatohor', indicating conditions characteristic of desert environments in the southern part of the Steppe zone. During the interphase period "beginning of flowering – beginning of bean formation", the total duration of which for the variety 'Diona' was 14 days, at an average temperature of 26.5°C and relative air humidity of 40.0%, evaporation increased to 286.4 mm and the moisture deficit reached 267.4 mm. The amount of precipitation during cultivation of the variety 'Diona' in this interphase period was 19.0 mm, or 18.9% of the total precipitation received during the crop growing season. Under these weather conditions, the moisture coefficient for the variety 'Diona' was 0.07, which is characteristic of desert conditions, while for the variety 'Sviatohor' it was 0.09. During the interphase period "beginning of bean formation – beginning of seed filling", the amount of atmospheric precipitation during cultivation of the variety 'Diona' was 9.3 mm, or 9.2% of the total precipitation received during the variety's growing season. At an average temperature of 28.3°C and relative air humidity of 41.0%, evaporation during this interphase period under cultivation of the variety 'Diona' increased to 301.7 mm, while the moisture deficit reached 292.4 mm. During cultivation of the medium-early variety 'Sviatohor' in the interphase period "beginning of bean formation – beginning of seed filling", the total duration of which was 22 days, adverse weather conditions negatively affected crop yield formation. At an average daily temperature of 26.3°C and relative air humidity of 43.0%, evaporation amounted to 270.0 mm and the moisture deficit reached 263.6 mm. Under these weather conditions, the moisture coefficient was 0.03, which is characteristic of desert conditions. During the interphase period "beginning of seed filling – full seed maturity", the moisture coefficient during cultivation of the variety 'Diona' over 16 days did not exceed 0.02, which is characteristic of desert conditions, while for the variety 'Sviatohor' it amounted to 0.11. The moisture coefficient during cultivation of the variety 'Diona' was 0.08 (desert conditions) and, correspondingly, 0.32 for the variety 'Sviatohor' (semi-arid zone). The total duration of both interphase periods during cultivation of the variety 'Diona' was 30 days, while for the variety 'Sviatohor' it was 38 days.

The reduction of the negative effects of extreme weather conditions observed during the cultivation of both soybean varieties in the interphase periods "emergence – beginning of branching", "beginning of seed filling – beginning of seed ripening" and "beginning of seed ripening – full seed maturity" was achieved through irrigation during the growing season using drip irrigation. During cultivation of the ultra-early variety 'Diona', 18 irrigations were carried out during the growing season with an irrigation rate of 5,315 m^3/ha, while for the medium-early variety 'Sviatohor', 20 irrigations were applied with an irrigation rate of 5,548 m^3/ha. The abundance of microorganisms and their metabolic activity are regarded as indicators of soil biological activity (Jezierska-Tys *et al.*, 2020). Determination of the abundance of microorganisms belonging to different ecological-functional groups in the soybean rhizosphere, depending on inoculation with rhizobia and endophytic bacteria, was carried out during the "flowering – beginning of bean formation" stage. It was established that soybean seed inoculation influenced the abundance of microorganisms belonging to different ecological-functional groups in the rhizosphere soil of both soybean varieties under investigation (Table 2).

Table 2. Abundance of microorganisms in rhizosphere soil (CFU g^{-1} soil), average for 2024-2025

Treatment	Phosphate-mobilising microorganisms	Pedotrophic microorganisms	Oligotrophic microorganisms	Nitrogen-fixing microorganisms
'Diona' variety				
1	9.9 ± 1.1	47.7 ± 5.7	46.6 ± 9.3	16.1 ± 2.9
2	30.8 ± 2.4	56.4 ± 2.2	88.9 ± 18.5	17.1 ± 1.8
3	13.9 ± 3.2	49.1 ± 1.1	110.7 ± 17.9	22.9 ± 3.7
4	16.3 ± 2.1	57.7 ± 9.7	28.2 ± 4.7	22.5 ± 3.3
5	13.7 ± 1.0	25.5 ± 3,9	64.7 ± 6.2	17.2 ± 2.8
'Sviatohor' variety				
1	26.1 ± 1.2	42.7 ± 7.7	67.7 ± 11.1	73.9 ± 16.8
2	33.2 ± 2.4	44.8 ± 6.1	72.7 ± 11.7	75.4 ± 11.0
3	32.7 ± 6.7	41.1 ± 6.7	58.0 ± 6.7	40.9 ± 4.5
4	31.6 ± 6.3	50.2 ± 7.5	57.3 ± 11.8	72.1 ± 13.7
5	25.1 ± 2.5	45.2 ± 8.1	78.4 ± 6.9	68.9 ± 7.5

Note: 1 – Control (without treatment); 2 – Ryzobin^K; 3 – Ryzobin^K + *B. velezensis* IMV B-8134; 4 – Ryzobin^K + *B. velezensis* IMV B-8135; 5 – RyzobinK + *Pseudomonas* sp. 6
Source: compiled by the authors

Soils frequently contain large amounts of insoluble mineral and organic phosphorus compounds, although phosphorus is essential for the growth of most plants and microorganisms. One approach to improving phosphorus nutrition of plants is the use of microorganisms capable of increasing the mobility of phosphorus compounds (Malynovska, 2021). The highest abundance of phosphate-mobilising microorganisms was recorded following the application of Ryzobin^K alone and in combination with the endophytic strains *B. velezensis* IMV B-8134 and *B. velezensis* IMV B-8135. In these treatments, their abundance reached 13.9-33.2 million CFU/g of soil. At the same time, the best values exceeded the control by 3.1 times for the variety 'Diona' and by 1.3 times for the variety 'Sviatohor'. Pedotrophic microorganisms participate in the transformation of organic matter and humus formation, while an increase in their abundance indicates intensification of these processes (Maslovska *et al.*, 2025). The greatest abundance of pedotrophic microorganisms in the rhizosphere soil of both studied varieties was promoted by inoculation with the complex Ryzobin^K + *B. velezensis* IMV B-8135. In these treatments, the abundance of pedotrophs was higher than in the non-inoculated control by 21.0% for the variety 'Diona' and 17.6% for the variety 'Sviatohor'. Oligotrophic microorganisms are adapted to ecological niches with low substrate concentrations and limited energy flow, and an increase in their abundance in soil, according to N.B. Dragone *et al.* (2024), may indicate more active utilisation of residual available nutrients. The highest abundance of oligotrophic microorganisms in the rhizosphere soil of soybean variety 'Diona' was promoted by inoculation with the complex Ryzobin^K + *B. velezensis* IMV B-8134, which exceeded the control value by 2.4 times. In the rhizosphere soil of soybean variety 'Sviatohor', the highest abundance of oligotrophic microorganisms was observed under inoculation with Ryzobin^K + *Pseudomonas* sp. 6, which was 1.2 times higher than the control.

Nitrogen is an essential macronutrient for plants; however, most of it is present in the atmosphere in the inaccessible form of N_2. Thus, replenishment of nitrogen reserves occurs, among other mechanisms, through biological fixation by nitrogen-fixing microorganisms, which convert it into compounds available to plants (Zulfarina *et al.*, 2017). An increase in their abundance in rhizosphere soil contributes to the supply of available nitrogen to plants, improvement of plant growth and maintenance of soil fertility. In the rhizosphere soil of soybean variety 'Diona', the highest abundance of nitrogen-fixing microorganisms (22.9 million CFU/g of soil) was recorded in the treatment Ryzobin^K + *B. velezensis* IMV B-8134, which was 42.2% higher than in the control treatment. In the rhizosphere of soybean variety 'Sviatohor', the differences between treatments averaged over the years of study were not statistically significant. Thus, the rhizosphere soil of both soybean varieties under complex endophyte-rhizobial inoculation was characterised by increased biological activity, as evidenced by the high abundance of microorganisms belonging to the main ecological-functional groups. This contributed to increased plant resistance to arid weather and climatic conditions and to higher productivity.

Determination of nutrient content in the 0-20 cm layer of dark chestnut soil in the experimental field showed that the complex microbial preparations had a positive residual effect on the soil, enriching it with nutrients available to plants. In the soil of treatments where inoculation with nodule-forming and endophytic bacteria was applied, the content of mineral nitrogen increased. During cultivation of soybean variety 'Diona' in the interphase period "beginning of branching – beginning of flowering", the mineral nitrogen content under bacterial inoculation increased by 4.75-5.72 mg/kg compared with the control treatments. It was also established that during cultivation of soybean varieties under irrigation with complex endophyte-rhizobial seed inoculation, the content of available phosphorus changed during the interphase period "beginning of branching – beginning of flowering". In the 0-20 cm soil layer, its maximum content was recorded in the treatment Ryzobin^K + *B. velezensis* IMV B-8134 at 38.4 mg/kg, which was higher than in the soil of the control treatments (Table 3).

Table 3. Agrochemical composition of dark chestnut soil under soybean plants in the 0-20 cm layer during the "beginning of branching – beginning of flowering" stage, mg/kg

Inoculants	Mineral nitrogen content ($N\text{-}NO_3$ + $N\text{-}NH_4$)	Available phosphorus content (P_2O_5)	Exchangeable potassium content (K_2O)
'Diona' variety			
Control 1 (without seed treatment)	23.67	23.4	155
Control 2 (seed treatment with water)	22.69	26.3	162
Ryzobin^K	28.82	38.0	200
Ryzobin^K + *B. velezensis* IMV B-8134	27.62	38.4	242
Ryzobin^K + *B. velezensis* IMV B-8135	28.42	37.6	230
Ryzobin^K + *Pseudomonas* sp. 6	26.00	37.3	216
$LSD_{0.05}$	2.27	5.58	28.4
'Sviatohor' variety			
Control 1 (without seed treatment)	15.77	26.4	225
Control 2 (seed treatment with water)	17.58	246	202
Ryzobin^K	27.75	37.8	228
Ryzobin^K + *B. velezensis* IMV B-8134	30.22	38.2	235
Ryzobin^K + *B. velezensis* IMV B-8135	32.06	37.3	207
Ryzobin^K + *Pseudomonas* sp. 6	24.80	37.4	218
$LSD_{0.05}$	5.83	5.08	10.1

Source: compiled by the authors

In the other treatments, the values of this parameter ranged within 37.6-38.3 and 37.3-38.0 mg/kg of soil. The content of exchangeable potassium during the interphase period "beginning of branching – beginning of flowering" in the 0-20 cm soil layer under the treatment Ryzobin^K + *B. velezensis* IMV B-8134 was the highest for the 'Diona' variety, reaching 242.75 mg/kg; correspondingly, for the 'Sviatohor' variety it amounted to 235 mg/kg, whereas in the control treatments its content was the lowest, amounting to 202 and 225 mg/kg of soil. During the interphase period "beginning of bean formation – beginning of bean filling", the content of mineral nitrogen increased in the soil under plants inoculated with nodule-forming and endophytic bacteria. During cultivation of the soybean variety 'Diona', the mineral nitrogen content under bacterial inoculation increased by 18.0-22.4 mg/kg compared with the control treatments. In the cultivation of the soybean variety 'Sviatohor', the mineral nitrogen content in the soil of inoculated treatments increased by 1.8-3.4 mg/kg compared with the control values (Table 4).

Table 4. Agrochemical composition of dark chestnut soil under soybean plants in the 0-20 cm layer during the "beginning of bean formation – beginning of bean filling" stage, mg/kg

Inoculants	Mineral nitrogen content ($N\text{-}NO_3 + N\text{-}NH_4$)	Available phosphorus content (P_2O_5)	Exchangeable potassium content (K_2O)
'Diona' variety			
Control 1 (without seed treatment)	12.27	62.5	163
Control 2 (seed treatment with water)	12.75	62.7	163
Ryzobin^K	19.72	64.3	197
Ryzobin^K + *B. velezensis* IMV B-8134	35.11	64.8	201
Ryzobin^K + *B. velezensis* IMV B-8135	30.30	69.2	246
Ryzobin^K + *Pseudomonas* sp. 6	29.10	60.6	176
$LSD_{0.05}$	7.77	2.34	25.0
'Sviatohor' variety			
Control 1 (without seed treatment)	38.8	61.8	227
Control 2 (seed treatment with water)	38.9	61.9	227
Ryzobin^K	42.20	63.5	154
Ryzobin^K + *B. velezensis* IMV B-8134	40.60	62.2	231
Ryzobin^K + *B. velezensis* IMV B-8135	40.00	68.4	208
Ryzobin^K + *Pseudomonas* sp. 6	41.90	69.3	187
$LSD_{0.05}$	1.16	4.22	28.0

Source: compiled by the authors

During the cultivation of soybean varieties under irrigation conditions with complex endophyte-rhizobial seed inoculation, the content of available phosphorus changed during the interphase period "beginning of bean formation – beginning of bean filling". In the 0-20 cm soil layer, its maximum content was recorded in the treatment Ryzobin^K + *B. velezensis* IMV B-8135, reaching 69.2 mg/kg, which was higher than in the control treatments. In the remaining treatments, the values of this parameter ranged within 63.2-64.8 and 54.6-68.4 mg/kg of soil. The content of exchangeable potassium compounds in the 0-20 cm layer under the treatment Ryzobin^K + *B. velezensis* IMV B-8135 during cultivation of the soybean variety 'Diona' was the highest, amounting to 246 mg/kg, whereas in the control treatments its content was the lowest at 163 mg/kg of soil; correspondingly, for the variety 'Sviatohor' it amounted to 231 mg/kg. The obtained data regarding the increase in mineral nitrogen and available phosphorus content in the soil under complex inoculation are consistent with the findings of P. Xing *et al.* (2022), who demonstrated that co-inoculation of *B. japonicum* 5038 with *Bacillus aryabhattai* MV35-5 promoted an increase in soil nitrogen content and had a significant effect on its accumulation in soybean plants, while inoculation with *B. japonicum* 5038 and *Paenibacillus mucilaginosus* 3016 contributed to an increase in the content of available phosphorus in the soybean rhizosphere soil. Thus, the effectiveness of bacterial preparations is determined by the ability of the bacteria on which they are based to fix atmospheric nitrogen and promote mineralisation of soil organic matter, thereby improving the mineral nutrition of plants. Modern bacterial preparations contribute to the formation of a powerful nitrogen-fixing apparatus (nodules) on roots, intensive development of leguminous plants and protection against diseases, which helps to obtain high seed yields with superior sowing qualities. For obtaining consistently high soybean seed yields, optimum plant density is of considerable importance, as it promotes intensive crop growth and development. In the present study, the sowing rate for the variety 'Diona' was 800 thousand seeds/ha and, correspondingly, for the variety 'Sviatohor' 600 thousand viable seeds/ha (Table 5).

Table 5. Plant density, field germination and survival of soybean plants depending on variety and application of bacterial preparations

Inoculants	Seed sowing rate, thousand/ha	Emerged plants, thousand/ha	Field seed germination, %	Plants at full maturity stage, thousand/ha	Plant survival, %
'Diona' variety					
Control 1 (without seed treatment)	800,000	400,000	50.0	340,000	85.0
Control 2 (seed treatment with water)	800,000	400,000	50.0	340,000	85.0
Ryzobin^{K}	800,000	480,000	60.0	470,000	97.9
Ryzobin^{K} + *B. velezensis* IMV B-8134	800,000	570,000	71.3	560,000	98.2
Ryzobin^{K} + *B. velezensis* IMV B-8135	800,000	560,000	70.0	540,000	96.4
Ryzobin^{K} + *Pseudomonas* sp. 6	800,000	520,000	65.0	510,000	98.1
	$LSD_{0.05}$	64,1	8.02	82.7	5.42
'Sviatohor' variety					
Control 1 (without seed treatment)	600,000	310,000	51.7	290,000	93.5
Control 2 (seed treatment with water)	600,000	310,000	51.7	290,000	93.5
Ryzobin^{K}	600,000	360,000	60.0	350,000	97.2
Ryzobin^{K} + *B. velezensis* IMV B-8134	600,000	380,000	63.3	370,000	97.4
Ryzobin^{K} + *B. velezensis* IMV B-8135	600,000	380,000	63.3	370,000	97.4
Ryzobin^{K} + *Pseudomonas* sp. 6	600,000	360,000	60.0	350,000	97.2
	$LSD_{0.05}$	26.3	4.33	29.7	1.62

Source: compiled by the authors

During the growing season of both soybean varieties under study, part of the plants died off, resulting in a reduction in crop density within the field experiment plots. Complex endophyte-rhizobial seed inoculation had a significant effect on plant preservation. The highest survival rate of plants of the variety 'Diona' was observed under seed inoculation with Ryzobin^{K} + *B. velezensis* IMV B-8134 at 98.2% and Ryzobin^{K} + *B. velezensis* IMV B-8135 at 96.4%, while for the variety 'Sviatohor' the highest survival rate was recorded under inoculation with the composition Ryzobin^{K} + *B. velezensis* IMV B-8134 at 97.4%. The lowest plant survival was observed in the control treatments, ranging from 85.0 to 93.5%. Soybean inoculation with rhizobia promotes improved nitrogen nutrition of plants, their growth and yield formation. During the experiments, the application of inoculation with the preparation Ryzobin^{K}, containing three strains of *B. japonicum*, resulted in a yield increase compared with the non-inoculated treatment of 9.23% (0.18 t/ha) for the soybean variety 'Diona' and 14.2% (0.30 t/ha) for the variety 'Sviatohor'. Considerably better results were achieved under complex endophyte-rhizobial inoculation. During the years of research, the maximum seed yield of both studied varieties was obtained in the treatment with complex seed inoculation Ryzobin^{K} + *B. velezensis* IMV B-8134, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early maturing variety 'Sviatohor', which exceeded the control values by 38-40% (Table 6).

Table 6. Seed yield of soybean varieties differing in maturity group under endophyte-rhizobial inoculation on irrigated lands of the Southern Steppe of Ukraine, t/ha, 2024-2025

Inoculants (B)	Yield, t/ha			Average increase relative to Control 1	
	2024	2025	average	t/ha	%
'Diona' variety (A_1)					
Control 1 (without seed treatment)	2.77	1.13	1.95	–	–
Control 2 (seed treatment with water)	2.80	1.15	1.97	–	–
Ryzobin[K]	3.02	1.25	2.13	0.18	9.23
Ryzobin[K] + *B. velezensis* IMV B-8134	3.57	1.82	2.69	0.74	37.9
Ryzobin[K] + *B. velezensis* IMV B-8135	3.26	1.77	2.51	0.56	28.7
Ryzobin[K] + *Pseudomonas* sp. 6	3.01	1.66	2.33	0.38	19.4
'Sviatohor' variety (A_2)					
Control 1 (without seed treatment)	2.84	1.37	2.10	–	–
Control 2 (seed treatment with water)	2.85	1.38	2.11	–	–
Ryzobin[K]	3.11	1.69	2.40	0.30	14.2
Ryzobin[K] + *B. velezensis* IMV B-8134	3.77	2.14	2.95	0.85	40.4
Ryzobin[K] + *B. velezensis* IMV B-8135	3.39	1.98	2.68	0.58	27.6
Ryzobin[K] + *Pseudomonas* sp. 6	3.11	1.98	2.54	0.44	20.9

Note: $LSD_{0.05}$ for partial effects: factor A – 0.154 t/ha, factor B – 0.091 t/ha; for mean (main) effects: factor A – 0.058 t/ha, factor B – 0.065 t/ha
Source: compiled by the authors

High yields were also obtained in the treatment Ryzobin[K] + *B. velezensis* IMV B-8135, amounting to 2.51 t/ha for the variety 'Diona' and 2.68 t/ha for the variety 'Sviatohor'. The yield increase of the variety 'Diona' under complex seed inoculation in the different experimental treatments, compared with Control 1, was as follows: Ryzobin[K] – 0.18 t/ha; Ryzobin[K] + *B. velezensis* IMV B-8134 – 0.74 t/ha; Ryzobin[K] + *B. velezensis* IMV B-8135 – 0.56 t/ha; and Ryzobin[K] + *Pseudomonas sp*. 6 – 0.38 t/ha. During cultivation of the medium-early variety 'Sviatohor', the yield increase was also sufficiently high and, in the respective experimental treatments, reached: Ryzobin[K] – 0.30 t/ha; Ryzobin[K] + *B. velezensis* IMV B-8134 – 0.85 t/ha; Ryzobin[K] + *B. velezensis* IMV B-8135 – 0.58 t/ha; and Ryzobin[K] + *Pseudomonas sp*. 6 – 0.44 t/ha. The lowest yield of conditioned seed for both soybean varieties was obtained in the control treatments (Control 1 and Control 2), amounting to 1.95-1.97 t/ha for the variety 'Diona' and 2.10-2.11 t/ha for the variety 'Sviatohor'. During the years of research, the highest seed yield for both soybean varieties was obtained in the treatment with complex seed inoculation Ryzobin[K] + *B. velezensis* IMV B-8134, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early variety 'Sviatohor', which exceeded the control values by 38-40%.

Increasing the productivity of grain legumes through the use of the potential of symbiotic and non-symbiotic microorganisms is an economically and environmentally justified practice in modern agriculture. In the study by E. Szpunar-Krok *et al.* (2023), under rhizobial inoculation with *B. japonicum* and in the absence of nitrogen fertilisation, the soybean variety Aldana showed an average increase in seed yield of 7.1% (0.28 t/ha), while the variety Annushka showed an increase of 14.6% (0.50 t/ha). In experiments conducted by M. Leggett *et al.* (2017) in the United States of America, inoculation of soybean with *B. japonicum* resulted in an average increase in soybean grain yield of 1.67% over four years of trials, while in Argentina the increase reached 6.39% over five years of trials, equivalent to 60 and 190 kg/ha respectively. Positive effects of complex seed inoculation using several strains simultaneously have also been reported. Thus, T.C. Tu *et al*. (2021) demonstrated that co-inoculation with the *Bradyrhizobium* isolate Bra6 and various *Pseudomonas* isolates enhanced the formation of active symbiotic systems, nutrient uptake and soybean biomass accumulation. However, it should be noted that despite the general approaches, the application of different inoculants requires adjustment taking into account the characteristics of indigenous microbiota, soil conditions and especially climate change. The experiments demonstrated the positive effect of the multistrain seed inoculation applied in the present study – a consortium of microorganisms consisting of three strains of nodule-forming bacteria and one of the newly isolated strains of endophytic bacteria.

CONCLUSIONS

It was established that the development of climate-oriented agrotechnologies is an important prerequisite for increasing the efficiency of agriculture in arid regions. The expediency of using plant growth-promoting bacteria as a promising approach to enhancing crop resilience and productivity was demonstrated. The obtained data indicated an increase in the physiological activity of microorganisms belonging to the main ecological-functional groups, as well as in the content of mineral nitrogen and available phosphorus in soybean rhizosphere soil under endophyte-rhizobial inoculation. Owing to the effectiveness of complex inoculation and the active development of agronomically beneficial groups within the rhizosphere microbiome, plant density and the intensity of crop growth and development increased. Complex endophyte-rhizobial seed inoculation promoted soybean plant survival under unfavourable climatic factors. The highest survival rate of plants of both studied varieties was observed under inoculation with the composition Ryzobin^{K} + *B. velezensis* IMV B-8134. In the same treatment, the maximum yield of both soybean varieties was obtained, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early variety 'Sviatohor', which exceeded the control values by 38-40%. Thus, the effectiveness of bacterial preparations is determined by the ability of the bacteria on which they are based to fix atmospheric nitrogen, promote mineralisation of soil organic matter, improve plant mineral nutrition and stress resistance, and enhance the biological activity of the rhizosphere microbiocenosis. In summary, the present study established the stimulatory and stress-protective effects of endophyte-rhizobial inoculation on the biological activity of rhizosphere microbiota, enrichment of soil with essential nutrients, adaptive potential and productivity of soybean varieties differing in maturity group under stressful weather and climatic conditions. It was concluded that co-inoculation with nodule-forming and non-rhizobial endophytic bacteria is an environmentally safe stress-protective strategy and a powerful natural resource for modern agricultural biotechnologies. Future research perspectives include investigating the effectiveness of endophyte-rhizobial inoculation in other climatic zones of Ukraine during the cultivation of grain legumes, as well as studying the prospects for the application of endophytic bacteria in the cultivation of cereal and industrial crops.

ACKNOWLEDGEMENTS

Dedicated to the bright memory of Doctor of Biological Sciences, Professor S.P. Goloborodko, with gratitude for his theoretical and practical contribution to this work.

FUNDING

This research was supported by the governmental scientific programme of the National Academy of Sciences of Ukraine, the fundamental sectoral project "Ecosystem functions of the soil microbiome in regenerative agrophytocenoses" (2025-2029).

CONFLICT OF INTEREST

None.

REFERENCES

[1] Convention on Biological Diversity. (1992, May). Retrieved from https://www.cbd.int/doc/legal/cbd-en.pdf.

[2] Dragone, N.B., Hoffert, M., Strickland, M.S., & Fierer, N. (2024). Taxonomic and genomic attributes of oligotrophic soil bacteria. *ISME Communications*, 4(1), article number ycae081. doi: 10.1093/ismeco/ycae081.

[3] Iutynska, H., Goloborodko, S., Tytova, L., & Dubynska, O. (2022). Effectiveness of endophytic-rhizobial seed inoculation of *Glycine max* (L.) Merr. cultivated in irrigated soil. *Journal of Central European Agriculture*, 23(1), 40-53. doi: 10.5513/JCEA01/23.1.3397.

[4] Jezierska-Tys, S., Wesołowska, S., Gałązka, A., Joniec, J., Bednarz, J., & Cierpiała, R. (2020). Biological activity and functional diversity in soil in different cultivation systems. *International Journal of Environmental Science and Technology*, 17, 4189-4204. doi: 10.1007/s13762-020-02762-5.

[5] Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., & Leister, T. (2017). Soybean response to inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. *Agronomy Journal*, 109(3), 1031-1038. doi: 10.2134/agronj2016.04.0214.

[6] Malynovska, I.M. (2021). The determination of specific phosphatmobilizing activity of soil microorganisms. *Agriculture and Plant Sciences: Theory and Practice*, 1, 43-47. doi: 10.54651/agri.2021.01.06.

[7] Marquez, I.S., Griesbaum, K., Clark, L.V., Ainsworth, E.A., Christian, N., & Heath, K.D. (2025). Dominant foliar endophytes influence soybean yield and transcriptome. *FEMS Microbiology Ecology*, 101(6), article number fiaf053. doi: 10.1093/femsec/fiaf053.

[8] Maslovska, O., Komplikevych, S., Zinchuk, D., Hnatush, A., Zaritska, Y., Telehuz, O., & Hnatush, S. (2025). Bacteriome of the soil affected by hostilities as a source of isolation of plant growth-promoting metal-resistant bacteria. *Annals of Microbiology*, 75(1), article number 19. doi: 10.1186/s13213-025-01807-9.

[9] Mayhood, P., & Mirza, B.S. (2021). Soybean root nodule and rhizosphere microbiome: Distribution of rhizobial and nonrhizobial endophytes. *Applied and Environmental Microbiology*, 87(10), article number e02884-20. doi: 10.1128/AEM.02884-20.

[10] Romanchuk, I.F. (2020). *Method for determining soil moisture using remote sensing data*. (Doctoral dissertation, Institute of Geological Sciences, Kyiv, Ukraine).

[11] Shangguan, L. (2023). Impact of climate change on agriculture and its countermeasures. *Geographical Research Bulletin*, 2, 73-74. doi: 10.50908/grb.2.0_73.

[12] Shevchuk, N., Tytova, L., Iutynska, G., Sergiienko, V., & Katrii, V. (2025). Influence of rhizobia and endophytic bacteria on adaptation and productivity of *Glycine max* (L.) Merr. under hyperthermia and drought conditions. *Microbiological Journal*, 87(5), 12-24. doi: 10.15407.

[13] Szpunar-Krok, E., Bobrecka-Jamro, D., Pikuła, W., & Jańczak-Pieniążek, M. (2023). Effect of nitrogen fertilization and inoculation with *Bradyrhizobium japonicum* on nodulation and yielding of soybean. *Agronomy*, 13(5), article number 1341. doi: 10.3390/agronomy13051341.

[14] Tariq, H., Viau, C., Subramanian, S., Geitmann, A., & Smith, D.L. (2025). Exo-metabolome profiling of soybean endophytes: A road map of antagonism against *Fusarium oxysporum*. *MSystems*, 10(10), article number e00927-25. doi: 10.1128/msystems.00927-25.

[15] Thenveettil, N., Bheemanahalli, R., Kharel, T.P., Reddy, K.N., Gao, W., & Reddy, K.R. (2025). Drought and high nighttime temperature impact on soybean seed yield and quality under ambient and elevated CO_2 environments. *Scientific Reports*, 15, article number 36679. doi: 10.1038/s41598-025-20392-0.

[16] Tu, T.C., Lin, S.H., & Shen, F.T. (2021). Enhancing symbiotic nitrogen fixation and soybean growth through co-inoculation with *Bradyrhizobium* and *Pseudomonas* isolates. *Sustainability*, 13(20), article number 11539. doi: 10.3390/su132011539.

[17] Ushkarenko, V.O., Chaban, V.O., Shepel, A.V., & Kokovikhin, S.V. (2019). Conditional consumption of nutrients by clary sage plants under cultivation in the conditions of the Southern Steppe of Ukraine under drip irrigation. *Irrigated Agriculture. Collection of Scientific Papers*, 71, 176-179.

[18] Xia, C., Huang, Z., Lu, B., Geng, Z., Wang, S., & Xu, C. (2026). Physiological and molecular response of soybean to combined drought and heat stresses during flowering. *BMC Plant Biology*, 26, article number 319. doi: 10.1186/s12870-025-08081-6.

[19] Xing, P., Zhao, Y., Guan, D., Li, L., Zhao, B., Ma, M., Jiang, X., Tian, C., Cao, F., & Li, J. (2022). Effects of *Bradyrhizobium* co-inoculated with *Bacillus* and *Paenibacillus* on the structure and functional genes of soybean *Rhizobacteria* community. *Genes*, 13(11), article number 1922. doi: 10.3390/genes13111922.

[20] Yanagi, M. (2024). Climate change impacts on wheat production: Reviewing challenges and adaptation strategies. *Advances in Resources Research*, 4(1), 89-107. doi: 10.50908/arr.4.1_89.
[21] Zeffa, D.M., Fantin, L.H., Koltun, A., de Oliveira, A.L., Nunes, M.P., Canteri, M.G., & Gonçalves, L.S. (2020). Effects of plant growth-promoting rhizobacteria on co-inoculation with *Bradyrhizobium* in soybean crop: A meta-analysis of studies from 1987 to 2018. *PeerJ*, 8, article number e7905. doi: 10.7717/peerj.7905.
[22] Zhakypbek, Y., Kossalbayev, B.D., Tursbekov, S., Tursbekova, G., Berdaliyeva, Z., & Belkozhayev, A.M. (2026). Application of beneficial bacteria to enhance plant drought resilience. *Plants*, 15(5), article number 753. doi: 10.3390/plants15050753.
[23] Zhu, W., Li, J., & Xie, T. (2024). Impact of climate change on soybean production: Research progress and response strategies. *Advances in Resources Research*, 4(3), 474-496. doi: 10.50908/arr.4.3_474.
[24] Zulfarina, Z., Rusmana, I., Mubarik, N.R., & Santosa, D.A. (2017). The abundance of nitrogen fixing, nitrifying, denitrifying and ammonifying bacteria in the soil of tropical rainforests and oil palm plantations in Jambi. *Makara Journal of Science*, 21(4), 181-187. doi: 10.7454/mss.v21i4.8841.

Підвищення адаптаційного потенціалу та врожайності сої за допомогою ендофітно-різобіальної інокуляції

Людмила Титова

Кандидат біологічних наук, старший науковий співробітник
Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України
03143, вул. Академіка Заболотного, 154, м. Київ, Україна
https://orcid.org/0000-0003-3131-4355

Олена Дубинська

Доктор філософії з біології, старший науковий співробітник
Інститут кліматично орієнтованого сільського господарства НААН
67667, вул. Маяцька дорога, 24, смт Хлібодарське, Україна
https://orcid.org/0000-0002-5572-0094

Надія Шевчук

Аспірант
Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України
03143, вул. Академіка Заболотного, 154, м. Київ, Україна
https://orcid.org/0000-0001-5574-4961

Галина Іутинська

Доктор біологічних наук, професор, член-кореспондент НАН України
Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України
03143, вул. Академіка Заболотного, 154, м. Київ, Україна
https://orcid.org/0000-0001-6692-2946

Анотація. Метою дослідження було вивчення впливу передпосівної комплексної інокуляції ендофітними та бульбочковими бактеріями на ризосферний мікробіоценоз, вміст макроелементів у ґрунті, адаптивний потенціал і продуктивність сої за дії стресових погодних і кліматичних чинників. Для проведення двофакторного польового досліду використано метод розщеплених ділянок із сортами сої різних груп стиглості: ультраранніми ‘Діона’ та середньораннім ‘Святогор’. Мікробіологічні методи застосовували для отримання інокулянтів на основі бульбочкових і ендофітних бактерій (*Bradyrhizobium japonicum* у складі комплексного біопрепарату Ризобін^{K} та їх сумішей з ендофітними бактеріями *Bacillus velezensis* IMB B-8134, B-8135, *Pseudomonas* sp. 6), а також для визначення чисельності мікроорганізмів різних еколого-функціональних груп шляхом висіву ґрунтових суспензій на селективні агаризовані середовища. Агрохімічні методи використовували для визначення вмісту макроелементів у ґрунті, статистичні – для обробки отриманих даних. Показано стимулювальний і стрес-протекторний ефекти коінокуляції насіння бульбочковими та ендофітними бактеріями на мікробні угруповання ризосферного ґрунту, а також на ріст і врожайність різних сортів сої на зрошуваних землях Південного Степу України. Встановлено зростання чисельності мікроорганізмів основних еколого-функціональних груп і підвищення вмісту доступних форм азоту, фосфору та калію в ризосферному ґрунті. Найефективнішим варіантом за синергічним впливом на сою виявилося оброблення насіння препаратом Ризобін^{K} у поєднанні з *Bacillus velezensis* ІМВ В-8134. Середня врожайність для сорту ‘Діона’ становила 2,69 т/га, для сорту ‘Святогор’ – 2,95 т/га, що перевищувало контроль на 38-40 %. Практична цінність роботи полягає в ефективності передпосівної ендофітно-ризобіальної коінокуляції насіння сої, що забезпечує підвищення продуктивності та стабільності агрофітоценозів в умовах кліматичних змін

Ключові слова: *Glycine max* (L.) Merr.; симбіотичні бактерії; стресові кліматичні чинники; зрошення; ризосферна мікробіота; макроелементи; ефективність

Received: 29.11.2025 Revised: 22.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 106-124

UDC 633.34:631.847:632.937

DOI: 10.31548/biologiya/2.2026.106

Efficacy of the combined application of biological products in soybean cultivation under laboratory and field conditions

Yuliia Kolomiiets*

Doctor of Agricultural Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-1919-6336

Nataliia Serhiichuk

PhD Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0001-6690-0114

Tetiana Rozhkova

PhD in Biological Sciences, Senior Researcher
D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine
03143, 154 Akademik Zabolotny Str., Kyiv, Ukraine
https://orcid.org/0000-0002-0791-9736

Abstract. The aim of the study was to evaluate the efficacy of biological products through the soybean response across sowing, growth, phytosanitary, symbiotic, and productivity parameters under laboratory and field conditions. The study employed spectrophotometry, the Kjeldahl method, Soxhlet extraction, and analysis of variance. Under laboratory conditions, the combined seed treatment with Phytovit and Avercom yielded the highest germination energy of 88.7%, exceeding the control by 10.1 percentage points. Laboratory germination under this treatment was 94.3%, which was 7.9 percentage points higher than the control, whilst seedling infection by microflora decreased to 4.2%, representing a reduction of 7.6 percentage points. Under field conditions, the application of biological products had a positive effect on seedling emergence, plant stand density, development of the leaf apparatus, and root system. Under the combined application of the products, field germination was 87.1%, and the number of nodules at the V5 stage reached 20.6 per plant. The phytosanitary condition of the crop also shifted towards a reduction in disease incidence and pest damage: the incidence of root rot decreased to 6.3%, and the biological efficacy of protection was 59.6%. At the flowering stage, the combined treatment promoted the formation of a more developed assimilatory and symbiotic apparatus in the plants. The leaf area index reached 4.0 m^2/m^2, the number of active nodules was 25.9 per plant, and their mass was 0.58 g per plant, reflecting more active functioning of the leaf apparatus and root system during the transition of plants to reproductive development. The preservation of the leaf apparatus and nodule formation were accompanied by subsequent seed filling and accumulation of productive mass. Seed yield under the combined application of Phytovit

Suggested Citation:
Kolomiiets, Yu., Serhiichuk, N., & Rozhkova, T. (2026). Efficacy of the combined application of biological products in soybean cultivation under laboratory and field conditions. *Biological Systems: Theory and Innovation*, 17(2), 106-124. doi: 10.31548/biologiya/2.2026.106.

*Corresponding author (julyja@i.ua)

and Avercom was 3.01 t/ha, the increase over the control was 24.4%, protein content was 39.1%, and oil content was 21.0%. The practical value of the study lies in the possibility of using its findings by agronomists, farmers, seed enterprises, and researchers to substantiate biological treatment of soybean, taking into account germination, phytosanitary condition, nodulation, yield, and seed quality

Keywords: phytosanitary condition; plant stand density; root system; number of nodules; reproductive organs

INTRODUCTION

The relevance of this study is determined by the need to clarify the factors that govern seed germination, seedling emergence, root system development, leaf apparatus development, and the formation of productive organs in soybean under both laboratory and field conditions. Soybean belongs to the group of crops in which ultimate productivity is formed through the successive passage of early, vegetative, and reproductive developmental phases. At these stages, the quality of the seed material, the phytosanitary condition of the crop, the activity of nodule formation, plant survival throughout the growing season, and the completeness of seed filling are all of significance. Therefore, studying the soybean response across a complex of laboratory, field, physiological, and productivity indicators makes it possible to detail the specific features of yield formation. The problem addressed by this study stems from the occurrence of non-uniform seed germination, seedling infection by microflora, thinning of plant stands following emergence, incidence of root and foliar diseases, pest damage to plants, and uneven development of the nodular apparatus. A separate challenge is the inadequate concordance between laboratory germination indicators and subsequent field growth and yield parameters. For this reason, evaluating soybean by a unified system of indicators – from laboratory germination through to seed quality – allows a more accurate description of the process of productivity formation in the crop.

With regard to the laboratory assessment of sowing quality, K. Kozhushko (2025) established that the effect of biostimulants on soybean seeds depended on the type of product and the concentration of the working solution. Some treatments increased germination and seedling development, whilst certain concentrations suppressed the initial formation of seed material. In an assessment of pre-sowing seed treatment, S. Berdin *et al*. (2024) demonstrated that the combination of biological products altered leaf area, nodule formation, and soybean yield. The most pronounced outcome was associated with combined seed preparation, which influenced not only initial development but also the subsequent formation of productivity. In the field of crop production research, S. Dymytrov & V. Sabluk (2023) established that seed treatment with biological products enhanced the development of the leaf apparatus, root system, and soybean yield. The results obtained demonstrated that the effect of biological treatment manifested through simultaneous changes in growth and productivity indicators. From the perspective of biological crop protection, A. Bueno *et al*. (2023) showed that biological control could constitute a component of protection against the principal phytophages of the crop. The results of their review demonstrated that biological agents are capable of reducing the dependence of crops on chemical pest control.

In the context of nutrition and physiological efficacy, C. Sible & F. Below (2023) established that the effect of biological products depended on product composition, crop, and the stage of application. The most pronounced effects were associated with improved uptake of nutrients, root system development, and the maintenance of productivity. In the area of combining biological and fungicidal protection, F. Santos *et al*. (2022) demonstrated that a Bacillus-based product in combination with fungicides reduced the development of foliar diseases in soybean. This approach also contributed to the preservation of productivity, since the protection of the leaf apparatus was associated with yield formation. In the context of combining bioformulations with mineral nutrition, A. Dass *et al*. (2025) established that the integration of bioformulations with fertilisers enhanced soybean productivity, nutrient use efficiency, and economic returns. The results showed that the biological components performed better not in isolation but within a nutritional system, where they supported plant growth and nutrient utilisation. From the perspective of soil-physiological resilience of the agrocoenosis, J. Gonçalves e Silva *et al*. (2024) established that an integrated crop-livestock system improved soil condition and the physiological parameters of soybean. The positive changes manifested through better plant development, more active metabolism, and more stable functioning of the crop within the agroecosystem.

In the area of high-yield soybean cultivation, Y. Zhong & S. Zhong (2024) showed that crop yield was determined by the combination of sowing dates, plant stand density, nutrition, water regime, and crop protection. The summarised results confirmed that soybean productivity is formed not by a single technological factor but by their coordinated action throughout the growing season. When assessing soil microbiota and soil quality, Z. Wu *et al*. (2025) established that organic amendments combined with mineral fertilisers reduced microbial limitations in the soil and increased soybean yield. Changes in the microbial environment were associated with nutrient availability, soil quality, and the growth response of the crop.

From the perspective of plant growth-promoting rhizobacteria, D. Nagrale *et al*. (2023) showed that these microorganisms facilitated nutrient mobilisation, suppression of harmful organisms, and development of field crops. Their action manifested through support of nutrition, reduction of phytosanitary pressure, and improvement of growth processes. In the area of field testing of growth-promoting microorganisms, D. Neuhoff *et al*. (2024) substantiated the need for standardised evaluation of such products under actual field conditions. The proposed approaches demonstrated that reliable assessment must combine a controlled experimental design, clearly defined treatment variants, and comparison of laboratory and field indicators. The relationship between germination, nodulation, phytosanitary condition, and soybean productivity remains insufficiently studied, as these parameters have not previously been examined in a comprehensive manner within a single laboratory-field experiment. The aim of the study was to evaluate the efficacy of biological product application through the soybean response across indicators of germination, early development, phytosanitary condition, nodulation, yield, and seed quality. The objectives of the study included evaluating laboratory, field, and productivity indicators of soybean, comparing treatment variants, and determining changes in seed quality.

MATERIALS AND METHODS

Conditions for establishing and conducting the laboratory-field experiment. The laboratory-field experiment was conducted in 2025 at the Uman National University of Horticulture: the laboratory phase was carried out in the educational and research seed analysis laboratory, and the field phase was conducted on the university's experimental field under the conditions of the Right-Bank Forest-Steppe of Ukraine. The methodological design of the experiment did not include an economic evaluation of biological product application or determination of their residual effects on soil microbiota in the following growing season. The experiment used seed material of soybean cultivar 'Annushka', obtained from a certified seed lot of the Uman National University of Horticulture. The cultivar belongs to the early-maturing group and was selected for its suitability to Forest-Steppe conditions, short growing season, and the possibility of evaluating the plant response to biological products within a complete development cycle. The laboratory phase involved germination of soybean seeds following treatment with biological products in four replications of 100 seeds per treatment. Germination was carried out in a Memmert IN55 thermostat (Germany) at a constant temperature of 24 ± 1°C on moistened Whatman Grade 1 filter paper (United Kingdom). The experiment was conducted in accordance with the provisions of ISTA (n.d.) and the Convention on Biological Diversity (2016).

The field experiment was established using a randomised block design with four replications per treatment. The area of each experimental plot was 48 m², of which the accounting area was 32 m². Within each treatment, 30 plants were analysed for phytosanitary and biometric determinations; 12 plants were selected for the study of nodulation; yield structure was determined from 20 representative plants; seed quality was assessed from three composite samples of 500 g each. No specific rhizobial inoculation of seed was carried out; nodules were formed from the natural population of nodule bacteria present in the soil, and

Phytovit and Avercom were evaluated as biological treatments without additional introduction of rhizobia. The experimental design comprised four treatments: untreated control; Phytovit – pre-sowing seed treatment at 0.05 L/t, applied by coating the seed with the working solution, mixing, and drying in the shade, followed by foliar application at 0.20 L/ha at the 3-4 trifoliate leaf stage; Avercom – pre-sowing seed treatment at 25 mL/t in 10 L of water, applied the day before sowing or on the day of sowing; foliar application at 0.30 L/ha was carried out at the 3-4 trifoliate leaf stage of soybean, upon the appearance of the first signs of pest damage, in the morning between 07:00 and 09:00 or in the evening between 18:00 and 20:00 at an air temperature not exceeding 24°C. Phytovit + Avercom – a combination of the same pre-sowing and foliar application rates in a tank mixture. Phytovit was selected as a metabolic biological product based on biologically active substances of the soil streptomycete *Streptomyces netropsis*, without viable cells of the producer organism; its mode of action is associated with bioprotective, growth-stimulating, and anti-stress effects on plants. Avercom was used as a biological insecto-acaronematicide based on a complex of natural avermectins produced by *Streptomyces avermitilis*; its action is associated with contact-intestinal effects on pests and nematodes, as well as additional growth-stimulating and adaptogenic properties. The experiment was established in early-maturing soybean crops with a row spacing of 45 cm and a seeding rate of 620 thousand viable seeds per hectare. The previous crop was winter wheat cultivar 'Podolianka'. The soil of the experimental plot was typical chernozem of light loamy texture. In the plough layer of 0-30 cm, humus content was 3.18%, pH of the salt extract was 6.7, nitrate nitrogen content was 21.4 mg/kg, mobile phosphorus content was 74.6 mg/kg, and exchangeable potassium content was 108.3 mg/kg. Primary tillage comprised stubble cultivation to a depth of 7-9 cm and ploughing to 23-25 cm. Prior to sowing, cultivation to a depth of 4-5 cm with levelling of the seedbed was carried out.

Fertilisation in all experimental treatments was uniform and provided for the application of a mineral background of $N_{30}P_{60}K_{60}$. Phosphorus and potassium fertilisers were applied during primary tillage, whilst nitrogen fertilisers were applied prior to pre-sowing cultivation. Sowing was carried out in the first decade of May, when the soil temperature at seed placement depth reached 10-12°C. Sowing depth was 4-5 cm. For weed control, an identical crop management system was applied across all treatments so that the effect of the biological products could be evaluated without the influence of differing agronomic backgrounds. The growing season was characterised by moderately dry conditions: the mean air temperature for May-September was 19.1°C, total precipitation was 286.4 mm, and the greatest moisture deficit was observed in the second half of June and in July, i.e. during the periods of budding, flowering, and early seed filling. Foliar applications of biological products were carried out at the 3-4 trifoliate leaf stage and at the onset of budding using a Marolex Profession 12 Plus knapsack sprayer (Poland); the working solution volume was 250 L/ha. Spraying was performed in the morning, approximately between 07:00 and 09:00, at an air temperature not exceeding 24°C, in the absence of precipitation, and with wind speed below 3 m/s. All other elements of soybean cultivation technology were identical across the control and experimental treatments.

Sowing properties of soybean seeds following biological treatment. Germination energy (%) was determined under laboratory conditions on the fourth day of germination by counting the number of seeds that had formed normally developed seedlings. Seeds were placed in Petri dishes or germination containers on moistened Whatman Grade 1 filter paper (United Kingdom) in a Memmert IN55 thermostat (Germany) at a constant temperature of 25°C. Laboratory germination (%) was determined following completion of the standard germination period by counting normally formed seedlings. Normally developed seedlings were defined as those with an intact primary root, a formed shoot, and no signs of pronounced growth suppression. The calculation was performed using formula (1):

$$G_l = \frac{n_g}{N} \times 100, \quad (1)$$

where G_l is laboratory germination (%); n_g is the number of normally germinated seeds (units); N is the total number of seeds in the sample (units). Primary root length (cm) was measured from the base of the seedling to the tip of the primary root using a Topex 30 cm laboratory ruler (Poland). Shoot length (cm) was determined from the point of transition of the root into the hypocotyl to the uppermost point of the seedling using a Mitutoyo 500-196-30 Absolute Digimatic Caliper digital calliper (Japan). Seedling dry weight (mg) was determined after drying of the plant material to constant weight in a Binder ED 56 drying oven (Germany) at 65°C. Following drying, samples were weighed on a Radwag AS 220.R2 analytical balance (Poland) to an accuracy of 0.0001 g. Seedling vigour index (conventional units) was calculated as the sum of root length and shoot length, taking into account laboratory germination:

$$I_s = G_l \times (L_r + L_s), \quad (2)$$

where I_s is seedling vigour index; G_l is laboratory germination (%); L_r is mean primary root length (cm); L_s is mean seedling shoot length (cm). Seedling infection by microflora (%) was determined as the proportion of seedlings exhibiting mould, tissue darkening, root or cotyledon necrosis. A Levenhuk Zeno Handy ZH7 5×/16 mm hand lens (USA) was used to clarify the condition of surface tissues.

Early field development of soybean following biological treatment. Field germination (%) was determined by counting the number of plants that had emerged on fixed row segments, with subsequent recalculation relative to the number of viable seeds sown. Counts were carried out at the stage of full emergence on permanently marked plots. The calculation was performed using formula (3):

$$G_f = \frac{n_s}{N_s} \times 100, \quad (3)$$

where G_f is field germination (%); n_s is the number of seedlings that emerged (units); N_s is the number of viable seeds sown (units). Plant height at the V3 stage (i.e. the stage of three fully developed trifoliate leaves) (cm) was measured from the soil surface to the uppermost point of the plant using a Stanley 2 m measuring rod (USA). Plant stand density at the V3 stage (plants/m²) was determined by direct counting of plants on the accounting area, with subsequent recalculation per square metre. The calculation was performed using formula (4):

$$D = \frac{N_p}{A}, \quad (4)$$

where D is plant stand density (plants/m²); N_p is the number of plants on the accounting area (units); A is the accounting area (m²). Leaf area (cm²/plant) was determined from the length and maximum width of leaf blades using a Topex 30 cm measuring ruler (Poland). Chlorophyll content (SPAD units) was determined using a Konica Minolta SPAD-502Plus portable chlorophyll meter (Japan) on fully expanded leaves of the middle tier. Root dry weight (g/plant) was determined after washing the root system free of soil, drying to constant weight in a Binder ED 56 drying oven (Germany) at 65°C, and weighing on a Radwag PS 1000.R2 laboratory balance (Poland). The number of nodules at the V5 stage (i.e. the stage of five fully developed trifoliate leaves) (nodules/plant) was established by direct counting of nodular formations on the main and lateral roots following washing of the root system in running water. Counting was carried out using an Eschenbach mobilux 6× hand lens (Germany).

Assessment of diseases and pest damage in soybean plants. The incidence of root rot (%) was determined as the proportion of plants exhibiting darkening of the root zone, necrosis of the root collar, or damage to the primary roots. Assessments were made using a Levenhuk Zeno Handy ZH7 5×/16 mm hand lens (USA). The development of foliar diseases (%) was determined as the proportion of the affected leaf area on the accounting plants. A Carson LED Lighted LinenTest LT-30 11.5× hand lens (USA) was used to detail the symptoms. The proportion of plants with pest damage (%) was determined as the number of plants exhibiting disruption of leaf blade integrity, tissue punctures, leaf edge feeding, or other characteristic signs of phytophage feeding activity. The calculation was performed using formula (5):

$$P_d = \frac{n_d}{N} \times 100, \quad (5)$$

where P_d is the proportion of plants with pest damage (%); n_d is the number of damaged plants (units); N is the total number of plants examined (units). The density of sucking pests (individuals/plant) was determined by direct counting of individuals on the leaves and stems of accounting plants. A BioQuip Plastic Vial Set entomological container (USA) was used to facilitate counting.

Plant survival to the flowering stage (%) was calculated as the ratio of the number of plants at the flowering stage to the number of plants at the full emergence stage:

$$S_f = \left(1 - \frac{N_s - N_f}{N_s}\right) \times 100, \qquad (6)$$

where S_f is plant survival to the flowering stage (%); N_s is the number of plants at the full emergence stage (units); N_f is the number of plants at the flowering stage (units). Assessments were conducted by two specialists with higher agronomic education, one of whom held the degree of PhD in Agricultural Sciences. The calculation was performed using formula (7):

$$I_d = \frac{\sum(a_i \times n_i)}{N}, \qquad (7)$$

where I_d is the plant disease/damage index (score); a_i is the disease or damage score; n_i is the number of plants with the corresponding score (units); N is the total number of plants examined (units). The biological efficacy of protection (%) was calculated from the reduction in root rot incidence in the experimental treatments compared with the control:

$$E_b = \frac{R_k - R_d}{R_k} \times 100, \qquad (8)$$

where E_b is the biological efficacy of protection (%); R_k is disease or damage development in the control (%); R_d is disease or damage development in the experimental treatment (%). The plant disease/damage index (score) was determined as the weighted mean score of disease incidence and pest damage on a 5-point scale, where 0 = no symptoms; 1 = slight damage; 2 = moderate; 3 = medium; 4 = severe; 5 = very severe damage or pest injury.

Physiological characterisation of soybean plants during flowering. The leaf area index (m^2/m^2) was determined on the basis of the leaf area of a single plant and the plant stand density per unit area. The calculation was performed using formula (9):

$$LAI = \frac{S_l \times D}{10{,}000}, \qquad (9)$$

where LAI is the leaf area index (m^2/m^2); S_l is the leaf area of a single plant (cm^2); D is plant stand density (plants/m^2). Total chlorophyll content (mg/g fresh weight) was determined by spectrophotometry following extraction of pigments with 80% acetone. A fresh leaf tissue sample was ground in a porcelain mortar, the extract was filtered, and the optical density was measured on a Shimadzu UV-1800 spectrophotometer (Japan) at wavelengths of 645 and 663 nm. Photosynthetic potential (million $m^2 \cdot$ days/ha) was determined from the mean value of the leaf area index between two recording dates and the duration of the inter-recording period. The calculation was performed using formula (10):

$$FP = \frac{LAI_1 + LAI_2}{2} \times T \times 0.01, \qquad (10)$$

where FP is photosynthetic potential (million $m^2 \cdot$ days/ha, after conversion); LAI_1 is the leaf area index at the first recording date (m^2/m^2); LAI_2 is the leaf area index at the second recording date (m^2/m^2); T is the duration of the inter-recording period (days); 0.01 is the conversion factor from $m^2 \cdot$ days/m^2 to million $m^2 \cdot$ days/ha. For presentation in million $m^2 \cdot$ days/ha, the values obtained were recalculated with reference to the crop area. The number of active nodules (nodules/plant) was determined following washing of the root system and sectioning of the nodules. Nodules with pink or reddish colouration of the internal tissue were classified as active. The proportion of active nodules (%) was calculated using formula (11):

$$A_n = \frac{N_a}{N_t} \times 100, \qquad (11)$$

where A_n is the proportion of active nodules (%); N_a is the number of active nodules (units); N_t is the total number of nodules on the plant (units). Nodule mass (g/plant) was determined following separation of nodular formations from the roots and weighing on a Radwag AS 220.R2 analytical balance (Poland). Leaf canopy temperature (°C) was measured using a Testo 835-H1 infrared thermometer (Germany) between 12:00 and 14:00 under clear or partly cloudy conditions, with no shading of plants at the time of measurement.

Yield structure of soybean following application of biological products. The number of pods and seeds (units/plant) was determined by direct counting of all formed pods on representative plants prior to harvest, after which the mean value for each treatment was calculated using the arithmetic mean in Microsoft Excel 2019 (USA). The weight of 1,000 seeds (g) was determined using the standard method by selecting two sub-samples of 500 seeds each and weighing on a Radwag PS 1000. R2 laboratory balance (Poland). Seed yield (t/ha) was determined following harvest, cleaning, and weighing of the seed mass from the accounting area. Recalculation was performed to the standard soybean seed moisture content of 14%. The calculation was performed using formula (12):

$$Y = \frac{M \times 10{,}000}{A \times 1{,}000} \times \frac{100 - W}{100 - W_s}, \quad (12)$$

where Y is seed yield (t/ha); M is the seed mass from the accounting area (kg); A is the accounting area of the plot (m²); W is actual seed moisture content (%); W_s is standard seed moisture content (%). The yield increase over the control (%) was calculated as the ratio of yield in the experimental treatment to the yield in the control:

$$Y_i = \frac{Y_d - Y_k}{Y_k} \times 100, \quad (13)$$

where Y_i is the yield increase over the control (%); Y_d is yield in the experimental treatment (t/ha); Y_k is yield in the control treatment (t/ha). Protein content in the seed (%) was determined by the Kjeldahl method following mineralisation of samples and determination of total nitrogen on a Velp Scientifica UDK 159 automatic analyser (Italy). Oil content in the seed (%) was determined by extraction in a Gerhardt Soxtherm SOX 412 apparatus (Germany) using petroleum ether as the extractant. Following completion of extraction, the residual solvent was removed, and the mass of extracted oil was determined gravimetrically. Statistical analysis of the results was performed by analysis of variance using Statistica 13.3 software (USA).

RESULTS

The combined application of Phytovit and Avercom produced the most pronounced seed response at the laboratory stage: germination energy was 88.7%, exceeding the control by 10.1 percentage points. The separate application of Phytovit and Avercom also increased this indicator; however, their combination yielded more uniform seedling formation during the early recording period. This outcome was associated with a simultaneous enhancement of initial growth processes and a reduction in the proportion of seeds that were delayed at the swelling or primary root emergence stage. Seeds subjected to the combined treatment transitioned more rapidly to active growth, and the seedlings formed were more uniform in outward condition. Laboratory germination under this treatment reached 94.3%, whilst in the control it was 86.4%. Accordingly, the increase in germination was associated not only with a greater number of germinated seeds but also with the formation of normally developed seedlings with a distinct primary root, an initial shoot, and intact cotyledons. In the treatments with biological products, the proportion of weak and developmentally delayed seedlings was lower; however, it was precisely the combined treatment that united the growth-stimulating action of Phytovit with the bioprotective action of Avercom, thereby ensuring more complete completion of early morphogenesis. Seedlings subjected to this treatment were characterised by preservation of tissue integrity, absence of widespread growth suppression, and formation of more uniform plant material by the time of the final laboratory assessment.

Development of the root portion was also greatest under the combined treatment: primary root length reached 9.4 cm, exceeding the control by 2.2 cm. Separate application of the products yielded intermediate values, with Phytovit having a stronger effect on root elongation and Avercom on the reduction of signs of microbial damage. Under the combined treatment, the primary root formed without widespread shortening, darkening of the apical portion, or disruption of the growth direction, indicating that early growth proceeded without noticeable tissue suppression. In some seedlings, the initiation of lateral rootlets was observed, so the root system had a more fully formed appearance by the time the laboratory assessment was completed. Seedling infection by microflora under the combined treatment decreased to 4.2%, which was 7.6 percentage points lower than the control. Of the individual products, a lower level of infection was provided by Avercom, consistent with its bioprotective orientation, whilst Phytovit more effectively supported the growth activity of seedlings. In the combined treatment, these effects were united: mould, darkening, necrosis of individual sections, and tissue softening manifested less frequently on the seed surface and seedling tissues. Symptoms of infection remained localised and did not cause widespread loss of seedlings from the general sample (Table 1).

Table 1. Laboratory germination indicators of soybean seeds depending on treatment scheme

Indicator	Control	Phytovit	Avercom	Phytovit + Avercom
Germination energy, %	78.6	84.8	82.9	88.7
Laboratory germination, %	86.4	91.2	89.6	94.3
Primary root length, cm	7.2	8.5	8.1	9.4
Shoot length, cm	5.9	6.8	6.5	7.4
Seedling dry weight, mg	74.5	82.1	79.8	87.6
Seedling vigour index, conventional units	1,132	1,395	1,308	1,584
Seedling infection by microflora (fungal-bacterial infection), %	11.8	7.1	6.4	4.2

Note: statistical analysis was performed by one-way analysis of variance (ANOVA); differences between the biological treatment variants and the control are statistically significant at $p \leq 0.05$ for all indicators presented
Source: developed by the author on the basis of formulae (1-2)

Based on the results of the laboratory phase, it was established that pre-sowing treatment with biological products was accompanied by changes in the passage of the initial developmental phases of soybean seeds. In the combined application variant of Phytovit and Avercom, uniform germination was observed, along with formation of a greater proportion of normally developed seedlings, active elongation of the primary root, and lower expression of microflora on seedlings. The results of the laboratory assessment demonstrated that the treated seed material passed through early developmental stages with a lower proportion of visually suppressed, infected, or underdeveloped seedlings. The data obtained served as the basis for the subsequent field evaluation of the effect of biological products on germination, plant stand density, early growth, and soybean productivity formation.

In the control, field germination was 78.9%, whilst the combined treatment yielded an increase of +8.2 percentage points. Separate application of Phytovit and Avercom also improved seedling emergence, though less markedly. This difference between treatments was associated with the combined treatment uniting stimulation of early seed growth with a reduction in losses at the initial stage of field development. In the control, lower germination manifested through thinning of individual row sections and non-uniform emergence, whilst in the treatments with biological products the crop stand had better coverage and a smaller proportion of plants that were delayed in transitioning to the next developmental phase. Plant stand density was also higher under biological treatment, confirming better realisation of field germination following emergence. Under the combined application of Phytovit and Avercom, it reached 43.6 plants/m², whilst in the control it was 39.5 plants/m². Phytovit applied separately yielded slightly higher plant stand density than Avercom, consistent with its growth-stimulating action. At the same time, the combined treatment exceeded both individual treatments, as it united support of growth processes with lower biotic pressure on young plants. This reduced plant loss following emergence and contributed to more uniform formation of the early plant canopy.

Early development of the shoot was most active under the combined treatment, manifesting through greater plant height and leaf area. At the V3 stage, plant height under the Phytovit + Avercom combination was 18.3 cm, and leaf area was 151 cm²/plant. Separate application of Phytovit produced a stronger growth response than Avercom; however, the combined treatment yielded the higher overall result. The increase in leaf area was associated not with the visual appearance of the leaves but with the quantitative accumulation of the assimilatory apparatus. Description of the physical appearance of the leaf blades has been omitted, as no deviations indicative of widespread leaf tissue suppression were established within this assessment. The chlorophyll indicator confirmed the advantage of the combined treatment over the separate application of products. Under Avercom, chlorophyll content was 38.1 SPAD units; under Phytovit it was higher, and the maximum value was obtained in the Phytovit + Avercom treatment. This sequence demonstrated that Phytovit had a stronger influence on the formation of the leaf apparatus, whilst Avercom acted primarily through a reduction of phytosanitary pressure. In the combined treatment, these effects were united, so plants had a higher chlorophyll status at the early stage of the growing season (Table 2).

Table 2. Effect of biological products on field germination and early soybean growth

Indicator	Control	Phytovit	Avercom	Phytovit + Avercom
Field germination, %	78.9	83.6	82.4	87.1
Plant stand density at V3 stage, plants/m²	39.5	41.8	41.2	43.6
Plant height at V3 stage, cm	14.6	16.8	16.1	18.3
Leaf area, cm²/plant	118	134	129	151
Chlorophyll content, SPAD units	36.4	39.2	38.1	41.6
Root dry weight, g/plant	0.48	0.57	0.54	0.64
Number of nodules at V5 stage, nodules/plant	14.2	17.8	16.9	20.6

Note: statistical analysis was performed by one-way analysis of variance (ANOVA); differences between the biological treatment variants and the control are statistically significant at $p \leq 0.05$ for all indicators presented
Source: developed by the author on the basis of formulae (3-4)

Thus, all three biological treatment schemes improved the early development of soybean compared with the control; however, the combined application of Phytovit and Avercom yielded the highest values for field germination, plant stand density, leaf area, and nodulation. The data obtained confirmed that the initial changes recorded at the laboratory stage were realised in better field development of plants and formed the basis for the subsequent assessment of the phytosanitary condition of the crop. Biological treatment reduced the phytosanitary pressure on soybean crops, with the most pronounced limitation of diseases and pest damage obtained under the combination of Phytovit and Avercom. In the control treatment, the incidence of root rot was 15.6%, manifesting through infection of the root zone, weakening of some plants, and non-uniform resumption of growth following the initial vegetative phases. In the treatments with biological products, the proportion of such plants was lower, as treatment reduced the pressure of soil microflora on young roots and the root collar. In practical terms, this meant less thinning of the plant stand, better plant survival, and more uniform formation of the above-ground mass during the early growth period. Foliar diseases also manifested with less intensity following application of biological products, but the mode of action of the products differed. Under Phytovit treatment, development of foliar diseases was 8.6%, meaning that infection remained predominantly localised and did not progress to widespread destruction of the assimilatory surface. Avercom manifested more prominently through limitation of pest damage: the proportion of damaged plants in this treatment was 10.5%. This difference between the products demonstrated that Phytovit more effectively maintained the condition of the leaf apparatus, whilst Avercom had a stronger effect on the entomological pressure. Under combined application, these directions of action were united, so disease incidence and phytophage damage did not form extensive foci of plant suppression.

The plant disease/damage index most effectively summarised the phytosanitary differences between treatments, as it accounted not only for the presence of symptoms but also for the degree of their expression. In the control it was 3.1 score, corresponding to a medium level of damage with a noticeable effect on plant condition. Under the combined treatment, the index decreased to 1.3 score, meaning symptoms remained predominantly slight and localised. In practical terms, this meant less loss of functional leaf area, a lower level of root system suppression, and better plant survival within the crop stand. The disease/damage index thus reflected the overall phytosanitary risk for subsequent crop development. The combined application of Phytovit and Avercom provided the highest biological efficacy of protection – 59.6%. This result was associated with the simultaneous reduction of root zone infection, localisation of foliar symptoms, and a lower proportion of plants with pest damage. In this treatment, the phytosanitary condition of the crop was not limited to a single protective direction but encompassed several levels: the root system, the leaf apparatus, and plant survival through to subsequent developmental stages. This created more favourable conditions for the further formation of the assimilatory surface, the nodular apparatus, and the reproductive organs (Table 3).

Table 3. Disease incidence and pest damage in soybean plants

Indicator	Control	Phytovit	Avercom	Phytovit + Avercom
Incidence of root rot, %	15.6	10.8	9.7	6.3
Development of foliar diseases, %	12.4	8.6	7.8	5.1
Proportion of plants with pest damage, %	18.2	14.7	10.5	7.8
Density of sucking pests, individuals/plant	4.6	3.8	2.5	1.9
Plant survival to flowering stage, %	87.5	91.6	92.2	94.8
Plant disease/damage index, score	3.1	2.2	1.9	1.3
Biological efficacy of protection, %	0.0	30.8	37.8	59.6

Note: statistical analysis was performed by one-way analysis of variance (ANOVA); differences between the biological treatment variants and the control are statistically significant at $p \leqslant 0.05$ for all indicators presented
Source: developed by the author on the basis of formulae (5-8)

The phytosanitary condition of soybean crops under different biological product application schemes was associated with the expression of root rot, foliar diseases, pest damage, and the overall efficacy of the protective action. In the control treatment, symptoms of root zone infection were most prominent. Under Phytovit treatment, attention was directed to the condition of the leaf apparatus, where diseases manifested locally. In the Avercom treatment, assessment was focused on plant pest damage during the early growth phase. The combined treatment encompassed the overall phytosanitary condition of the crop through the total reduction in disease and damage expression. The data obtained were used for the subsequent analysis of physiological plant indicators and the formation of soybean productivity.

The combined biological treatment provided more pronounced development of the assimilatory apparatus at the flowering stage compared with the control and separate application of the products. The leaf area index under the Phytovit + Avercom combination was 4.0 m^2/m^2, whilst in the control it was 3.2 m^2/m^2. The increase in this indicator reflected a greater area of functional leaf surface per unit of crop stand, which was associated with better plant survival, more active accumulation of vegetative mass, and lower phytosanitary pressure during the preceding developmental period. Separate application of Phytovit and Avercom also contributed to an increase in the leaf area index; however, the combined treatment ensured more complete canopy closure and more uniform formation of the assimilatory area. Photosynthetic potential also increased under biological treatment, with the highest level obtained in the Phytovit + Avercom treatment. In the combined treatment it was 2.03 million $m^2 \cdot$ days/ha, whilst in the control it was 1.64 million $m^2 \cdot$ days/ha. This difference demonstrated that plants not only formed a greater leaf area but also maintained its functional contribution to organic matter accumulation for a longer period. In practical terms, this meant a higher capacity of the crop stand to sustain photosynthetic activity during the flowering stage, when the basis for subsequent seed filling is formed. The individual products produced an intermediate effect; however, their combined application provided a more complete integration of leaf development, pigment status, and the duration of assimilatory apparatus activity. Leaf canopy temperature decreased under the combined treatment compared with the control: in the control it was 30.6°C, whilst in the Phytovit + Avercom treatment it was 29.1°C. The lower leaf surface temperature indicated reduced thermal loading on the plants and a more stable water-physiological status during the period of active vegetation. This indicator complemented the results regarding the leaf area index and photosynthetic potential, since greater assimilatory area was of significance only provided its functional activity was preserved. Accordingly, at the flowering stage, the combined treatment ensured not an isolated improvement of a single parameter but simultaneous changes in the leaf apparatus, pigment status, symbiotic activity, and the thermal regime of the plants. Symbiotic activity of soybean also intensified following application of biological products, manifesting in a greater number of active nodules and a higher proportion thereof. Following Avercom application, 21.8 active nodules per plant were formed on the root system, whilst under the combined treatment this indicator was higher. The proportion of active nodules under the Phytovit + Avercom combination exceeded the Avercom treatment by 6.6 percentage points. This response was associated with better root system development and a lower level of plant infection during the early vegetative phases. Phytovit promoted more active growth of the root portion, whilst Avercom reduced the action of factors that could limit the formation of the symbiotic apparatus. In the combined treatment, these directions of action were united, so nodulation was part of the overall physiological condition of the plants during the flowering stage (Table 4).

Table 4. Physiological condition of soybean plants at the flowering stage

Indicator	Control	Phytovit	Avercom	Phytovit + Avercom
Leaf area index, m^2/m^2	3.2	3.6	3.5	4.0
Total chlorophyll content, mg/g fresh weight	1.82	2.04	1.98	2.21
Photosynthetic potential, million m^2·days/ha	1.64	1.82	1.78	2.03
Active nodules, nodules/plant	18.4	22.6	21.8	25.9
Proportion of active nodules, %	62.1	68.4	66.9	73.5
Nodule mass, g/plant	0.42	0.51	0.49	0.58
Leaf canopy temperature, °C	30.6	29.8	29.9	29.1

Note: statistical analysis was performed by one-way analysis of variance (ANOVA); differences between the biological treatment variants and the control are statistically significant at $p \leqslant 0.05$ for all indicators presented
Source: developed by the author on the basis of formulae (9-11)

Thus, at the flowering stage, the combined treatment simultaneously improved four interrelated parameters: a greater assimilatory surface (LAI 4.0 m^2/m^2), higher photosynthetic potential (2.03 million m^2 · days/ha), more active symbiotic apparatus (25.9 active nodules/plant), and lower thermal loading on the leaves (29.1°C). It was precisely this combination of changes that formed the physiological basis for subsequent seed filling and accumulation of productive mass. The combined application of Phytovit and Avercom ensured the greatest formation of reproductive organs, manifesting in an increase in the number of pods per plant. In the control, this indicator was 31.4 pods/plant, whilst under the combined application of the products it reached 40.5 pods/plant. Separate application of Phytovit and Avercom also increased the number of pods, but less markedly. This difference was associated with the better condition of plants during the preceding stages: higher field germination, more developed leaf area, lower phytosanitary pressure, and more active root system formation. As a result, a greater proportion of flower buds progressed to formed pods, and the reproductive portion of the plant was denser compared with the control. Seed filling of the pods was also most fully realised under the combined treatment. Under Phytovit application, 78.6 seeds per plant were recorded, whilst in the Phytovit + Avercom treatment this indicator was higher, at 89.4 seeds/plant. The increase relative to Phytovit alone was 10.8 seeds/plant, demonstrating the additional effect of combining the products. Phytovit more effectively supported growth and physiological processes, whilst Avercom reduced losses associated with plant damage and phytosanitary pressure. In the combined treatment, these effects were united, so the productive portion of the plant formed more completely.

Seed weight increased in all treated variants, but the maximum value was obtained under the combined application of the products. Under Avercom, the weight of 1,000 seeds was 157.1 g, and under the combined treatment it was 164.6 g. The increase in this indicator was associated with more favourable passage of the filling period, preservation of the assimilatory surface, and translocation of photosynthates to the seed. The individual products yielded an intermediate result, whilst their combined application produced a higher level of seed filling. The weight of 1,000 seeds in this case reflected the completeness of dry matter accumulation in the seed. Seed yield most clearly reflected the cumulative effect of the preceding growth, phytosanitary, and physiological changes. Under the Phytovit + Avercom combination, it was 3.01 t/ha, corresponding to an increase over the control of 24.4%. Separate application of Phytovit yielded a higher yield increase than Avercom; however, the combined treatment provided the highest result. This was explained by the simultaneous increase in the number of pods, better seed filling, greater seed weight, and plant survival through to the ripening period. That is, yield was formed not through a single structural element but through the accumulation of effects at several stages of soybean development. Qualitative seed indicators changed less sharply than the yield structure components; however, the combined treatment provided the highest protein and oil values among all treatments. Protein content under the Phytovit + Avercom combination was 39.1%, which was 1.3 percentage points higher than the control, and oil content reached 21.0%. The changes obtained demonstrated that biological products exerted their primary influence on yield formation and seed filling, whilst qualitative parameters responded more moderately. In the combined treatment, the increase in protein and oil content was accompanied by greater seed weight and higher yield; therefore, the final outcome was characterised not only by an increase in the quantity of produce but also by the preservation of its qualitative properties (Table 5). Analysis of variance confirmed the statistical significance of differences between treatments for yield, field germination, and the number of active nodules ($p \leqslant 0.05$). The least pronounced, though nonetheless significant, differences were recorded for protein and oil content in the seed.

Table 5. Soybean productivity depending on biological product application

Indicator	Control	Phytovit	Avercom	Phytovit + Avercom
Number of pods, pods/plant	31.4	36.2	35.1	40.5
Number of seeds, seeds/plant	67.2	78.6	75.9	89.4
Weight of 1,000 seeds, g	151.8	158.9	157.1	164.6
Seed yield, t/ha	2.42	2.72	2.66	3.01
Yield increase over control, %	0.0	12.4	9.9	24.4
Protein content in seed, %	37.8	38.5	38.3	39.1
Oil content in seed, %	20.3	20.7	20.6	21.0

Note: statistical analysis was performed by one-way analysis of variance (ANOVA); differences between the biological treatment variants and the control are statistically significant at $p \leqslant 0.05$ for all indicators presented
Source: developed by the author on the basis of formulae (12-13)

The yield structure of soybean in the experiment encompassed the number of pods, seed filling, seed weight, and the overall output of produce per unit area. These indicators were formed successively throughout the growing season and reflected the transition of plants from vegetative growth to reproductive development. Following emergence, the basis of future productivity was constituted by plant survival within the crop stand, as it was this that determined the number of individuals capable of progressing to flowering, flower bud formation, and pod ripening. Subsequent development of the leaf area ensured the accumulation of photosynthates, which were translocated to the stem, root system, and reproductive organs. The condition of the root system influenced the uptake of water and nutrients, whilst nodule formation supported nitrogen nutrition of the plants during the period of active growth and seed filling. The phytosanitary condition of the crop determined the preservation of functional tissues of leaves, stems, and roots, so the level of disease incidence or pest damage was reflected in the completeness of formation of the productive portion of the plants. The number of pods indicated the result of the initiation and survival of reproductive organs; the number of seeds indicated pod filling; the weight of 1,000 seeds indicated the completion of filling and dry matter accumulation; and yield indicated the total seed mass output following harvest. In this sequence, the early condition of the crop stand, development of the assimilatory apparatus, functioning of the roots, and preservation of reproductive organs translated into the quantitative and qualitative parameters of the harvest.

DISCUSSION

The results obtained demonstrated that the application of biological products influenced the successive developmental stages of soybean – from seed germination through to yield formation. At the laboratory stage, the combined application of Phytovit and Avercom was associated with more uniform seedling formation, preservation of tissue integrity, and lower expression of microflora. This response created the preconditions for a greater proportion of the seed material to transition to subsequent developmental phases without pronounced suppression. Under field conditions, the effect of the products manifested through the condition of seedlings, development of the leaf apparatus, formation of the root system, and nodular formations. Plant survival following emergence was accompanied by more active accumulation of above-ground mass and better functioning of the assimilatory surface. The phytosanitary condition of the crop also changed: disease incidence and pest damage did not attain widespread distribution, which supported further plant development throughout the growing season. Yield formation reflected the preceding growth stages, as the number of reproductive organs, seed filling, and seed quality depended on plant condition throughout the growing season. The combined application of biological products ensured a successive connection between germination, field development, phytosanitary condition, symbiotic activity, and soybean productivity.

In the context of biological seed treatment and integrated control of harmful organisms, J. Lamichhane *et al*. (2022) and W. Zhou *et al*. (2024) examined how biological and combined approaches altered the initial development of crops, phytosanitary condition, and yield. In a meta-analysis of 396 studies, J. Lamichhane *et al*. demonstrated that biological seed treatment increased seedling emergence, plant biomass, disease control, and yield; for yield, the mean increase was 21 ± 2% compared with untreated seed. In the present study, yield under the combined treatment increased by 24.4%, which was close to the generalised effect established in the meta-analysis. This indicates that the action of Phytovit and Avercom was realised not only through germination but also through subsequent plant survival, reduced root rot incidence, development of the nodular apparatus, and seed filling. W. Zhou *et al*. considered integrated protection as a system combining biological control, monitoring, and several protective measures, rather than as the action of a single agent. This is consistent with the result obtained: the Phytovit + Avercom combination yielded a higher effect than the individual products, as Phytovit supported initial growth and the assimilatory apparatus whilst Avercom reduced biotic pressure; together, these actions translated into a lower disease/damage index, more active nodulation, and higher seed productivity.

In the context of microbial nutrition, rhizosphere activity, and plant biostimulation, H. Khosravi *et al*. (2024) and R. Johnson *et al*. (2024) focused on the role of PGPR biofertilisers and biostimulants in crop growth, nutrition, and stress tolerance. H. Khosravi *et al*. described the action of rhizobacteria through nitrogen fixation, hormone production, and solubilisation of nutrient compounds, though with greater emphasis on the general prospects of application. R. Johnson *et al*. considered biostimulants as agents influencing plant metabolism, productivity, and resistance to adverse factors. In the present study, the microbial-physiological aspect was revealed through the relationship between the leaf apparatus, chlorophyll status, nodulation, and subsequent seed formation. In particular, the increase in chlorophyll content to 2.21 mg/g fresh weight and the rise in the proportion of active nodules to 73.5% in the combined treatment is consistent with the mechanisms of PGPR action described by H. Khosravi *et al*. through hormonal stimulation and nutrient mobilisation, whilst R. Johnson *et al*. associated similar changes in pigment status with a reduction in abiotic stress loading on plants.

In the area of biocontrol mechanisms and the practical application of biological agents, A. Tyagi *et al*. (2024) and M. Villavicencio-Vásquez *et al*. (2025) analysed the mechanisms of action, selection, formulations, and limitations of biocontrol agents in plant protection. M. Villavicencio-Vásquez *et al*. examined *Trichoderma* and *Bacillus* through the mechanisms of mycoparasitism, competition, antibiosis, enzymatic activity, and induced resistance. A. Tyagi *et al*. reviewed the role of bacterial, fungal, and viral agents in the control of crop diseases, drawing attention to the factors that alter their efficacy in the field. In the present study, the biocontrol component was not limited to a phytopathological description alone, as the phytosanitary condition was linked to plant development, preservation of the leaf surface, and formation of yield structure. The reduction of the plant disease/damage index from 3.1 to 1.3 score and the biological efficacy of protection of 59.6% are consistent with the mechanisms described by M. Villavicencio-Vásquez *et al*.: Avercom, based on *S. avermitilis*, exerted contact-intestinal action on phytophages, whilst Phytovit, acting through metabolites of *S. netropsis*, could induce systemic plant resistance to pathogens – which explains the different directions of action of the two products when used in combination. This made it possible to demonstrate that the action of the biological products manifested not in isolation but through the interrelationship of protective, physiological, and productivity-related changes in soybean crops.

To characterise the relationship between nutrition, symbiotic activity, and yield, A. Mirriam *et al*. (2022) and V. Paramesh *et al*. (2023) demonstrated that the combination of several agronomic factors yielded a higher result than the action of a single component. V. Paramesh *et al*. summarised that integrated nutrient management increased crop yield across a broad range – from 1.3 to 66.5% – whilst simultaneously improving soil properties and soil microbiological activity. A. Mirriam *et al*. established that *Bradyrhizobium* inoculation combined with phosphorus nutrition provided a soybean yield increase of up to 30%, with the best result obtained at an application rate of 15 kg P/ha. In the present study, the yield increase under the combined application of Phytovit and Avercom was 24.4%, which was lower than the maximum reported by A. Mirriam *et al*. but higher than some of the effects summarised by V. Paramesh *et al*. The difference lay in the fact that here the result was formed not through a mineral nutrition system or Bradyrhizobium inoculation but through the combination of the growth-stimulating and bioprotective action of the products. It was precisely for this reason that the combined treatment yielded a better result than the separate application of Phytovit or Avercom: the former product supported early growth and the leaf apparatus, the latter reduced phytosanitary pressure, and their combined action translated into higher nodulation, better seed filling, and greater yield.

With regard to production constraints of soybean and the role of microbial bioformulations, A. Khan *et al*. (2023) and P. Majidian *et al*. (2024) analysed different levels of the problem: from production dependence on external factors to the technological stability of microbial products. P. Majidian *et al*. noted that Iran covers more than 90% of its soybean requirements through imports, and attributed the production decline to biotic and abiotic stresses, a narrow cultivar base, and technological limitations. A. Khan *et al*. considered microbial bioformulations as a system in which efficacy depends on the viability of the microorganisms, the carrier, the delivery method, and the capacity to support plant nutrition, growth, and protection; for a stable crop response, a sufficient number of viable cells was indicated as necessary, specifically not below 10^6-10^7. In the present study, these principles were tested not at the level of general technological prospects but through specific soybean indicators: the combined treatment provided a yield of 3.01 t/ha, an increase over the control of 24.4%, and lower seedling infection by microflora. The superior result of the combined treatment was explained by the combination of two actions: Phytovit supported early growth and leaf apparatus formation, whilst Avercom reduced biotic pressure, so the effect progressed from germination through to nodulation, seed filling, and increased productivity.

In the analysis of organic nutrient sources and the plant microbiome, M. Khan *et al*. (2024) and S. Compant *et al*. (2025) focused on soil organic matter, microbial activity, rhizosphere processes, and the role of microorganisms in crop growth. M. Khan *et al*. considered organic fertilisers and cover crops as factors improving the soil environment and microbial biomass, whilst S. Compant *et al*. reviewed the functions of the plant microbiome in nutrition, stress tolerance, and protection from pathogens. In the present study, this area was made specific through the soybean response to biological treatment: seedling infection by microflora decreased to 4.2%, the incidence of root rot decreased to 6.3%, and the number of nodules at the V5 stage reached 20.6 per plant. In contrast to studies where the emphasis is placed on the general functions of soil microbiota, the present study demonstrates the transition from lower microbial infection to more active nodulation, more stable plant development, and yield formation. The Phytovit + Avercom combination yielded a higher result than the individual products through the combination of growth support and reduction of biotic pressure.

In terms of biotic pressure and long-term agronomic background, A. Afzal & T. Mukhtar (2024) and S. Panday *et al*. (2024) examined different sources of productivity constraints: phytoparasitic nematodes and long-term fertilisation systems in crop rotations. A. Afzal & T. Mukhtar reviewed approaches to nematode management as a factor of yield loss, focusing on protective strategies and food security. S. Panday *et al*. demonstrated that long-term fertilisation schemes altered productivity, profitability, and water use in a soybean-wheat system. In the present study, the biotic and production components were linked through the condition of the root system, the phytosanitary background, and the subsequent formation of seeds. In contrast to studies where harmful organisms or long-term nutrition are analysed separately, the present study traces how early reduction of seedling infection and more stable root development translated into more active nodulation and formation of the reproductive portion of soybean.

For the assessment of agroecosystem resilience to climatic and biodiversity-related factors, N.-F. Wan *et al*. (2024) and A. Raza *et al*. (2025) directed attention to broader mechanisms of agricultural adaptation. A. Raza *et al*. considered strategies for developing climate-smart crops oriented towards the preservation of productivity under stress conditions. N.-F. Wan *et al*. analysed the cascading socio-ecological benefits of biodiversity for agricultural production, including pest regulation and the maintenance of agroecosystem functions. In the present study, resilience was examined not through breeding or landscape-level approaches but through the soybean response to biological treatment under laboratory and field conditions. The results obtained detailed how the condition of seedlings, the leaf apparatus, the root system, and the phytosanitary background formed the foundation for yield structure. This approach narrowed the scale of analysis to a specific crop but more deeply revealed the internal sequence of growth and productivity changes.

In the examination of initial seed quality and the role of beneficial bacteria, L. Reed & B. Glick (2023) and D. Patyal *et al*. (2025) analysed technologies that influence the initial development of plants. D. Patyal *et al*. described seed coating as a method of improving seed quality, protecting seedlings, and enhancing the field realisation of crop potential. L. Reed & B. Glick reviewed the application of plant growth-promoting bacteria to support agricultural crop development. In the present study, this area was made specific through the soybean response to biological treatment from the moment of germination through to seed ripening. Whereas in the cited works the main emphasis fell on coating technology or the general role of bacteria, the present study demonstrated the subsequent transition of initial changes into field germination, symbiotic activity, preservation of the leaf apparatus, and formation of seed mass. The present study thus detailed the action of biological treatment of soybean through the successive connection between germination, field development, phytosanitary condition, symbiotic activity, and yield formation.

CONCLUSIONS

The study involved a laboratory-field assessment of the effect of Phytovit, Avercom, and their combination on the sowing, growth, phytosanitary, physiological, and productivity indicators of soybean. The laboratory phase demonstrated that the combined seed treatment most markedly affected the initial seedling development: germination energy was 88.7%, laboratory germination was 94.3%, root length was 9.4 cm, and seedling infection by microflora decreased to 4.2%. The data indicated more uniform seedling formation, preservation of tissue integrity, and lower expression of microbial infection during the laboratory germination period. Under field conditions, the application of biological products was reflected in seedling emergence, plant stand density, development of the leaf apparatus, and formation of the root system. Under the Phytovit + Avercom combination, field germination reached 87.1%, leaf area was 151 cm^2/plant, and the number of nodules at the V5 stage was 20.6 per plant. This plant response demonstrated the successive transition of laboratory changes into field development, as the treated plants formed more uniform crop stands, accumulated above-ground mass more actively, and possessed a formed nodular apparatus at the early stage of the growing season. The phytosanitary condition of the crop also changed depending on the treatment scheme. Under the combined application of the products, the incidence of root rot decreased to 6.3%, the development of foliar diseases decreased to 5.1%, and the biological efficacy of protection was 59.6%. This demonstrated a reduction in disease expression, a lower level of plant pest damage, and better crop survival through to the flowering stage. The reduction in phytosanitary pressure created conditions for the further development of the leaf apparatus, root system, and reproductive portion of the plants. At the flowering stage, the combined treatment promoted the formation of a more developed assimilatory and symbiotic apparatus in the plants. The leaf area index was 4.0 m^2/m^2, the number of active nodules was 25.9 per plant, and their mass was 0.58 g per plant. Nodule formation and preservation of the leaf apparatus were associated with subsequent seed filling and accumulation of productive mass. Soybean productivity confirmed the succession of changes recorded at the preceding stages of the study. Under the combined application of Phytovit and Avercom, seed yield was 3.01 t/ha, the increase over the control was 24.4%, the weight of 1,000 seeds was 164.6 g, protein content was 39.1%, and oil content was 21.0%. The combination of the products thus ensured the coordinated formation of the sowing, phytosanitary, symbiotic, and productivity parameters of soybean, which manifested in better plant condition throughout the growing season and higher quality of the seed formed. A limitation of the study was that it did not encompass an economic evaluation of biological product application or their residual effects on soil microbiota in the following growing season. Further research should be conducted for other cultivars, regions, and growing years, and with consideration of economic efficiency.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Afzal, A., & Mukhtar, T. (2024). Revolutionizing nematode management to achieve global food security goals: An overview. *Heliyon*, 10(3), article number e25325. doi: 10.1016/j.heliyon.2024.e25325.

[2] Berdin, S.I., Murach, O.M., Kolisnyk, O.M., & Tryus, V.O. (2024). Soybean yield depending on pre-sowing seed treatment schemes with biological preparations of different action. *Agrarian Innovations*, 24, 24-30. doi: 10.32848/agrar.innov.2024.24.3.

[3] Bueno, A.D.F., Sutil, W.P., Jahnke, S.M., Carvalho, G.A., Cingolani, M.F., Colmenarez, Y.C., & Corniani, N. (2023). Biological control as part of the soybean integrated pest management (IPM): Potential and challenges. *Agronomy*, 13(10), article number 2532. doi: 10.3390/agronomy13102532.

[4] Compant, S., Cassan, F., Kostić, T., Johnson, L., Brader, G., Trognitz, F., & Sessitsch, A. (2025). Harnessing the plant microbiome for sustainable crop production. *Nature Reviews Microbiology*, 23, 9-23. doi: 10.1038/s41579-024-01079-1.

[5] Convention on Biological Diversity. (2016, May). Retrieved from https://www.cbd.int/convention/text.

[6] Dass, A., Singh, A., Nithinkumar, K., Rajanna, G.A., Verma, R.K., Rathore, S.S., & Meena, V.S. (2025). Synergies and trade-offs of integrating bio-formulations with mineral fertilizers in soybean under the climate crisis: Implications for productivity, profitability, and nutrient-use efficiency. *Journal of Agriculture and Food Research*, 24, article number 102474. doi: 10.1016/j.jafr.2025.102474.

[7] Dymytrov, S., & Sabluk, V. (2023). Effectiveness of seed treatment with biopreparations for increasing soybean (*Glycine max* (L.) Merr.) productivity. *International Science Journal of Engineering & Agriculture*, 2(3), 67-81. doi: 10.46299/j.isjea.20230203.07.

[8] Gonçalves e Silva, J.A., *et al*. (2024). Integration crop-livestock system increases the sustainability of soybean cultivation through improved soil health and plant physiology. *Agriculture, Ecosystems & Environment*, 359, article number 108770. doi: 10.1016/j.agee.2023.108770.

[9] ISTA. (n.d.). *International rules for seed testing*. Retrieved from https://www.seedtest.org/en/international-rules-for-seed-testing-_content---1--1083.html.

[10] Johnson, R., Joel, J.M., & Puthur, J.T. (2024). Biostimulants: The futuristic sustainable approach for alleviating crop productivity and abiotic stress tolerance. *Journal of Plant Growth Regulation*, 43(3), 659-674. doi: 10.1007/s00344-023-11144-3.

[11] Khan, A., Singh, A.V., Gautam, S.S., Agarwal, A., Punetha, A., Upadhayay, V.K., Kukreti, B., Bundela, V., Jugran, A.K., & Goel, R. (2023). Microbial bioformulation: A microbial assisted biostimulating fertilization technique for sustainable agriculture. *Frontiers in Plant Science*, 14, article number 1270039. doi: 10.3389/fpls.2023.1270039.

[12] Khan, M.T., Aleinikovienė, J., & Butkevičienė, L.-M. (2024). Innovative organic fertilizers and cover crops: Perspectives for sustainable agriculture in the era of climate change and organic agriculture. *Agronomy*, 14(12), article number 2871. doi: 10.3390/agronomy14122871.

[13] Khosravi, H., Khoshru, B., Nosratabad, A.F., & Mitra, D. (2024). Exploring the landscape of biofertilizers containing plant growth-promoting rhizobacteria in Iran: Progress and research prospects. *Current Research in Microbial Sciences*, 7, article number 100268. doi: 10.1016/j.crmicr.2024.100268.

[14] Kozhushko, K.S. (2025). Comparative analysis of the effect of biostimulants on soybean sowing qualities under laboratory conditions. *Agrarian Innovations*, 33, 147-151. doi: 10.32848/agrar.innov.2025.33.24.

[15] Lamichhane, J.R., Corrales, D.C., & Soltani, E. (2022). Biological seed treatments promote crop establishment and yield: A global meta-analysis. *Agronomy for Sustainable Development*, 42, article number 45. doi: 10.1007/s13593-022-00761-z.

[16] Majidian, P., Ghorbani, H.R., & Farajpour, M. (2024). Achieving agricultural sustainability through soybean production in Iran: Potential and challenges. *Heliyon*, 10(4), article number e26389. doi: 10.1016/j.heliyon.2024.e26389.

[17] Mirriam, A., Mugwe, J., Raza, M.A., Seleiman, M.F., Maitra, S., & Gitari, H.H. (2022). Aggrandizing soybean yield, phosphorus use efficiency and economic returns under phosphatic fertilizer application and inoculation with *Bradyrhizobium*. *Journal of Soil Science and Plant Nutrition*, 22, 5086-5098. doi: 10.1007/s42729-022-00985-8.

[18] Nagrale, D.T., Chaurasia, A., Kumar, S., Gawande, S.P., Hiremani, N.S., Shankar, R., Gokte-Narkhedkar, N., Renu, & Prasad, Y.G. (2023). PGPR: The treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. *World Journal of Microbiology and Biotechnology*, 39, article number 100. doi: 10.1007/s11274-023-03536-0.

[19] Neuhoff, D., Neumann, G., & Weinmann, M. (2024). Testing plant growth promoting microorganisms in the field: A proposal for standards. *Frontiers in Plant Science*, 14, article number 1324665. doi: 10.3389/fpls.2023.1324665.

[20] Panday, S.C., *et al*. (2024). Long-term fertilization strategies for improving productivity, profitability and water-use efficiency of soybean-wheat cropping systems. *The Journal of Agricultural Science*, 162(5), 444-457. doi: 10.1017/S0021859624000558.

[21] Paramesh, V., Kumar, R.M., Rajanna, G.A., Gowda, S., Nath, A.J., Madival, Y., Jinger, D., Bhat, S., & Toraskar, S. (2023). Integrated nutrient management for improving crop yields, soil properties, and reducing greenhouse gas emissions. *Frontiers in Sustainable Food Systems*, 7, article number 1173258. doi: 10.3389/fsufs.2023.1173258.

[22] Patyal, D., Sachdeva, K., Sharma, K., Renuka, Panigrahi, C.K., Tutlani, A., & Khan, R.R. (2025). An innovative and sustainable seed coating technology for improving seed quality and crop performance. *Journal of Scientific Research and Reports*, 31(5), 597-607. doi: 10.9734/jsrr/2025/v31i53056.

[23] Raza, A., Khare, T., Zhang, X., Rahman, M., Hussain, M., Gill, S.S., Chen, Z.-H., Zhou, M., Hu, Z., & Varshney, R.K. (2025). Novel strategies for designing climate-smart crops to ensure sustainable agriculture and future food security. *Journal of Sustainable Agriculture and Environment*, 4(2), article number e70048. doi: 10.1002/sae2.70048.

[24] Reed, L., & Glick, B.R. (2023). The recent use of plant-growth-promoting bacteria to promote the growth of agricultural food crops. *Agriculture*, 13(5), article number 1089. doi: 10.3390/agriculture13051089.

[25] Santos, F.M., Viera, L.S., Camargo, D.P., Muniz, M.F.B., Costa, I.F.D., Guedes, J.V.C., Santos, J.R.P., & Silva, J.C.P. (2022). Integrating a *Bacillus*-based product with fungicides by foliar application to protect soybean: A sustainable approach to avoid exclusive use of chemicals. *Pest Management Science*, 78(11), 4832-4840. doi: 10.1002/ps.7104.

[26] Sible, C., & Below, F. (2023). Role of biologicals in enhancing nutrient efficiency in corn and soybean. *Crops & Soils*, 56(2), 13-19. doi: 10.1002/crso.20263.

[27] Tyagi, A., *et al*. (2024). A review on biocontrol agents as sustainable approach for crop disease management: Applications, production, and future perspectives. *Horticulturae*, 10(8), article number 805. doi: 10.3390/horticulturae10080805.

[28] Villavicencio-Vásquez, M., Espinoza-Lozano, F., Espinoza-Lozano, L., & Coronel-León, J. (2025). Biological control agents: Mechanisms of action, selection, formulation and challenges in agriculture. *Frontiers in Agronomy*, 7, article number 1578915. doi: 10.3389/fagro.2025.1578915.

[29] Wan, N.-F., Dainese, M., Wang, Y.-Q., & Loreau, M. (2024). Cascading social-ecological benefits of biodiversity for agriculture. *Current Biology*, 34(12), 587-603.

[30] Wu, Z., Chen, X., Lu, X., Zhu, Y., Han, X., Yan, J., Yan, L., & Zou, W. (2025). Impact of combined organic amendments and chemical fertilizers on soil microbial limitations, soil quality, and soybean yield. *Plant and Soil*, 507, 317-334. doi: 10.1007/s11104-024-06733-4.

[31] Zhong, Y.T., & Zhong, S.L. (2024). Advances in agronomic practices for high-yield soybean cultivation. *Bioscience Methods*, 15(6), 337-347. doi: 10.5376/bm.2024.15.0033.

[32] Zhou, W., Arcot, Y., Medina, R.F., Bernal, J., Cisneros-Zevallos, L., & Akbulut, M.E.S. (2024). Integrated pest management: An update on the sustainability approach to crop protection. *ACS Omega*, 9(40), 41130-41147.

Ефективність комплексного застосування біопрепаратів при вирощуванні сої в лабораторних та польових умовах

Юлія Коломієць

Доктор сільськогосподарських наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-1919-6336

Наталія Сергійчук

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0001-6690-0114

Тетяна Рожкова

Кандидат біологічних наук, старший науковий співробітник
Інститут мікробіології та вірусології ім. Д. К. Заболотного НАН України
03143, вул. Академіка Заболотного, 154, м. Київ, Україна
https://orcid.org/0000-0002-0791-9736

Анотація. Метою дослідження було оцінити ефективність біопрепаратів через реакцію сої за посівними, ростовими, фітосанітарними, симбіотичними та продуктивними параметрами в лабораторних і польових умовах. У дослідженні використовували спектрофотометрію, метод К'єльдаля, екстракцію Сокслета та дисперсійний аналіз. У лабораторних умовах комплексна обробка насіння Фітовітом і Аверкомом забезпечувала найвищу енергію проростання – 88,7 %, що перевищувало контроль на 10,1 в. п. Лабораторна схожість за цієї схеми становила 94,3 % і була вищою за контроль на 7,9 в. п., тоді як ураженість проростків мікрофлорою знижувалася до 4,2 %, тобто була меншою на 7,6 в. п. У польових умовах застосування біопрепаратів позитивно позначалося на формуванні сходів, густоті рослин, розвитку листкового апарату та кореневої системи. За комплексного використання препаратів польова схожість становила 87,1 %, а кількість бульбочок у фазі V5 досягала 20,6 шт./рослину. Фітосанітарний стан посівів також змінювався в напрямі зменшення ураження хворобами й пошкодження шкідниками: поширення кореневих гнилей знижувалося до 6,3 %, а біологічна ефективність захисту становила 59,6 %. У фазі цвітіння комплексна обробка сприяла формуванню більш розвиненого асиміляційного та симбіотичного апарату рослин. Індекс листкової поверхні досягав 4,0 $м^2/м^2$, кількість активних бульбочок – 25,9 шт./рослину, а їхня маса – 0,58 г/рослину, що відображало активніше функціонування листкової поверхні та кореневої системи в період переходу рослин до генеративного розвитку. Збереження листкового апарату й формування бульбочок поєднувалися з подальшим наливом насіння та накопиченням продуктивної маси. Урожайність насіння за поєднання Фітовіту й Аверкому становила 3,01 т/га, приріст до контролю – 24,4 %, вміст білка – 39,1 %, а олії – 21,0 %. Практична цінність дослідження полягає в можливості використання його результатів агрономами, фермерами, насіннєвими господарствами та дослідниками для обґрунтування біологічної обробки сої з урахуванням проростання, фітосанітарного стану, бульбочкоутворення, урожайності та якості насіння

Ключові слова: фітосанітарний стан; густота рослин; коренева система; кількість бульбочок; генеративні органи

Received: 09.12.2025 Revised: 20.04.2026 Accepted: 21.05.2026 Published: 27.05.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 2, 2026, 125-139

UDC 632.4:633.34:631.461(477.41)

DOI: 10.31548/biologiya/2.2026.125

Species composition and structure of the phytoneamatode complex of soybean in the Right-Bank Forest-Steppe of Ukraine

Valerii Pashkovskyi

Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-1127-9361

Oleksandr Babych*

PhD in Biological Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-4988-5009

Abstract. The aim of the study was to determine the species composition as well as the taxonomic and ecological structure of the phytoneamatode complex associated with soybean under conditions of the Right-Bank Forest-Steppe of Ukraine. The study employed field route surveys, laboratory methods for nematode extraction from soil and root systems (Baermann funnel technique and sieving method), morphological and morphometric identification using light microscopy, as well as structural-ecological analysis of communities and methods of variation statistics. It was established that the nematode complex of the soybean rhizosphere comprises 26 species belonging to 17 genera, 10 families, 5 orders, and 2 classes, indicating a high taxonomic diversity of soil biota in the agrocenosis. The class Chromadorea dominated (over 80% of the total structure), determining the functional orientation of the community under intensive agricultural use. The order Tylenchida was the most abundant, forming the main share of the phytoparasitic block and including economically important species such as *Pratylenchus penetrans*, *Tylenchorhynchus dubius*, *Helicotylenchus dihystera*, and *Paratylenchus nanus*. Their dominance indicated the formation of a stable invasion potential in the soybean rhizosphere and the presence of critical phytosanitary pressure. At the same time, a significant proportion of saprobiotic and bacteriotrophic nematodes (Rhabditida and Cephalobidae) indicated intensive processes of organic matter mineraliiation and active functioning of the soil microbial component. The combination of a high proportion of phytoparasitic and decomposer forms creates a functionally heterogeneous community structure, reflecting simultaneous processes of ecological stress and biological compensation in the agroecosystem. Structural analysis revealed an intermediate level of ecological stability of the system, characteristic of intensively used agricultural lands with periodic disturbance of soil structure and simplification of trophic relationships. The results obtained can be used for bioindication assessment of soil condition and for the development of ecologically oriented monitoring systems and protection of soybean crops against phytonematodes

Keywords: species composition; taxonomic structure; agrocenoses; *Pratylenchus penetrans*; *Tylenchorhynchus dubius*; soil bioindication

Suggested Citation:
Pashkovskyi, V., & Babych, O. (2026). Species composition and structure of the phytoneamatode complex of soybean in the Right-Bank Forest-Steppe of Ukraine. *Biological Systems: Theory and Innovation*, 17(2), 125-139. doi: 10.31548/biologiya/2.2026.125.

*Corresponding author (babichoa@nubip.edu.ua)

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most strategically important leguminous crops in both global and regional agriculture, combining high food, feed, and agroecological value. Its importance is determined not only by its high protein and vegetable oil content, but also by its ability to fix atmospheric nitrogen symbiotically, which significantly affects soil nutrient balance and reduces the need for mineral fertilisers. However, under current trends of global warming, soil degradation, and intensification of agricultural production, achieving consistently high soybean yields is impossible without effective control of harmful organisms, among which phytoneamatodes are among the most problematic. Recent studies indicate that plant-parasitic nematodes cause significant yield losses in many agricultural crops. A. Pulavarty *et al*. (2021) noted that phytoneamatodes have global importance as biotic stressors capable of substantially reducing crop productivity across different climatic zones. At the same time, the authors emphasised that damage at the initial stages of field infestation may not be visually apparent, complicating their diagnosis and, consequently, phytosanitary control. F.A. Adamu *et al*. (2024) demonstrated that phytoneamatodes significantly affect the physiological condition of soybean plants by disrupting water balance, reducing nutrient uptake efficiency, and causing secondary infections due to root tissue damage. According to F.A. Adamu *et al*., even at relatively low population densities, nematodes can lead to a considerable reduction in plant biomass and productivity. Representatives of the genus *Pratylenchus* play a particularly important role in modern nematology. S.H. Abdel-Baset *et al*. (2025) reported that *Pratylenchus* spp. are among the most damaging nematodes of soybean, capable of penetrating root tissues and causing necrotic lesions, which disrupt water and nutrient transport. These nematodes form stable soil populations and can survive for extended periods even in the absence of typical host plants.

In addition, cyst-forming nematodes and other parasitic groups play an important role in shaping the phytosanitary status of agrocenoses, with their distribution largely depending on agronomic practices and land-use structure. In this context, the findings of A. Babych *et al*. (2024) are of particular importance, demonstrating that populations of cyst-forming nematodes in field agroecosystems are characterised by high stability and the ability to persist in anabiosis for many years in the absence of suitable host plants. The authors found that key factors regulating their abundance include scientifically grounded anti-nematode crop rotations and the level of saturation of agrocenoses with susceptible crops. Furthermore, it was emphasised that disruption of agronomic balance promotes the accumulation of invasion potential of cyst-forming nematodes, creating long-term risks for crop productivity. These findings complement current understanding of the spatiotemporal dynamics of plant-parasitic nematodes and confirm the need to consider their population resilience when developing integrated pest management systems. A separate line of research concerns the influence of agricultural practices on the structure of nematode communities. H.-W. Wang *et al*. (2022) found that intensive farming systems, particularly deep mechanical soil tillage and simplified crop rotations, lead to reduced trophic diversity of nematodes and an increased proportion of plant-parasitic forms. Such changes indicate disruption of trophic links and reduced ecological stability of agroecosystems. E.M. Furmanczyk *et al*. (2025) showed that nematode communities are sensitive indicators of land-use change. They demonstrated that monoculture systems, particularly long-term soybean cultivation without proper crop rotation, promote the accumulation of plant-parasitic nematodes and reduce the functional diversity of soil biota, negatively affecting agroecosystem stability.

The article by U. Shrestha *et al*. (2020) summarised modern approaches to the use of nematodes as bioindicators of soil health, emphasising that the structure of nematode communities is an integral indicator of soil ecological condition. U. Shrestha *et al*. argued that the ratio between trophic groups allows assessment of both anthropogenic pressure and the level of functional maturity of ecosystems. J. Kim *et al*. (2024) drawn attention to the genetic variability of plant-parasitic nematodes, which provides them with high adaptability to climatic and technological conditions. The authors outlined that this characteristic complicates the effective control of nematodes under field conditions and highlights the importance of taking these features into account when developing integrated plant protection systems. Recent syntheses by S. Lazarova *et al*. (2021) indicated that nematodes are both important regulators of soil processes and significant phytopathogens that can determine the economic efficiency of crop production. The authors emphasised the need for a comprehensive approach to evaluating their role in soil ecosystems.

Thus, the current scientific literature demonstrates the high ecological and economic significance of phytoneamatodes in soybean agrocenoses. They are a relevant component of soil systems, while simultaneously acting as a major limiting factor for crop productivity. Despite certain advances in understanding their biology and ecology, questions regarding species structure, dominance of individual taxa, and the functional organisation of nematode communities in soybean agrocenoses of the Right-Bank Forest-Steppe of Ukraine remain insufficiently studied, highlighting the relevance of this research. The aim of the study was to investigate the diversity, taxonomic composition, and ecological structure of the phytonematode complex associated with soybean in the Right-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The study was conducted during 2023-2025 under stationary field conditions at the Agronomic Research Station of the National University of Life and Environmental Sciences of Ukraine, located in the Right-Bank Forest-Steppe zone of Ukraine. The soils of the experimental plots were typical low-humus chernozems with a medium loamy texture, characterised by high natural fertility and favourable physicochemical properties for soybean cultivation. The object of the study was the regulatory mechanisms of phytoneamatodes in the soybean (*Glycine max* (L.) Merr.) rhizosphere. The subject of the study included species composition, taxonomic structure, and the dominance level of individual taxa within the agrocenosis. Soil and plant samples were collected throughout the soybean growing season (emergence, budding, and pod-filling stages) following the standard route survey methodology. Each composite sample consisted of 10-15 subsamples collected using the "envelope" method within an area of up to 1 hectare. The total weight of a composite sample ranged from 500 to 1,000 g. Root samples were collected simultaneously with soil samples and transported to the laboratory in sealed containers to prevent desiccation. Nematodes were extracted from soil using a modified Baermann funnel technique with an exposure time of 24-48 hours. To improve extraction efficiency, a sieving method was also applied using a set of sieves with mesh sizes of 1.0, 0.5 and 0.25 mm. Nematodes from plant material were extracted by macerating root tissues followed by incubation in water using Baermann funnels (Hooper *et al*., 2005). For fixation, nematodes were preserved in a 4% formalin solution or TAF (triethanolamine-formalin). Permanent slides were prepared using the J.W. Seinhorst (1959) method (standard glycerine dehydration method).

Nematode identification was carried out based on morphological and morphometric characteristics using a light microscope at magnifications ranging from ×100 to ×1,000. Species identification was carried out using taxonomic keys and identification manuals (Mai, 2018), while modern systematic criteria described by T.J. Jones *et al*. (2013) were applied to ensure taxonomic consistency. For each sample, species composition, abundance, and taxonomic affiliation were determined. The taxonomic structure of nematode communities was analysed at the levels of class, order, family, genus, and species. Community structure was assessed using indices of species diversity and relative abundance (%). Species dominance was determined according to the scale commonly used in soil zoology: dominant species (>10%), subdominant species (1-10%), and rare species (<1%). The ecological structure of the nematode complex was evaluated based on trophic classification by G.W. Yeates *et al*. (1993), categorising species into phytoparasitic, bacterivorous, and fungivorous groups. This approach allowed determination of the functional role of the community within the agrocenosis. All experimental procedures, including plant sampling, were conducted in accordance with the principles of the Convention on Biological Diversity (2010). The mathematical processing of the obtained results was performed using methods of variation statistics. Mean values, standard deviations, and relative indicators were calculated. To assess the significance of differences between samples, both parametric and non-parametric tests were applied, including the Student's t-test for independent samples and the χ^2 test for the analysis of frequency distributions, depending on the nature of data distribution. The level of statistical significance was set at $p < 0.05$, with the corresponding p-values calculated for each test. Graphical models of the research results were developed using Microsoft Excel.

RESULTS AND DISCUSSION

The taxonomic analysis of the nematode complex in soybean agrocenoses indicates its considerable diversity and hierarchical organisation (Fig. 1).

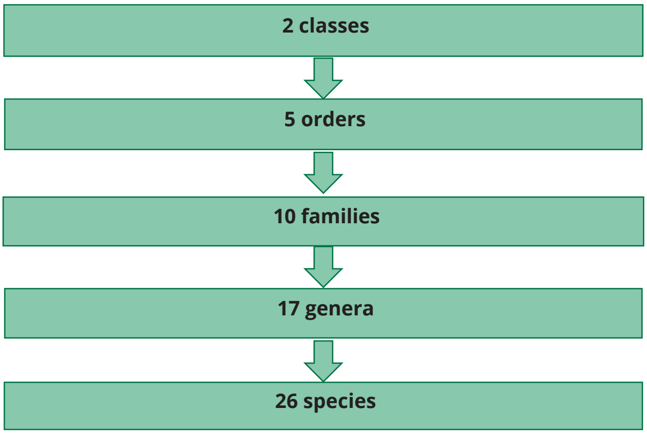

Figure 1. Taxonomic structure of the phytoneamatode complex in soybean agrocenoses
Source: developed by the authors

The highest number of nematode species belongs to the class Chromadorea, whereas the class Enoplea is represented by a smaller number of taxa. The dominance of particular families and genera of plant-parasitic and saprobiotic nematodes reflects the ecological heterogeneity of the soybean agrocenosis. The established taxonomic structure characterises the soybean agrocenosis as a relatively stable ecosystem with a high level of functional differentiation of nematode fauna. A total of 26 species were identified within the community, belonging to 17 genera, 10 families, 5 superfamilies, and 5 orders, grouped into 2 classes – Enoplea and Chromadorea (Table 1).

Table 1. Systematic position of nematode species in the soybean rhizosphere

Class	Order	Superfamily	Family	Subfamily	Genus	Species
Enoplea	Araeolaimida de Coninck et Sch. Stekhoven, 1933	–	Plectidae Oerley, 1880	–	*Plectus*	*Plectus elongatus* Bastian, 1865
Enoplea	Enoplida Chitwood, 1933	–	Alaimidae Micoletzky, 1922	–	*Alaimus*	*Alaimus primitivus* de Man, 1880
Enoplea	Dorylaimida Pearse, 1936	Dorylaimoidea de Man, 1876; Thorne, 1934	Dorylaimidae de Man, 1876	Mesodorylaiminae Andrassy, 1959	*Mesodorylaimus*	*Mesodorylaimus bastiani* (Butschli, 1873) Andrassy, 1959
Chromadorea	Rhabditida Chitwood, 1933	Rhabditoidea Oerley, 1880; Travassos, 1920	Rhabditidae Oerley, 1880	Rhabditinae Micoletzky, 1922	*Pelodera*	*Pelodera teres* Schneider, 1866

Table 1. Continued

Class	Order	Superfamily	Family	Subfamily	Genus	Species
Chromadorea	Rhabditida Chitwood, 1933	Rhabditoidea Oerley, 1880; Travassos, 1920	Rhabditidae Oerley, 1880	Rhabditinae Micoletzky, 1922	*Mesorhabditis*	*Mesorhabditis monohystera* (Butschli, 1873) Dougherty, 1955
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Cephalobinae Filipjev, 1934	*Cephalobus*	*Cephalobus persegnis* Bastian, 1865
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Cephalobinae Filipjev, 1934	*Eucephalobus*	*Eucephalobus oxiuroides* (de Man, 1876) Steiner, 1936
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Cephalobinae Filipjev, 1934	*Eucephalobus*	*Eucephalobus mucronatus* (Koslowska, Rogińska-Wasilewska, 1963) Andrassy, 1967
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Acrobelinae Thorne, 1937	*Acrobeloides*	*Acrobeloides butschlii* (de Man, 1884) Steiner & Buhrer, 1933
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Acrobelinae Thorne, 1937	*Acrobeles*	*Acrobeles ciliatus* von Linstow, 1877
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Acrobelinae Thorne, 1937	*Chiloplacus*	*Chiloplacus symmetricus* (Thorne, 1925) Thorne, 1937
Chromadorea	Rhabditida Chitwood, 1933	Cephaloboidea Paramonov, 1952	Cephalobidae (Filipjev, 1934) Chitwood et Chitwood, 1934	Acrobelinae Thorne, 1937	*Cervidellus*	*Cervidellus devimucronatus* Sumencova, 1964
Chromadorea	Rhabditida Chitwood, 1933	–	Panagrolaimidae Thorne, 1937; Paramonov, 1956	Panagrolaiminae Thorne, 1937	*Panagrolaimus*	*Panagrolaimus rigidus* (Schneider, 1866) Thorne, 1937
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Aphelenchoidea Fuchs, 1970; Thorne, 1949	Aphelenchidae Bastian, 1865	–	*Aphelenchus*	*Aphelenchus avenae* Bastian, 1865
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Aphelenchoidea Fuchs, 1970; Thorne, 1949	Aphelenchoididae Skarbilovich, 1947; Paramonov, 1953	Aphelenchoidinae Skarbilovich, 1947	*Aphelenchoides*	*Aphelenchoides asterocaudatus* Das, 1960

Table 1. Continued

Class	Order	Superfamily	Family	Subfamily	Genus	Species
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Aphelenchoidea Fuchs, 1970; Thorne, 1949	Aphelenchoididae Skarbilovich, 1947; Paramonov, 1953	Aphelenchoidinae Skarbilovich, 1947	*Aphelenchoides*	*Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev & Sch. Stekhoven, 1941
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Tylenchoidea Filipjev, 1934; Chitwood & Chitwood, 1947	Tylenchidae Oerley, 1880	Tylenchinae Filipjev, 1934	*Aglenchus*	*Aglenchus agricola* (de Man, 1884) Andrassy, 1954
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Tylenchoidea Filipjev, 1934; Chitwood & Chitwood, 1947	Tylenchidae Oerley, 1880	Tylenchinae Filipjev, 1934	*Aglenchus*	*Aglenchus costatus* de Man, 1921
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Filipjev, 1934; Paramonov, 1967	Tylenchorhynchidae (Fliava, 1964) Golden, 1971	Tylenchorhynchinae Fliava, 1964	*Tylenchorhynchus*	*Tylenchorhynchus dubius* (Butschli, 1873) Filipjev, 1936
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Filipjev, 1934; Paramonov, 1967	Pratylenchidae Thorne, 1949; Siddigi, 1963	Pratylenchinae Thorne, 1949	*Pratylenchus*	*Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Filipjev, 1934; Paramonov, 1967	Pratylenchidae Thorne, 1949; Siddigi, 1963	Pratylenchinae Thorne, 1949	*Pratylenchus*	*Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Filipjev, 1941; Wieser, 1953	Hoplolaimidae (Filipjev, 1941) Wieser, 1953	Rotylenchoidinae Whitehead, 1958	*Helicotylenchus*	*Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Thorne, 1949	Paratylenchidae Thorne, 1949; Raski, 1962	–	*Paratylenchus*	*Paratylenchus nanus* (Cobb, 1923) Brzeski, 1936
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Jairajpuri, 1965	Qudsianematidae Jairajpuri, 1965; Siddigi, 1969	Qudsianematinae Jairajpuri, 1965	*Eudorilaimus*	*Eudorilaimus obtusicaundatus* (Bastian, 1865) Andrassy, 1959
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Jairajpuri, 1965	Qudsianematidae Jairajpuri, 1965; Siddigi, 1969	Qudsianematinae Jairajpuri, 1965	*Eudorilaimus*	*Eudorilaimus monohystera* (Bastian, 1865) Andrassy, 1959

Table 1. Continued

Class	Order	Superfamily	Family	Subfamily	Genus	Species
Chromadorea	Tylenchida Filipjev, 1934; Thorne, 1949	Hoplolaimoidea Jairajpuri, 1965	Qudsianematidae Jairajpuri, 1965; Siddigi, 1969	Qudsianematinae Jairajpuri, 1965	*Eudorilaimus*	*Eudorilaimus projectus* (de Man, 1880) Andrassy, 1959

Note: domain: *Eukaryota*; Kingdom: *Animalia*; Subkingdom: *Eumetazoa*; Superphylum: *Bilateria*; Clade: *Protostomia*; Phylum: *Nematoda*
Source: developed by the authors

The greatest species diversity was characteristic of the order Tylenchida, which includes both plant-parasitic nematodes (in particular representatives of the genera *Pratylenchus*, *Tylenchorhynchus*, and *Helicotylenchus*) and facultatively saprobiontic forms that play an important role in soil processes. Alongside them, bacteriophagous and saprobiontic nematodes were also represented (including representatives of Rhabditida and Cephalobidae), as well as more primitive dorylaimid species of the class Enoplea, indicating the coexistence of different ecological groups ranging from soil decomposers to specialised plant-parasitic species. In general, the community structure indicates a complex functional organisation of the nematode complex in the soybean agrocenosis and its dependence on trophic conditions. In the taxonomic structure, the class Chromadorea was dominant, comprising 22 species (84.6%). Representatives of this class form the basis of soil nematode communities in agrocenoses and include both saprobiontic and plant-parasitic forms. The class Enoplea was represented by only 4 species (15.4%), indicating its subordinate role in the formation of the studied complex (Fig. 2).

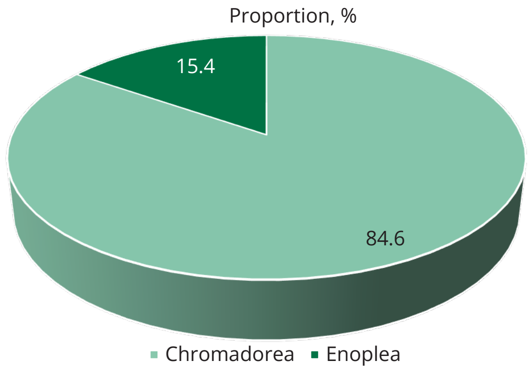

Figure 2. Structure of the nematode complex (by classes)
Source: developed by the authors

The class Enoplea is represented by only 4 species (15.4%), indicating its subordinate role in the formation of the studied complex. The most abundant order is Tylenchida, which includes 14 species (53.8%) and plays a key role in shaping the phytoparasitic component of the nematode community. It includes economically important species such as *Tylenchorhynchus dubius*, *Pratylenchus penetrans*, *Pratylenchus pratensis*, *Helicotylenchus dihystera*, and *Paratylenchus nanus*. A subdominant position is occupied by the order Rhabditida with 10 species (38.5%), represented mainly by bacteriotrophic and saprobiontic forms (*Cephalobus*, *Acrobeloides*, *Panagrolaimus*, etc.), which play an important role in the mineralisation of organic matter (Fig. 3).

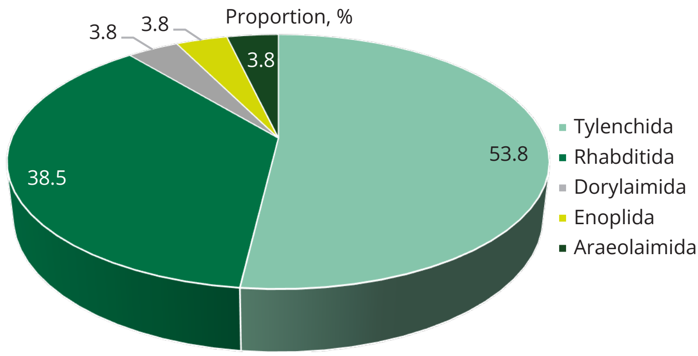

Figure 3. Structure of the nematode complex (by orders)

Note: other orders are represented insignificantly: Dorylaimida – 1 species; Enoplida – 1 species; Araeolaimida – 1 species

Source: developed by the authors

Thus, the structure is characterised by a pronounced polydominant-monodominant pattern with the absolute predominance of Tylenchida. Such a structure of the nematode community can be explained by the agrotechnical conditions associated with intensive agricultural use of the soil, particularly the cultivation of crops in monoculture or short-rotation crop systems, which promote the accumulation of plant-parasitic species of the order Tylenchida. An additional factor is the systematic application of organic fertilisers, which supports high populations of bacteriotrophic forms (Rhabditida) but does not limit the development of plant-parasitic nematodes. The highest species richness is observed in the families *Cephalobidae*, *Rhabditidae*, *Aphelenchoididae*, *Tylenchidae*, and *Pratylenchidae*. A considerable proportion is also represented by *Qudsianematidae* and *Panagrolaimidae*. This indicates the coexistence of both saprobiontic and phytoparasitic elements within the structure of the complex (Fig. 4).

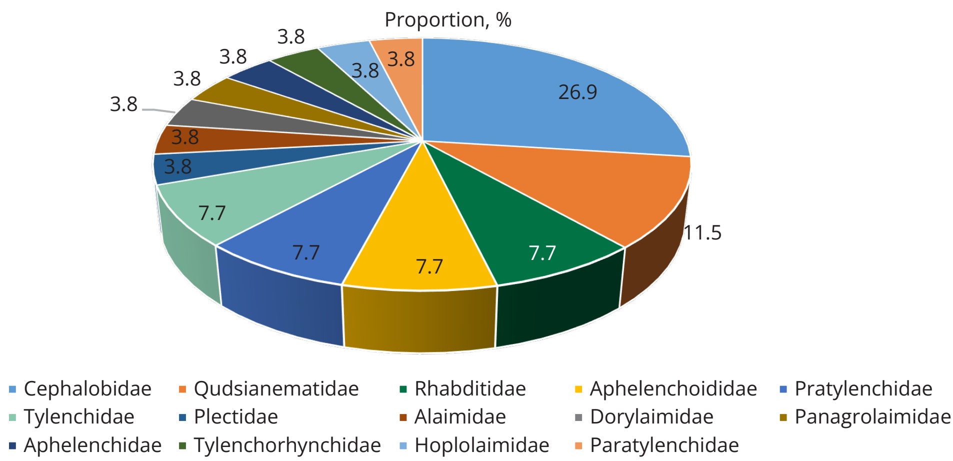

Figure 4. Structure of the nematode complex (by families)

Source: developed by the authors

The generic structure of the nematode complex is characterised by a high level of diversity (20 genera) and the absence of clearly expressed dominant genera. The leading position is occupied by the genus Eudorilaimus (11.5%) and a group of subdominants (*Eucephalobus*, *Pratylenchus*, *Aglenchus*, *Aphelenchoides* – 7.7% each). The lowest species richness is observed in genera represented by a single species (3.8% each), which belong to the group of decomposers, including: *Plectus*, *Alaimus*, *Mesodorylaimus*, *Pelodera*, *Mesorhabditis*, *Cephalobus*, *Acrobeloides*, *Acrobeles*, *Chiloplacus*, *Cervidellus*, *Panagrolaimus*, *Aphelenchus*, *Tylenchorhynchus*, *Helicotylenchus*, and *Paratylenchus*. Their combined share constitutes 57.7% of the total number of genera, indicating high taxonomic diversity and a relatively even structure of the nematode community (Fig. 5).

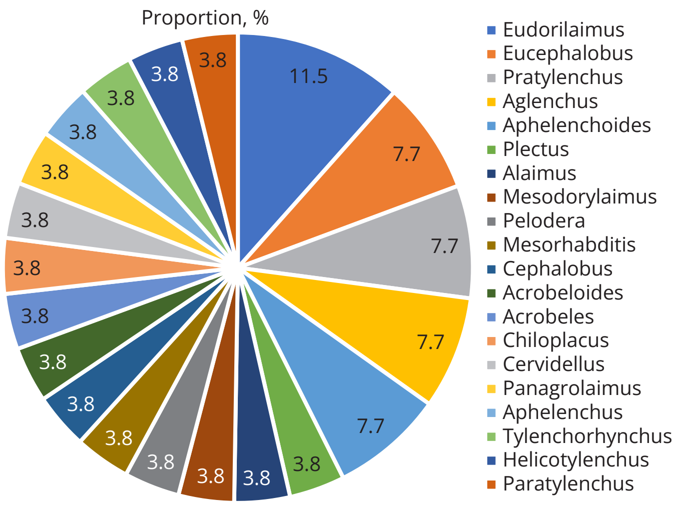

Figure 5. Structure of the nematode complex (by genera)

Source: developed by the authors

The diagram indicates a relatively uniform generic structure of the nematode complex without pronounced dominance of individual taxa. The genus *Eudorylaimus* constituted the largest proportion (11.5%), whereas the genera *Eucephalobus*, *Pratylenchus*, *Aglenchus*, and *Aphelenchoides* each accounted for 7.7%. The remaining genera were represented by lower proportions (3.8%), indicating a moderately balanced taxonomic diversity. Based on the conducted analysis, phytoneamatodes in soybean agrocenoses were categorised into three dominance groups. Based on the conducted analysis, phytoneamatodes in soybean agrocenoses were categorised into three dominance groups. The dominant species, which are the most abundant and ecologically influential in the agrocenosis, include *Pratylenchus penetrans* (Pratylenchidae, Tylenchida/Chromadorea) and *Tylenchorhynchus dubius* (Tylenchorhynchidae, Tylenchida/Chromadorea). The subdominant species, although less abundant, are locally capable of affecting plant health, and these include *Pratylenchus pratensis*, *Helicotylenchus dihystera*, *Paratylenchus nanus*, *Aphelenchoides bicaudatus*, and *Aphelenchus avenae*. The rare species, which are mostly saprophytic or poorly studied, include *Eudorylaimus*, *Aglenchus*, *Plectus*, *Alaimus*, *Mesodorylaimus*, *Pelodera*, *Mesorhabditis*, *Cephalobus*, *Eucephalobus*, *Acrobeloides*, *Acrobeles*, *Chiloplacus*, *Cervidellus*, and *Panagrolaimus* (Table 2).

Table 2. Dominance of phytoneamatodes

Status	Species	Genus	Order/Class
Dominant	*Pratylenchus penetrans*	Pratylenchus	Tylenchida/Chromadorea
Dominant	*Tylenchorhynchus dubius*	Tylenchorhynchus	Tylenchida/Chromadorea
Subdominant	*Pratylenchus pratensis*	Pratylenchus	Tylenchida/Chromadorea
Subdominant	*Helicotylenchus dihystera*	Helicotylenchus	Tylenchida/Chromadorea

Table 2. Continued

Status	Species	Genus	Order/Class
Subdominant	*Paratylenchus nanus*	Paratylenchus	Tylenchida/Chromadorea
Subdominant	*Aphelenchoides bicaudatus*	Aphelenchoides	Tylenchida/Chromadorea
Subdominant	*Aphelenchus avenae*	Aphelenchus	Tylenchida/Chromadorea
Rare	*Eudorilaimus obtusicaundatus*	Eudorilaimus	Tylenchida/Chromadorea
Rare	*Eudorilaimus monohystera*	Eudorilaimus	Tylenchida/Chromadorea
Rare	*Eudorilaimus projectus*	Eudorilaimus	Tylenchida/Chromadorea
Rare	*Aglenchus agricola*	Aglenchus	Tylenchida/Chromadorea
Rare	*Aglenchus costatus*	Aglenchus	Tylenchida/Chromadorea
Rare	*Plectus elongatus*	Plectus	Araeolaimida/Enoplea
Rare	*Alaimus primitivus*	Alaimus	Enoplida/Enoplea
Rare	*Mesodorylaimus bastiani*	Mesodorylaimus	Dorylaimida/Enoplea
Rare	*Pelodera teres*	Pelodera	Rhabditida/Chromadorea
Rare	*Mesorhabditis monohystera*	Mesorhabditis	Rhabditida/Chromadorea
Rare	*Cephalobus persegnis*	Cephalobus	Rhabditida/Chromadorea
Rare	*Eucephalobus oxiuroides*	Eucephalobus	Rhabditida/Chromadorea
Rare	*Eucephalobus mucronatus*	Eucephalobus	Rhabditida/Chromadorea
Rare	*Acrobeloides butschlii*	Acrobeloides	Rhabditida/Chromadorea
Rare	*Acrobeles ciliatus*	Acrobeles	Rhabditida/Chromadorea
Rare	*Chiloplacus symmetricus*	Chiloplacus	Rhabditida/Chromadorea
Rare	*Cervidellus devimucronatus*	Cervidellus	Rhabditida/Chromadorea
Rare	*Panagrolaimus rigidus*	Panagrolaimus	Rhabditida/Chromadorea

Source: developed by the authors

Table 2 reflects a clearly expressed structural differentiation of the phytonematode complex in soybean agrocenoses along the dominance gradient. The dominant block is represented by two species of the genera *Pratylenchus* and *Tylenchorhynchus*, belonging to the order Tylenchida, and forms the main plant-parasitic core of the community, determining the potential level of damage to the soybean root system. The subdominant group is more diverse and includes both migratory endoparasites (*Pratylenchus pratensis*) and epiphytic as well as semi-endoparasitic forms (*Helicotylenchus, Paratylenchus*), together with fungimycotrophic and mixotrophic species (*Aphelenchoides*, *Aphelenchus*), indicating an increased complexity of the trophic structure of the nematode complex. Rare species are represented mainly by saprobiontic and soil-dwelling forms of different ecological groups (bacteriotrophs, fungivores, and primitive dorylaimids), which characterise the general condition of the soil biocenosis and the level of its biodiversity. Thus, the community structure shows a pronounced shift toward a plant-parasitic assemblage of the order Tylenchida while maintaining a significant saprotrophic component, which overall reflects the agrocenotic nature of the environment and its anthropogenic transformation. Overall, the taxonomic structure of the nematode complex is characterised by a high level of species and generic diversity, the dominance of representatives of the class Chromadorea, the predominance of the order Tylenchida, which forms the basis of the phytoparasitic complex, and a significant proportion of saprobiontic forms (Rhabditida) that ensure the functioning of the soil ecosystem. The ratio of taxa from different trophic groups indicates ecological balance and functional completeness of the nematode community (Table 3).

Table 3. Dominance by families and orders

Order/Class	Family	Number of species	Status
Tylenchida/Chromadorea	Pratylenchidae	2	Dominant/Subdominant
Tylenchida/Chromadorea	Tylenchorhynchidae	1	Dominant
Tylenchida/Chromadorea	Hoplolaimidae	1	Subdominant

Table 3. Continued

Order/Class	Family	Number of species	Status
Tylenchida/Chromadorea	Paratylenchidae	1	Subdominant
Tylenchida/Chromadorea	Qudsianematidae	3	Rare
Tylenchida/Chromadorea	Tylenchidae	2	Rare
Tylenchida/Chromadorea	Aphelenchoididae	2	Subdominant
Tylenchida/Chromadorea	Aphelenchidae	1	Subdominant
Rhabditida/Chromadorea	Rhabditidae	2	Rare
Rhabditida/Chromadorea	Cephalobidae	7	Rare
Rhabditida/Chromadorea	Panagrolaimidae	1	Rare
Araeolaimida/Enoplea	Plectidae	1	Rare
Enoplida/Enoplea	Alaimidae	1	Rare
Dorylaimida/Enoplea	Dorylaimidae	1	Rare

Source: developed by the authors

The results presented in the Table 3 demonstrate not only the taxonomic structure of the nematode complex but also its functional organisation within the soybean agrocenosis. The predominance of the order *Tylenchida* (*Chromadorea*) indicates the dominance of plant-parasitic forms that directly affect the phytosanitary condition of the crop. In particular, migratory endoparasites of the family *Pratylenchidae* are among the factors negatively influencing soybean productivity through damage to the root system, disruption of water and nutrient balance in plants, and the creation of secondary infection sites for pathogens. Species of the family *Tylenchorhynchidae* form the ectoparasitic component that suppresses root growth and reduces the overall vitality of plants, whereas representatives of the families *Hoplolaimidae* and *Paratylenchidae* are of considerable phytosanitary importance, especially in short-rotation crop systems with a high proportion of related crops. Nematodes of the families *Aphelenchoididae* and *Aphelenchidae*, although mainly belonging to fungimycotrophic forms, may act as indirect regulators of the soil mycobiota, influencing the balance between beneficial and pathogenic fungi, which is also reflected in the condition of the rhizosphere. At the same time, the high representation of the families *Rhabditidae*, *Cephalobidae*, and *Panagrolaimidae* within *Rhabditida* indicates intensive processes of organic matter decomposition and mineralisation, which constitutes a positive factor for the functioning of the soil ecosystem, since these groups participate in the transformation of organic matter and nutrient cycling. Representatives of *Enoplea* (*Plectidae*, *Alaimidae*, *Dorylaimidae*), despite their relatively low abundance, perform an important indicator function by reflecting the level of stability of the soil environment. Their presence indicates a certain degree of ecological diversity and the partial preservation of natural mechanisms regulating the community. Thus, the structure presented in the table is characterised by a combination of a pronounced plant-parasitic complex (*Tylenchida*) and a well-developed saprobiontic block (*Rhabditida*), which together determine both the negative impact on soybean productivity through parasitic pressure and the maintenance of fundamental soil biocenosis processes through active mineralisation of organic matter.

The conducted study demonstrated that the nematode complex of soybean agrocenoses under the conditions of the Right-Bank Forest-Steppe of Ukraine is characterised by high taxonomic richness and clear functional differentiation, which is typical of intensively exploited agricultural landscapes of the temperate zone. Similar patterns of structural organisation of soil communities under different land-use systems were reported by E.M. Furmanczyk *et al*. (2025), who showed that the type of agroecosystem is a key factor determining both species diversity and the dominance of particular trophic groups of nematodes. The authors emphasised that intensive farming systems contribute to a reduction in community complexity and an increase in the role of plant-parasitic forms. The general concept of using nematodes as indicators of soil condition was comprehensively discussed by J.L. Garland *et al*. (2010), who demonstrated that the structure of nematode communities reflects the level of ecological maturity of the soil system. The author emphasised that the ratio of trophic groups allows assessment of the degree of ecosystem disturbance and the intensity of anthropogenic pressure. In the present study, the dominance of plant-parasitic forms combined with a high proportion of decomposers indicates an intermediate and unstable state of the agroecosystem. The high pathogenicity of certain groups was confirmed by S.R. Koenning & J.A. Wrather (2010), who established significant soybean yield losses caused by nematode invasions. Similar findings were reported by T.W. Allen *et al*. (2017), who emphasised that nematodes are among the dominant negative factors affecting global soybean production. The significant influence of agrotechnical measures, particularly the optimal saturation of crop rotations with host plants as the main limiting factor preventing the mass reproduction of cyst-forming nematodes, was demonstrated by A. Babych *et al*. (2024). In contrast, disruption of scientifically based crop rotation structures leads to the accumulation of nematodes in the soil and the formation of long-term invasive pressure.

Additionally, G.L. Tylka & C.C. Marett (2014) demonstrated the spatial heterogeneity of cyst-forming nematode distribution in crop rotations, confirming the influence of local agroecological conditions on the formation of community structure. From the perspective of functional ecology, T. Bongers & H. Ferris (1999) demonstrated that nematodes are key regulators in nutrient cycling. They ensure the transformation of organic matter and the transfer of energy between specific trophic levels. In this analysis, the high proportion of Rhabditida indicates active mineralisation processes and an intensive detrital flow. H. Ferris (2010) further developed the concept of "metabolic footprints", which allows assessment of the contribution of nematodes to ecosystem functioning. In the studied agrocenosis, the ratio of trophic groups indicates an intermediate level of ecological stability characteristic of systems subjected to regular anthropogenic disturbance. The structural organisation of nematode food chains was also described by G.W. Yeates *et al*. (1993), who proposed a trophic classification as the basis for ecological analysis of soil communities. The results obtained herein confirm the mixed nature of the community, with dominance of plant-parasitic and bacteriotrophic forms. Modern studies on the ecological condition of soil ecosystems demonstrate the substantial role of anthropogenic factors in shaping the structure of soil biota. In particular, the study by N. Kosovska *et al*. (2022) showed that the application of nanopreparations and biologically active substances significantly affected the composition and functioning of the soil microbiome, leading to restructuring of microbial communities and changes in the intensity of biogeochemical processes in soil. The authors emphasised that even relatively moderate agrochemical pressure may cause shifts in the structure of soil biocenoses, which is reflected in the functional organisation of the ecosystem. In turn, contemporary studies on the phytosanitary pressure within agroecosystems indicate that chemical plant protection products may exert indirect effects on soil biota. In the work of T. Stefanovska *et al*. (2025), it was established that neonicotinoids and butenolides affect not only target phytophagous organisms but also associated components of soil mesofauna, including nematodes. The authors noted that even at recommended application rates, these compounds are capable of modifying the structure of soil communities, indicating their indirect influence on trophic interactions within agroecosystems. The present findings regarding the dominance of plant-parasitic nematodes (Tylenchida) and the significant proportion of saprobiontic forms (Rhabditida) are consistent with these observations, since changes in the soil microbiocenosis, the intensity of mineralisation processes, and agrochemical pressure may collectively influence the structure of nematode communities. Thus, anthropogenic impact, including the use of modern agrochemical and biotechnological plant protection products, acts as an important factor shaping the functional organisation of soil biocenoses. Therefore, the results of the study confirm that the structure of the soybean nematode complex is the result of complex interactions among agrotechnical factors, edaphic conditions, and the biological characteristics of individual taxa. It simultaneously reflects processes of ecological transformation and partial compensation of the functional stability of the soil system.

CONCLUSIONS

The conducted research made it possible to systematically characterise the species composition, taxonomic structure, and ecological organisation of the phytoneamatode complex in soybean agrocenoses under conditions of the Right-Bank Forest-Steppe of Ukraine. It was established that the nematode community is characterised by a high level of taxonomic diversity and comprises 26 species belonging to 17 genera, 10 families, 5 orders, and 2 classes (Enoplea and Chromadorea), indicating a complex multilevel organisation of soil biota in soybean agroecosystems. The dominant taxonomic group is the class Chromadorea, accounting for more than 80% of the total community structure. The predominance of this class is determined by the high representation of the order Tylenchida, which forms the main phytoparasitic component. The most ecologically and agronomically significant species are *Pratylenchus penetrans* and *Tylenchorhynchus dubius*, which act as dominants and form a stable invasion background in the soybean rhizosphere. Their presence confirms a high phytopathogenic potential of the agrocenosis and an increased risk of reduced crop productivity. The subdominant level of the structure is represented by *Pratylenchus pratensis*, *Helicotylenchus dihystera, Paratylenchus nanus*, as well as certain aphelenchoid forms, which further complicate the trophic structure of the community and contribute to a multi-vector parasitic load on plant root systems. A significant proportion of saprobiontic and bacterivorous nematodes (Rhabditida) was recorded, indicating intensive organic matter decomposition processes and active functioning of the soil microbial component. This combination of phytoparasitic and decomposer elements reflects the simultaneous action of two contrasting processes: phytosanitary stress and biological compensation within the soil system. Ecological analysis revealed that the nematode community is polydominant with a clearly expressed balance between dominant, subdominant, and rare species. This indicates an intermediate level of ecological stability of the agroecosystem, typical of intensively used agricultural lands. Thus, the phytoneamatode complex of soybean in the Right-Bank Forest-Steppe of Ukraine is a sensitive indicator of agroecological soil conditions, reflecting both the level of anthropogenic pressure and the functional activity of soil biota. The obtained results confirm the feasibility of using nematodes as bioindicators of agroecosystem health and may be applied in the development of environmentally oriented monitoring systems and protection strategies for soybean crops against soil-borne phytopathogens. Future research perspectives include a more detailed analysis of the spatiotemporal dynamics of dominant phytoneamatodes, assessment of their interactions with rhizosphere microbial communities, and development of integrated pest management strategies aimed at regulating their populations in soybean agrocenoses.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Abdel-Baset, S.H., Abo-Rehab, M.E.A., & Mohamed, S.M.A. (2025). Pathogenicity and management of root-knot nematode, *Meloidogyne javanica* infecting soybean plants at different inoculum levels. *Pakistan Journal of Nematology*, 43(1), 23-31. doi: 10.17582/journal.pjn/2025/43.1.23.31.

[2] Adamu, F.A., Hundessa, W.B., Yimer, S.G., Aseffa, A.W., & Meressa, B.H. (2024). Plant parasitic nematodes on soybean (*Glycine max* (L.) Merr.) in the Metekel zone (Ethiopia) and varietal resistance evaluation to *Meloidogyne javanica*. *Journal of Phytopathology*, 172(2), article number e13287. doi: 10.1111/jph.13287.

[3] Allen, T.W., *et al*. (2017). Soybean yield loss estimates due to diseases. *Plant Health Progress*, 18(1), 19-27. doi: 10.1094/PHP-RS-16-0066.

[4] Babych, A., Babych, O., Havryliuk, O., Statkevych, O., Dziuman, Y., Litvinov, D., Zavgorodniy, V., Kaduk, V., & Prichodko, D. (2024). Managing populations of cyst-forming nematodes in agroecosystems of field crops. *Biosystems Diversity*, 32(2), 203-209. doi: 10.15421/012421.

[5] Bongers, T., & Ferris, H. (1999). Nematode bioindicators. *Trends in Ecology & Evolution*, 14, 224-228. doi: 10.1016/S0169-5347(98)01583-3.

[6] Convention on Biological Diversity. (2010, October). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[7] Ferris, H. (2010). Nematode metabolic footprints. *European Journal of Soil Biology*, 46, 97-104. doi: 10.1016/j.ejsobi.2010.01.003.

[8] Furmanczyk, E.M., *et al.* (2025). An analysis of soil nematode communities across diverse horticultural cropping systems. *Soil Systems*, 9(3), article number 77. doi: 10.3390/soilsystems9030077.

[9] Garland, J.L., Mackowiak, C.L., & Zabaloy, M.C. (2010). Organic waste amendment effects on soil microbial activity in a corn-rye rotation: Application of a new approach to community-level physiological profiling. *Applied Soil Ecology*, 44(3), 262-269. doi: 10.1016/j.apsoil.2010.01.003.

[10] Hooper, D.J., Hallmann, J., & Subbotin, S.A. (2005). Methods for extraction, processing and detection of plant and soil nematodes. In M. Luc, R.A. Sikora & J. Bridge (Eds.), *Plant parasitic nematodes in subtropical and tropical agriculture* (pp. 53-86). Wallingford: CABI Publishing. doi: 10.1079/9780851997278.0053.

[11] Jones, J.T., *et al*. (2013). Top 10 plant-parasitic nematodes. *Molecular Plant Pathology*, 14, 946-961. doi: 10.1111/mpp.12057.

[12] Kim, J., Sung, Y.W., Yang, J.-W., Nam, K.J., Lee, K.-L., Shim, D., & Kim, Y.-H. (2024). Genetic variations underlying root-knot nematode resistance in sweetpotato. *Gene*, 931, article number 148895. doi: 10.1016/j.gene.2024.148895.

[13] Koenning, S.R., & Wrather, J.A. (2010) Suppression of soybean yield potential in the continental united states by plant diseases from 2006 to 2009. *Plant Health Progress*, 11, 5-11. doi: 10.1094/PHP-2010-1122-01-RS.

[14] Kosovska, N., Makarenko, N., Bondar, V., Matviikiv, A., & Symochko, L. (2022). Soil microbiome under the influence of nano- and biopreparations. *International Journal of Ecosystems and Ecology Science*, 12(3). doi: 10.31407/ijees12.301.

[15] Lazarova, S., Coyne, D., Rodríguez, M.G., Peteira, B., & Ciancio, A. (2021). Functional diversity of soil nematodes in relation to the impact of agriculture – a review. *Diversity*, 13(2), article number 64. doi: 10.3390/d13020064.

[16] Mai, W.F. (2018). *Plant-parasitic nematodes: A pictorial key to genera*. Ithaca, NY: Cornell University Press.

[17] Pulavarty, A., Egan, A., Karpinska, A., Horgan, K., & Kakouli-Duarte, T. (2021). Plant parasitic nematodes: A review on their behaviour, host interaction, management approaches and their occurrence in two sites in the republic of Ireland. *Plants*, 10(11), article number 2352. doi: 10.3390/plants10112352.

[18] Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67-69. doi: 10.1163/187529259X00381.

[19] Shrestha, U., Dee, M.E., Piya, S., Ownley, B.H., & Butler, D.M. (2020). Soil inoculation with *Trichoderma asperellum*, *T. harzianum* or *Streptomyces griseoviridis* prior to anaerobic soil disinfestation (ASD) does not increase ASD efficacy against Sclerotium rolfsii germination. *Applied Soil Ecology*, 147, article number 103383. doi: 10.1016/j.apsoil.2019.103383.

[20] Stefanovska, T., Skwiercz, A.T., Zhukov, O., & Husieva, A. (2025). Effects of acetamiprid and flupyradifurone-based insecticides on soil fauna: Research on nematodes and earthworms. *Journal of Horticultural Research*, 33(1), 121-126. doi: 10.2478/johr-2025-0012.

[21] Tylka, G.L., & Marett, C.C. (2014). Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014. *Plant Health Progress*, 15(2), 85-87. doi: 10.1094/PHP-BR-14-0006.

[22] Wang, H.-W., Zhu, Y.-X., Xu, M., Cai, X.-Y., & Tian, F. (2022). Co-application of spent mushroom substrate and PGPR alleviates tomato continuous cropping obstacle by regulating soil microbial properties. *Rhizosphere*, 23, article number 100563. doi: 10.1016/j.rhisph.2022.100563.

[23] Yeates, G.W., Bongers, T., De Goede, R.G., Freckman, D.W., & Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera – an outline for soil ecologists. *Journal of Nematology*, 25(3), 315-331.

Видовий склад та структура комплексу фітонематод сої в умовах Правобережного Лісостепу України

Валерій Пашковський

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-1127-9361

Олександр Бабич

Кандидат біологічних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-4988-5009

Анотація. Метою дослідження було встановлення видового складу, таксономічної та екологічної структури комплексу фітонематод сої в умовах Правобережного Лісостепу України. У роботі застосовано польові маршрутні обстеження, лабораторні методи екстракції нематод із ґрунту та кореневої системи (лійковий метод Берманна та ситової сепарації), морфологічну і морфометричну ідентифікацію з використанням світлової мікроскопії, а також методи структурно-екологічного аналізу угруповань і варіаційної статистики. Встановлено, що комплекс нематод ризосфери сої включає 26 видів, які належать до 17 родів, 10 родин, п'яти рядів та двох класів, що засвідчило про високу таксономічну різноманітність ґрунтової біоти в агроценозі. Домінуючим був клас Chromadorea (понад 80 % від загальної структури), що вказує на функціональну спрямованість угруповання в умовах інтенсивного агровикористання. Найбільш чисельним був ряд Tylenchida, який визначає основну частку фітопаразитичного блоку та включає економічно значущі види *Pratylenchus penetrans*, *Tylenchorhynchus dubius*, *Helicotylenchus dihystera* та *Paratylenchus nanus*. Їх домінування засвідчило формування стабільного інвазійного потенціалу у ризосфері сої та наявність критичного фітосанітарного навантаження. Водночас суттєва частка сапробіонтних і бактеріотрофних нематод (Rhabditida та Cephalobidae) вказала на інтенсивні процеси мінералізації органічної речовини та активне функціонування мікробного блоку ґрунту. Поєднання високої частки фітопаразитичних і редуцентних форм формує функціонально неоднорідну структуру угруповання, що відображає одночасні процеси екологічного напруження та біологічної компенсації в агроекосистемі. Структурний аналіз показав проміжний рівень екологічної стабільності системи, характерний для інтенсивно використовуваних сільськогосподарських угідь із періодичним порушенням ґрунтової структури та звуженням трофічних зв'язків. Отримані результати можуть бути використані для біоіндикаційної оцінки стану ґрунтів та розробки екологічно орієнтованих систем моніторингу і захисту посівів сої від фітонематод

Ключові слова: видовий склад; таксономічна структура; агроценози; *Pratylenchus penetrans*; *Tylenchorhynchus dubius*; біоіндикація ґрунтів

БІОЛОГІЧНІ СИСТЕМИ: Теорія та Інновації

Науковий журнал
Том 17, № 2. 2026

Заснований у 2010 р. Періодичність випуску: щоквартально

Оригінал-макет видання виготовлено у відділі науково-технічної інформації
Національного університету біоресурсів і природокористування України

Відповідальний редактор:
О. Наумовська

Підписано до друку 21 травня 2026 р.
Формат 70*100/16
Умов. друк. арк. 11,4
Наклад 100 прим.

Адреса редакції:
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 13, м. Київ, Україна
Тел.: +38(044)-258-42-63
E-mail: bs@bioscience.com.ua
https://bioscience.com.ua/uk

BIOLOGICAL SYSTEMS: Theory and Innovation

Scientific Journal
Volume 17, No. 2. 2026

Founded in 2010. Frequency: quarterly

The original layout of the publication is made in the Department of Scientific and Technical Information of National University of Life and Environmental Sciences of Ukraine

Managing Editor:
O. Naumovska

Signed for print of May 21, 2026
Format 70*100/16
Conventional printed pages 11.4
Circulation 100 copies

Editors office address:
National University of Life and Environmental Sciences of Ukraine
03041, 13 Heroiv Oborony Str., Kyiv, Ukraine
Tel.: +38(044)-258-42-63
E-mail: bs@bioscience.com.ua
https://bioscience.com.ua/en