Національний університет біоресурсів і природокористування України

БІОЛОГІЧНІ СИСТЕМИ:
Теорія та Інновації

Том 16, № 2
2025

Київ 2025

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 № 11 від 22 травня 2025 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

Адреса редакції:
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https://bioscience.com.ua/uk

National University of Life and Environmental Sciences of Ukraine

BIOLOGICAL SYSTEMS: Theory and Innovation

Vol. 16, No. 2

2025

Kyiv 2025

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. 11 of May 22, 2025)

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

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Редакційна колегія

Editorial Board

Зміст/Contents

Received: 30.01.2025 Revised: 11.04.2025 Accepted: 22.05.2025

BIOLOGICAL SYSTEMS: Theory and Innovation

Journal homepage: https://bioscience.com.ua/en
Vol. 16, No. 2, 2025, 10-22

UDC 632.98:633.1 DOI: 10.31548/biologiya/2.2025.10

Aerial visual monitoring of potato globoderosis

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Abstract. The relevance of the problem under study lies in the need for timely detection of potato globoderosis foci in order to localise, eliminate and prevent further spread of the quarantine organism. The main task of the work was to improve the system of nematological monitoring of phytocenoses by combining the latest and traditional diagnostic methods. To detect focal lesions of solanaceous crops by globoderosis, the use of unmanned aerial vehicles (UAVs) was tested. The golden potato nematode *Globodera rostochiensis* (Wollenweber, 1923) Behrens remains one of the most dangerous quarantine organisms in Ukraine. The spread of monoculture of solanaceous crops in small households contributes to the mass accumulation of this phytoparasite and an increase in its harmfulness. It has been found that damage to crops not only leads to significant yield losses, but also complicates the sale of products due to the need to comply with quarantine restrictions. The results obtained confirm the need to improve traditional approaches and introduce new approaches to phytonematological monitoring. It has been determined that the optimal time for nematological monitoring of potatoes is the end of June to the first half of July, when visual symptoms of globoderosis appear and female

Suggested Citation:
Babych, A., Babych, O., Dzyuman, Ya., Prykhodko, D., & Pashkovsky, V. (2025). Aerial visual monitoring of potato globoderosis. *Biological Systems: Theory and Innovation*, 16(2), 10-22. doi: 10.31548/biologiya/2.2025.10.

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nematodes are found on the roots. Analysis of aerial photographs made it possible to refine survey routes and selectively collect plant samples from affected areas, which significantly reduced costs compared to traditional methods. If it was impossible to monitor during the growing season, the quarantine status of the fields was determined after harvesting by analysing the soil for the presence of nematode cysts. Establishing the spatial distribution, total area of detected foci, and level of potato globoderosis infestation allows for the differentiation and local application of phytosanitary measures, which significantly reduces plant protection costs. It should be noted that the developed methodology for aerial visual inspection of phytocenoses using modern UAVs equipped with high-resolution cameras can also be used to detect foci of damage to agricultural crops by other types of cyst-forming nematodes. The developed methodology for aerial visual monitoring should primarily be used by specialists in phytosanitary inspections for plant quarantine, as well as by specialists in large farms and agricultural holdings

Keywords: golden potato cyst nematode; nematodoses; advanced nematological diagnostic methods; phytocenoses; unmanned aerial vehicles

INTRODUCTION

Significant changes that have taken place in the structure of Ukraine's sown areas have led to a shift towards cultivating agricultural crops in short-rotation crop rotations, and potatoes are, in fact, grown as a monoculture in the household sector. Under such conditions, there is a massive accumulation of highly specialised harmful organisms, among which cyst-forming nematodes are one of the most problematic.

According to studies by Y. He *et al*. (2022), J.A. Price *et al*. (2022), M. Esquibet *et al*. (2024), the golden potato cyst nematode (GPCN) *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and the pale potato cyst nematode (PPCN) – *Globodera pallidae* (Stone, 1973) Behrens, 1975 represent a significant threat to potato production. Their parasitism in potato roots causes plant damage, which is called globoderosis. In addition to potatoes, a number of other crops of the Solanaceae family, in particular tomatoes and eggplants, are host plants for these nematode species. In the absence of susceptible cultivated plants for reproduction, they are able to complete their full development cycle and, accordingly, survive on weeds such as nightshade, henbane and some others.

C.M. Holguin *et al*. (2023) and R. Jiang *et al*. (2022) believe that since the potato originated in South America – the highlands of the Andes, where tuberous forms of Solanum still occur – the centre of origin of potato cyst nematodes is also the Central Andes, from where they were most likely brought to Europe along with planting material. The golden potato cyst nematode, *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975, was first discovered in Germany at the end of the 19th century. However, until the beginning of the 20th century, it was mistakenly considered a biological race of the beet nematode (Kuhn, 1874), which was widespread in many countries at that time. Only with time was their belonging to different biological species scientifically proven.

A. Babich *et al*. (2018) noted that the first mention in scientific literature of the discovery in Ukraine of the golden potato nematode – *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 – dates back to 1963. It is believed that the golden potato nematode most likely entered the Potato Wart Research Station in Chernivtsi region along with seed tubers. However, despite the introduction of quarantine measures, it was not possible to prevent the spread of the phytoparasite. In 2025, the golden potato nematode is found sporadically in most areas of modern potato cultivation in Ukraine. L.A. Pylypenko *et al*. (2008) at the beginning of the 21st century, from soil samples collected in the Zakarpattia region, the pale potato nematode *Globodera pallida* (Stone, 1973) Behrens, 1975, which is subject to external plant quarantine, was also isolated and identified for the first time. However, despite a number of scientific publications, there is no official registration of this species in Ukraine as of 2025 (Subbotin, 2025). One of the main obstacles to the effective detection of *Globodera pallida* is its low abundance in mixed populations with *Globodera rostochiensis*, where the proportion of the former usually does not exceed 2-5%.

The golden potato nematode in Ukraine is subject to internal plant quarantine, while the pale potato nematode is subject to external plant quarantine. A biological feature of the pale potato nematode is that, unlike the golden potato nematode, females form brown cysts without an intermediate yellow phase. The authors O.I. Borzykh *et al*. (2021) pointed out that the pale nematode is more aggressive because it is capable of infecting potato varieties that are resistant to the golden potato nematode. The ontogenesis of the golden potato nematode includes the following stages: egg, larvae (invasive second instar, parasitic third and fourth instars), adults: male and female, and cyst (dead female filled with eggs). Its microscopic size makes it difficult to detect its foci in a timely manner, especially in the early stages of colonisation of the land, and the cyst stage is a factor in protecting the offspring under conditions unfavourable for reproduction. J.M. Mwangi *et al.* (2021) noted that the cyst is also a source of passive dispersal of the quarantine organism along with contaminated planting material, soil cultivation tools, wind erosion of the soil, etc. Considering that traditional nematological monitoring of agrocenoses requires considerable physical effort and time, the aim of the study was to develop new methods for remote detection of globoderosis foci.

MATERIALS AND METHODS

The study was conducted over four growing seasons in 2021, 2022, 2023 and 2024 (repeated annually) using modern UAV models (DJI Phantom and DJI Spark) at the Voykov private lease enterprise (Chernihiv district, Chernihiv region), as well as on private plots in the village of Mykhailo-Kotsyubynske, Chernihiv district, Chernihiv region, on sod-podzolic soils typical for this region. The optimal periods for aerial visual diagnosis of potato plant damage by globoderosis were determined to be the third decade of June and the first and second decades of July. The research materials were potatoes susceptible to the golden potato nematode ('Lugovska', 'Svitanok Kyivskyi') and nematode-resistant varieties ('Dnipryanka', 'Zabava', 'Slovianka'), root systems, soil samples, cysts, eggs, larvae and adult golden potato nematodes.

Soil samples were collected with a soil auger before planting and after harvesting the tubers. At the same time, on land where seed potatoes were to be grown, as well as to determine the effectiveness of phytosanitary measures, 300 primary samples of 5 cm^3 each with a total volume of 1,500 cm^3 of soil or 6 average samples of 250 cm^3 of soil were collected from 1 ha. On fields intended for growing food potatoes, 100 primary samples were collected from 1 hectare, from which an average sample of at least 400 cm^3 of soil or 2 average samples of 200 cm^3 of soil were formed. From each household plot, 50 primary samples of 5 cm^3 of soil were collected from a uniform grid, with a total volume of at least 200 cm^3 of soil. To determine the potential harmfulness based on actual crop losses, the sizes of the survey areas ranged from 1-5 m^2 to 100 m^2. Control measurements of typical foci for this phytocenosis, repeated four times, made it possible to determine their average sizes, and counting the number of similar foci for each group made it possible to determine the total area of damage.

For flotation analysis, an average sample of 100 cm^3 of air-dry soil was poured into a 1-litre glass, filled with water and thoroughly mixed. The contents of the glass were left to settle until a sediment appeared. Since cysts are lighter than water, they float to the surface. The resulting mixture was poured into a funnel with a paper filter inserted beforehand. Each sample was stirred and drained twice. After the water had completely drained, the presence of cysts was determined, which were mostly distributed across the upper water level on the cone filter. The accuracy of the method was achieved by visually counting the number of eggs and larvae in the cysts using optical instruments (binoculars), isolated by flotation from the selected soil samples. In total, over 800 plant and soil samples were collected and analysed during the research period.

Aerial monitoring of agrocenoses was carried out using modern UAVs (DJI Phantom and DJI Spark) during the daytime in order to obtain high-resolution images. The percentage of affected plants and the average damage score were calculated using generally accepted formulas (1, 2), taking into account the specifics of nematological research.

$$P = \frac{100 * \mathrm{n}}{\mathrm{N}}, \tag{1}$$

where P – the nematological damage index, %; n – the number of plants with signs of damage; N – the total number of plants recorded, in pieces.

$$B = \frac{\sum n*b}{N}, \quad (2)$$

where B – the average damage index; $\sum n*b$ – the total index obtained by multiplying the number of damaged plants by the corresponding damage scores; N – the number of plants surveyed during the study, in pieces.

The level of plant damage, % (on the reference plot), was determined using the following formula (3):

$$Da = \frac{100*(M*n)}{P}, \quad (3)$$

where Da – degree of plant damage, %; M – average size of nematode foci, m^2; n – number of detected foci, pcs.; P – size of the area subject to accounting, m^2.

Experimental studies of plants, including the collection of plant material, complied with institutional, national and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992). Statistical processing of the experimental data obtained was performed using Microsoft Office Excel 2007, Statgrafics and Statistica 5.0 computer programs.

RESULTS AND DISCUSSION

With modern potato cultivation in Ukraine, which is essentially a monoculture of small households, it is quite difficult to comply with phytosanitary measures to control the golden potato cyst nematode, which causes its mass accumulation and leads to significant crop losses. In order to detect foci of this limited-spread quarantine organism in a timely manner and prevent its further spread to new areas, it is necessary to develop both new diagnostic methods and improve generally accepted monitoring methods. For commercial plantings, and in recent years for many household plots, a banded arrangement of potato bushes is typical when using a mechanised planting method. This is clearly visible when the surveyed area has uniform plant cover. At a low level of infestation, the banded structure of the cover is preserved, but unevenness may appear locally, and chlorotic or stunted plants may be found sporadically (Fig. 1).

Figure 1. Photo of potato globoderosis foci detected using UAV
Source: provided by the authors

In Ukraine, over 90% of potatoes are grown on private household plots, essentially within settlements. Under these conditions, the most effective method for aerial visual inspection of plantings for globoderosis infestation is the use of unmanned aerial vehicles (UAVs). Using a pre-planned flight route within a geographical information system (GIS) and flying at a height of 50 metres in a shuttle pattern, it's possible to survey dozens of hectares of potato plantings in just a few hours, whereas traditional ground inspections would take several days. An important advantage of using UAVs is the non-contact nature of the survey, which reduces the risk of spreading the quarantine nematode species to neighbouring plots – a risk that exists when people or equipment work in the field. If local irregularities are detected in the plant cover, a UAV can perform a repeat inspection of the designated areas from a height of 2-10 metres, providing detailed images for further analysis (Fig. 2).

At a moderate degree of damage, the row structure of potato plantings is generally preserved; however, localised foci with clearly pronounced plant suppression are diagnosed. These foci are visually distinguishable against the background of unaffected plants by such features as delayed growth and development, and mottled leaf colour ranging from pale green to yellowish or brown desiccated leaves. At the same time, it should be noted that under conditions of high crop density, due to row closure, the distinct strip structure of the crop may not be visible. However, in this case, a focal infestation of globoderosis can still be diagnosed visually. In cases of severe or very severe damage, globoderosis foci can extend for several dozen metres, with significant plant thinning, especially in the central part, and stunting at the edges of such foci. At the same time, small micro-foci of less affected, chlorotic plants may also appear in different parts of the field. This is due to the different times of initial infestation of the plots by the golden potato nematode.

Figure 2. Photo of potato globoderosis foci detected using UAV
Source: provided by the authors

According to studies by D. Sigareva *et al*. (2024) and A. Babich *et al*. (2018), nematodes parasitising the roots of susceptible crops cause morphological, physiological, and pathological changes in plant cells. The visual signs of plant damage (stunted growth, chlorosis, yellowing and dieback of lower leaves, wilting, and – in cases of severe infestation – localised plant death) become visually apparent and mostly coincide with the beginning of potato flowering. The root system of such affected plants often has a huge number of small rootlets. However, in cases of very severe infestation, the roots turn brown and die prematurely, which leads to the death of globoderosis-affected plants and the formation of "bald spots." The presence of bald spots indicates that these areas have been infested by the golden potato nematode for a long time, provided that females are found on the roots of the plants during selective route surveys. This also indicates a high probability of infestation of adjacent plots, even in the absence of typical visual signs of globoderosis. Specifically, it was found that the use of technical equipment during plowing or mechanical potato diggers during harvesting – with constant movement from one owner to another without thorough cleaning of the equipment – was one of the reasons for the passive dispersal of golden potato nematode cysts within the settlement. The optimal periods for aerial visual diagnosis of globoderosis damage in potato plantings are the third decade of June and the first and second decades of July. These calendar dates mostly coincide with the potato flowering phase. This is because during the period of mass appearance of females on the root system, plants suffer the most from the negative effects of both the parasitism of the females and unfavourable environmental factors, especially prolonged drought. Plants, even with a moderate level of infestation, wilt during the daytime, and at a high level, they dry out prematurely, which leads to the localised formation of bald spots. Table 1 provides the criteria for the remote visual assessment of globoderosis infestation in potato plantings, which are based on visual signs detected using a UAV.

Table 1. Criteria for remote visual assessment of potato plant damage caused by globoderosis

Score	Degree of damage	Visual manifestations of damage to plants	Proportion of affected area out of total, %	Predicted yield reduction, %
0	None	The plant cover is uniform. No signs of plant damage	0	0
1	Absent or mild	Slight chlorosis of individual plants or small foci	<10	<5
2-3	Mild	The plant cover is locally uneven. Chlorotic and sporadic patches of stunted plants	11-25	6-10
4-5	Moderate	Significant unevenness of the plant cover. Small and moderate foci with wilted leaves and localised plant death	26-50	11-30

Table 1. Continued

Score	Degree of damage	Visual manifestations of damage to plants	Proportion of affected area out of total, %	Predicted yield reduction, %
6-7	Severe	The plant cover is patchy. Significant depression and local plant death. Clearly visible areas of damage, so-called "bald spots"	51-75	31-50
8-9	Very severe	Very significant thinning of potato plantings. Large, often continuous areas with significant thinning of plants and the formation of "bald spots"	>75	>50

Note: formulas 1, 2, and 3 were used to calculate the degree of plant damage and the proportion of the surveyed area affected
Source: developed on the basis of authorial research

Globoderosis disrupts plant metabolism, suppresses photosynthesis, and impairs the uptake of water and nutrients, which manifests itself in symptoms that can be clearly diagnosed visually. The developed criteria represent a systematised approach to nematological assessment of potato plantings. It should also be noted that aerial visual monitoring enables the inspection of large areas of solanaceous crops for potato globoderosis at the most appropriate phenological stages.

For diagnosis, the degree of host plant infestation was assessed depending on the level of root invasion by females of Heteroderidae. The evaluation scale (0-9 points) takes into account visual manifestations, the proportion of affected area, and predicted yield losses. This approach combines qualitative observations with quantitative outcomes, forming the basis for management decisions in agricultural production. These criteria were developed in the course of the present study. For instance, at the first score, symptoms of infestation were weakly expressed, and chlorotic plants were mainly found in marginal field strips, with the proportion of affected area not exceeding 10%. At the 2-3 score, chlorotic and locally yellowed plants were diagnosed on up to 25% of the surveyed area. At a moderate damage level (scores 4-5), pronounced heterogeneity of plant cover encompassed up to 50% of the surveyed area, with locally diagnosed foci of severe suppression and plant death. Severe (scores 6-7) and very severe (scores 8-9) infestation indicate the long-term persistence of golden potato cyst nematode foci in certain agrocenoses. Marked thinning of stands and the formation of so-called "bald patches" over extensive areas resulted in yield losses ranging from 31-50% or even higher.

The developed criteria can be integrated into UAV software, satellite monitoring systems, or mobile applications for agro-scouting, aligning with modern trends in precision agriculture, and providing a foundation for economic and biological modelling of the feasibility of applying plant protection products (PPPs) or implementing crop rotation. In addition, the visual assessment scale may be used in agronomic expertise and in state monitoring of the phytosanitary condition of agricultural lands. It should be emphasised that conducting aerial visual monitoring at later stages may be complicated by potato late blight epiphytotics, particularly under prolonged humid conditions. However, during epiphytotic disease development, plant death is observed over large contiguous areas, in contrast to the predominantly focal damage caused by potato globoderosis. It has been established that globoderosis symptoms are more clearly expressed at daytime temperatures above 25°C. Therefore, the first trial flights are best carried out between 12:00 and 18:00, when affected plants, in addition to the above-mentioned diagnostic features, show the greatest wilting, especially if no precipitation has occurred for several days prior to the inspection.

Aerial visual detection of localised areas of heterogeneous plant cover subsequently narrowed down the survey area of suspected agrocenoses for examination by standard methods. To confirm that potato plants were affected specifically by globoderosis, it was appropriate to analyse samples of infested plants collected during selective route surveys. The detection of white females on the root system of suppressed plants served as confirmation that agrocenoses were colonised by the quarantine organism – the golden potato cyst nematode.

The use of UAVs in quarantine inspections of household plots provides significant advantages. Route surveys of plots were first conducted to clarify the phytosanitary status of households where aerial visual monitoring had diagnosed potato infestation by globoderosis. If the infestation was confirmed by conventional methods, adjacent plots in the settlement were also examined. Where it was not possible to survey all problem plots during the vegetation period of host plants, the nematological status of such land was determined after harvest by sampling and analysing soil for the presence of cysts containing viable offspring. Based on the remotely detected foci of plant suppression or local plant death, it was possible to plan the inspection routes of problem plots more precisely and collect nematological samples to confirm or refute the colonisation of a particular plot by the golden potato cyst nematode. Priority attention was paid to heterogeneity and patchiness of the plant cover, as well as differences in plant colouration (chlorosis, yellowing, browning, leaf desiccation). Technical errors in farming operations were mostly distinguished by sharper rectangular contours. By contrast, foci of locally suppressed plants usually had an elongated-elliptical shape in the predominant direction of soil cultivation operations, which is attributable to passive dispersal of cysts by machinery.

Aerial visual diagnosis substantially reduced the time and costs associated with sampling large numbers of soil specimens by the route method, their preparation for flotation analysis, cyst extraction from air-dried soil, and counting of eggs and larvae in each sample. Given that, at the initial stages of agrocenosis colonisation by cyst-forming nematodes, foci may be smaller than 1 m^2, whereas after long-term infestation they may extend over several hundred square metres, the dimensions of accounting plots ranged from 1-5 m^2 to 100 m^2, depending on the actual yield losses observed. Control measurements of typical foci for the given phytocoenosis, conducted in quadruplicate, enabled determination of their average size, while counting the number of similar foci in each group yielded the total affected area. The long-term persistence of foci within defined spatial boundaries and the slow course of the pathological process are the main advantages of diagnosing potato globoderosis compared with actively migrating phytophagous species or the spread of mycological diseases capable of epiphytotics.

Thus, the use of UAVs makes it possible to establish the spatial distribution of cyst-forming nematode foci across surveyed areas within a short timeframe while significantly reducing the costs compared to conventional route methods. Digital mapping of phytoparasitic nematode spread offers great prospects for the practical application of research results. Integrating nematological monitoring results into electronic databases with GPS-based spatial referencing enables reproduction of inspection routes and localisation of detected microfoci of cyst-forming nematodes in subsequent surveys. This in turn provides opportunities to track and, if necessary, adjust both the boundaries of their spread and the level of soil infestation, which is critically important for decision-making regarding the removal of quarantine restrictions from certain territories. Research demonstrates the effectiveness of UAVs equipped with thermal and multispectral sensors, as well as the use of machine learning and metabarcoding techniques, for the early detection of nematodes and assessment of plant condition in various crops.

According to J. Wainer & Q. Dinh (2021) and X. Wang *et al.* (2022), under modern classification the genus *Globodera* includes, besides the golden potato cyst nematode (*Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975), also the pale potato cyst nematode (*Globodera pallida* (Stone, 1973) Behrens, 1975), which is more aggressive as it can infest potato varieties that are resistant to the golden potato nematode. Data from V.P. Fedorenko *et al*. (2012) and A. Pulavarty *et al*. (2022) indicate that traditional nematological monitoring includes visual diagnosis of potato plantings; sampling and analysis of plants and soil; flotation-based cyst extraction; counting of eggs and larvae contained in cysts; species identification of nematodes; and compilation of nematological cartograms. Priority nematological surveys should be conducted in household sector plots, since under monoculture or with minimal intervals between repeated cultivation of host plants, populations of the golden potato cyst nematode accumulate much more rapidly than in crop rotation fields. As R.A. Sikora *et al*. (2023) note, confirmation of infestation specifically by globoderosis requires the examination of the root system of visually suppressed potato plants for the presence of spherical white females.

Given the labour intensity and significant time costs, particularly for soil sampling, flotation analysis, and counting the number of eggs and larvae in each sample, the search for alternative monitoring methods of PCN (potato cyst nematodes) has become essential. At the end of the 20th century, small aircraft were first used to detect foci of *Globodera* infestation. However, according to A.G. Babych *et al*. (2024), due to the high costs of such aerial visual surveys, as well as the difficulty of clearly delineating the distribution boundaries of infestation foci when interpreting aerial photographs, this method did not become widely implemented. The main advantage of developing nematological maps, including those produced with modern technical tools, compared with actively migrating phytophagous species, lies in the long-term persistence of foci within defined spatial boundaries, which is due to the ability of progeny to remain viable in the cyst stage for 10 years or more in a state of anabiosis.

The study by K. Jindo *et al*. (2023) demonstrated the effectiveness of UAV-derived data, particularly thermal sensors and vegetation indices, for early detection of potato infection with the pale cyst nematode *G. pallida*. A correlation was found between canopy temperature and infection density, linked to the physiological response of the plants. The chlorophyll content index proved most reliable for predicting yield losses. The results indicate the potential for integrating such methods into modern agromonitoring systems, with further refinement through multifactorial studies. In the research by M.P. Tangvik *et al*. (2025), metabarcoding (for nematodes, bacteria, fungi, and oomycetes), UAV-based multispectral imaging, and traditional nematode extraction from soil were combined to diagnose potato crop infestation in Norway. NDVI analysis identified areas of optimal and suppressed plant growth. Evidence pointed to phytonematodes as the likely cause, given their presence and the low alpha-diversity in affected zones. Metabarcoding showed high accuracy compared with traditional diagnostic methods. The combined approach proved promising for detecting growth differences across entire fields. However, no statistically significant correlation was found between the relative abundance of *Globodera* spp. and NDVI. Nonetheless, the dominance of this genus in metabarcoding results suggests its potential role in plant growth suppression. The absence of a direct link may be explained by the fact that after a certain threshold density of the phytoparasite is reached, further accumulation does not cause a proportional increase in damage.

In the study by V.P. Cavalcanti *et al*. (2023), the potential of UAV imagery was assessed for monitoring the impact of root-knot nematodes on lettuce (*Lactuca sativa*). The experiment determined the optimal flight altitude for mapping infestation and calculated vegetation indices and canopy parameters. It was established that UAV imagery, even from low-cost equipment, can be reliably used to detect plant stress and predict yield, despite some limitations related to lighting. In the study by B.H.T. Arantes *et al*. (2023), the effectiveness of UAV-based multispectral imaging was examined for detecting *Heterodera glycines* and *Pratylenchus brachyurus* during the soybean flowering stage (R1). Data from Sequoia, Sentera, and Phantom 4 Advanced cameras were used to analyse the relationship between spectral reflectance and nematode density in soil and roots. It was found that the red spectrum best indicated the presence of *H. glycines*, whereas for *P. brachyurus* the most informative were the RedEdge and NIR spectra. The results confirm the feasibility of early diagnosis of infestation using readily available sensors. In the context of aerial monitoring of potato *Globodera* infestations, the study by D. Komarchuk *et al*. (2020) is also relevant, as the authors developed algorithms and software for UAV flight planning aimed at monitoring plant stress. This aligns with the present study, in which aerial photographs were used to detect potato infestation foci caused by the golden nematode, refine survey routes, and enable targeted sampling. The application of the solutions described by D. Komarchuk *et al*. may improve the accuracy, efficiency, and timeliness of phytosanitary monitoring using UAVs.

The study by F.V. Pereira *et al*. (2022) focused on the application of machine learning and remote sensing for monitoring coffee plantations infested with nematodes. Using UAV and Planet satellite imagery, the authors assessed physiological plant parameters with the help of Random Forest and SVM algorithms. The results demonstrated high accuracy in predicting such indicators as chlorophyll content, plant height, branch length, and branch number. This approach enables spatial analysis of crop condition and, accordingly, the adoption of management decisions regarding protection against harmful organisms. S. Joalland *et al*. (2018) evaluated the effectiveness of imagery obtained from UAV-mounted sensors and handheld devices for phenotyping sugar beet cultivars infected with beet cyst nematode (BCN). The analysis included spectral, hyperspectral, thermal, and height-related parameters of crop canopies. It was established that spectral indices related to chlorophyll, nitrogen, and water content reliably distinguish nematode-resistant cultivars from susceptible ones, particularly in the later stages of vegetation. UAVs proved advantageous compared with traditional methods. The findings highlight the potential of integrating multiple sensor datasets for yield prediction and improvement of breeding processes.

A.J. Oliveira *et al*. (2019) presented a methodology for detecting nematodes in coffee plantations using RGB imagery acquired from UAVs and machine learning techniques. Visual plant features were employed to classify plots as infested or non-infested. Several approaches were tested, including segmentation. The best performance was achieved by the U-net model with manual segmentation, yielding an F-measure of 63%. The article by R. Sathya Priya *et al*. (2025) reviewed modern and traditional remote sensing technologies used for monitoring pests, plant diseases, parasitic nematodes, weeds, soil microorganisms, and environmental threats. The technology enables detection of infestation foci, localised pesticide application, and effective agronomic decision-making. Special attention was given to the integration of remote sensing with site-specific weed management (SSWM) and field survey data. In the context of Agriculture 4.0, such approaches enhance crop productivity, reduce costs, and contribute to the sustainable development of the agricultural sector.

In summary, these studies indicate the existence of correlations between spectral indices, canopy temperature, and infestation levels, which make it possible to predict yield losses and identify problem areas. New approaches that combine traditional and modern methods increase the accuracy of monitoring and contribute to the implementation of precision farming systems, which ensures effective control of harmful organisms and the sustainable development of agricultural production.

CONCLUSIONS

Considering the labour-intensive nature of traditional route-based nematological surveys of phytocenoses, the use of an effective and economically accessible method of remote diagnostics allows for the monitoring of large areas for globoderosis infestation in a short time. This enables the local and differentiated application of phytosanitary measures, thereby significantly reducing the material costs of plant protection.

The optimal times for aerial visual diagnosis of globoderosis damage in potato plantings are the last ten days of June and the first and second ten days of July. This period coincides with the mass appearance of females on the root system, which mostly overlaps with the flowering phase when plants suffer most from the negative effects of both phytoparasites and adverse environmental factors, especially prolonged drought. The use of a traditional monitoring system remains necessary to confirm or refute the suspicion of globoderosis in certain potato agrocoenoses that has been diagnosed by the aerial visual method. Since invasive larvae have minimal active migration, the centres of spread for the golden potato nematode remain within defined spatial boundaries for many years. This is a significant advantage for the development and practical use of nematological maps compared to actively migrating species of phytophages.

The research results also confirm the feasibility of applying the developed method of aerial visual monitoring to detect local damage to plants by other species of cyst-forming nematodes. It is promising to create internal farm maps (plans) with a detailed determination of the location of cyst-forming nematode foci on cartograms, their area, and soil infestation density to optimise the structure of sown areas and crop rotation. This will also aid in the organisation and implementation of effective and environmentally friendly control measures. New approaches that combine traditional and modern methods increase the effectiveness and accuracy of monitoring phytoparasitic nematodes, contribute to the implementation of precision farming systems, and ensure the differentiation of pest control measures. Prospects for further research include using the rapid development of UAV technologies and the implementation of artificial intelligence to improve methods of phytosanitary monitoring, which opens up new opportunities in the detection and control of quarantine organisms.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Аеровізуальний моніторинг глободерозу картоплі

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Анотація. Актуальність досліджуваної проблеми полягає в необхідності своєчасного виявлення осередків глободерозу картоплі з метою їх локалізації, ліквідації і запобіганню подальшому розселенню карантинного організму. Основним завданням роботи було вдосконалення системи нематологічного моніторингу фітоценозів за поєднання новітніх і традиційних методів діагностування. Для виявлення осередкового ураження пасльонових культур глободерозом було апробовано використання безпілотних літальних апаратів (БПЛА). Золотиста картопляна нематода *Globodera rostochiensis* (Wollenweber, 1923) Behrens залишається одним із найбільш небезпечних карантинних організмів в Україні. Поширення монокультури пасльонових культур у дрібних домогосподарствах сприяє масовому накопиченню цього фітопаразита та зростанню його шкідливості. Виявлено, що ураження посівів призводить не лише до значних втрат урожаю, а й ускладнює реалізацію продукції через необхідність дотримання карантинних обмежень. Отримані результати підтверджують необхідність удосконалення традиційних та впровадження новітніх підходів до фітонематологічного моніторингу. Визначено, що оптимальними строками нематологічного моніторингу картоплі є кінець червня – перша половина липня, коли проявляються візуальні симптоми глободерозу та виявляються самиці нематоди на коренях. Аналіз аерофотознімків дозволяв уточнювати маршрути обстеження та цільово відбирати рослинні зразки з уражених ділянок, що значно знижувало витрати порівняно з традиційними методами. У разі неможливості контролю під час вегетації карантинний статус полів визначали після збирання врожаю за аналізом ґрунту на наявність цист нематоди. Встановлення особливостей просторового поширення, загальної площі виявлених осередків та рівня ураженості картоплі глободерозом, дає змогу диференціювати та локально застосовувати фітосанітарні заходи, що суттєво скорочує витрати на захист рослин. Варто зазначити, що розроблена методологія аеровізуального обстеження фітоценозів сучасними БПЛА, обладнаних камерами з високою роздільною здатністю,

також може бути використана для виявлення осередків ураження сільськогосподарських культур іншими видами цистоутворюючих нематод. Розроблену методологію аеровізуального моніторингу доцільно першочергово використовувати спеціалістами фітосанітаних інспекцій з карантину рослин, а також фахівцями великих господарств та агрохолдингів

Ключові слова: золотиста картопляна цистоутворююча нематода; нематодози; новітні методи нематологічного діагностування; фітоценози; безпілотні літальні апарати

Received: 27.12.2024 Revised: 31.03.2025 Accepted: 22.05.2025

BIOLOGICAL SYSTEMS: Theory and Innovation

Journal homepage: https://bioscience.com.ua/en
Vol. 16, No. 2, 2025, 23-36

UDC 631.95:631.527.5:004.9

DOI: 10.31548/biologiya/2.2025.23

Principles of high-throughput phenotyping and sensor platforms for non-invasive research in plant protection

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Abstract. Climate change and the increasing resistance of pathogens are driving the need for innovative methods of plant disease diagnostics, particularly for high-risk pathogens such as *Fusarium graminearum*, which causes significant wheat yield losses. Traditional visual inspection methods suffer from low throughput and subjectivity, limiting their effectiveness in large-scale monitoring. This study aimed to explore the principles of high-throughput phenotyping and to evaluate the effectiveness of a sensor platform for the non-invasive investigation of *Fusarium* ear blight under field conditions in Ukraine (Kyiv region). A combination of multispectral imaging, thermal imaging, and machine learning algorithms was applied in a 1-hectare experimental field with 20% of the plots artificially infected. The results demonstrated that the proposed system achieved a 92% accuracy rate in early pathogen detection, representing a 37% improvement over visual assessment methods. Spectral indices showed a strong correlation with pathogen concentration: a decrease in the normalised difference vegetation index from 0.72 to 0.35 corresponded with an 80% increase in fungal biomass. Thermal imaging revealed a rise in leaf temperature of 2.5°C as early as 5-7 days after infection. The integration of all methods enabled an accuracy of 96% in processing one hectare within 2.5 hours, which is three times faster than traditional approaches. Polymerase chain reaction analysis confirmed the specificity of the techniques: 95% of infected samples contained *Fusarium* deoxyribonucleic acid, while sequencing revealed a 100% match for β-tubulin. Automated data processing required 2.5 hours per hectare, compared with 8 hours per hectare for visual inspection, and scaling up to 10 hectares reduced time expenditure by a factor of 12. The study confirmed the effectiveness of high-throughput phenotyping for precision plant protection and highlighted the need for further refinement of the methods in line

Suggested Citation:
Matkovskyi, R., Taran, O., & Kolomiets, Yu. (2025). Principles of high-throughput phenotyping and sensor platforms for non-invasive research in plant protection. *Biological Systems: Theory and Innovation*, 16(2), 23-36. doi: 10.31548/biologiya/2.2025.23.

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with local climatic conditions. The practical significance of this research lies in the potential to reduce fungicide use through targeted treatment of infected areas, minimise crop losses in regions with high infection pressure, and establish a foundation for automated monitoring systems compatible with precision agriculture technologies

Keywords: agriculture; pathogens; fungal infections; hyperspectral imaging; thermal imaging

INTRODUCTION

Global agriculture is facing a complex set of challenges, including climate change, increasing pathogen resistance, and the pressing need to ensure food security. In Ukraine, where the agricultural sector plays a critical role in the national economy, these challenges are intensified by the intensification of farming practices and shifting climatic conditions, which contribute to the spread of fungal infections – particularly *Fusarium graminearum*. This pathogen not only significantly reduces wheat yields but also poses a health risk due to the production of toxic compounds, underscoring the urgency of developing innovative methods for disease diagnosis and control.

Traditional diagnostic approaches, such as visual inspection or laboratory testing, are unsuitable for large-scale monitoring due to their low throughput, subjectivity (e.g. reliance on the agronomist's expertise), and delayed detection – since symptoms often appear only at advanced stages of infection. In this context, high-throughput phenotyping (HTP) is emerging as a key tool, as it integrates sensor technologies – including multispectral imaging, thermal imaging, and hyperspectral visualisation – with machine learning (ML) algorithms for non-invasive analysis of plant conditions down to the cellular level (Ma *et al*. 2022). However, despite recent advancements, challenges remain in adapting HTP to specific biotic threats, particularly in regions with complex climatic conditions such as Ukraine.

Between 2020 and 2024, significant advances were made in the field of HTP. The study by Z. Ma *et al*. (2022) systematised modern sensor-based methods, identifying three key categories: multispectral imaging, which employs indices such as Normalised Difference Vegetation Index (NDVI) and Normalised Difference Red Edge (NDRE) to assess the physiological state of plants based on light reflectance across various spectral bands; thermal imaging, which detects temperature anomalies linked to changes in transpiration under stress conditions; hyperspectral imaging, which analyses hundreds of spectral channels to reveal molecular-level changes, such as the accumulation of toxins.

While these technologies have proven effective in assessing abiotic stress factors (e.g. drought), their application to biotic threats – such as *Fusarium graminearum* – remains limited. For instance, the study by D. Li *et al.* (2021) demonstrated the efficacy of an HTP platform for evaluating yield and salinity tolerance but did not address pathogen-host interactions. This gap is critical, as fungal infections often mimic the symptoms of physiological stress, complicating accurate diagnosis.

A significant contribution to the understanding of HTP was made by Q. Xiao *et al.* (2022), who integrated phenotyping into genome-wide association studies (GWAS) to identify genes associated with resistance. However, their approach did not address the technical challenges of processing large volumes of data in real time – an essential requirement for precision agriculture. Similarly, H. Yuan *et al*. (2023) found that existing monitoring systems tend to underestimate the influence of microclimatic factors (e.g. local humidity fluctuations) on pathogen dynamics – an issue particularly relevant to Ukraine, where variable weather conditions increase the risk of epiphytotics. The study by X. Fu & D. Jiang (2022) examined the role of HTP as an innovative tool for enhancing the sustainability of agricultural systems in the context of climate change, with a focus on analysing plant adaptation mechanisms to stress factors. The authors highlight the integration of sensor technologies, machine learning algorithms, and large-scale data for real-time crop performance monitoring, offering new pathways for the optimisation of agricultural production.

Despite recent progress, significant gaps remain. A lack of specificity is evident in the fact that most studies – for example, T. Gill *et al*. (2022) –

focus on general plant responses to stress, whereas *Fusarium graminearum* requires tailored models that account for its unique biochemical markers. Limited validation also poses a challenge, as image-processing algorithms developed by J. Zhou *et al*. (2022) overlook thermal anomalies that may serve as early indicators of infection (e.g., localised temperature increases caused by the plant's immune response). Moreover, the sensor systems proposed by F. Tanner *et al*. (2022) have not been tested under conditions of high humidity – typical of southern regions of Ukraine, where soil moisture can reach 80%, thereby affecting the accuracy of thermal imaging data.

The integration of information technology into agricultural research has become critically important for optimising crop breeding and precision farming, particularly in the context of high-throughput phenotyping. The study by S. Vedmediev (2024) demonstrates the potential of specialised software to automate and enhance the accuracy of phenotypic trait analysis in sunflower breeding programmes. Building on this approach, the present study adopts similar principles to wheat pathology, combining sensor technologies and machine learning to develop a non-invasive diagnostic platform for detecting *Fusarium graminearum*. In doing so, it extends the application of IT-driven phenotyping to plant disease management. This study aimed to develop an integrated method for diagnosing *Fusarium* ear blight, incorporating multispectral indices (NDVI, NDRE) to assess photosynthetic activity, thermal data to detect localised temperature increases, and machine learning techniques – particularly deep neural networks – for classifying infection patterns.

MATERIALS AND METHODS

Experimental site and sampling. The study was conducted in the experimental field of the National Academy of Agrarian Sciences of Ukraine in Kyiv Region between May and July 2024. The site was selected due to the representative nature of the region's agroclimatic conditions – characterised by a moderate continental climate and chernozem soils – which are typical of Ukraine's main grain-producing areas, thus allowing for broader applicability of the results. The research focused on winter wheat (*Triticum aestivum*), cultivar Podolianka, sown on a 1-hectare plot. This variety was chosen due to its widespread use in agricultural production, moderate susceptibility to *Fusarium graminearum*, and adaptability to local conditions, enabling a reliable assessment of diagnostic method effectiveness. To ensure controlled conditions, the field was divided into 40 plots of 250 m^2 each. Of these, 8 plots (20%) were artificially inoculated with *Fusarium graminearum*, the primary causal agent of *Fusarium* ear blight in Ukraine. The fungal strain was sourced from the microbiological collection of the National Academy of Agrarian Sciences of Ukraine. This pathogen was selected due to its epidemiological importance and the significant economic losses it causes in the region. Laboratory analyses were also conducted to validate the results and perform necessary diagnostic tests, including PCR and DNA sequencing.

Plant sampling was conducted using a stratified randomisation method. Only plants at the tillering stage (4-6 weeks after emergence) without mechanical damage were included. Exclusion criteria comprised the presence of symptoms of other diseases (e.g. rust, powdery mildew), insect damage, or abnormal soil moisture levels (above 80% or below 30%). Soil moisture was measured using TEROS 12 sensors with an accuracy of ±3%. The presence of other diseases, such as rust or powdery mildew, was assessed through visual inspection by qualified agronomists and confirmed via laboratory-based PCR (polymerase chain reaction) using species-specific primers.

Infection protocol and monitoring. To ensure uniform distribution of the pathogen, infected plots were selected using a random number generator implemented in the "numpy" library in Python. The infection protocol began with the preparation of a spore suspension of *Fusarium graminearum*. The fungus was cultured on potato dextrose agar (Germany) at 25°C for seven days. Spores were then carefully removed from the medium's surface using a sterile spatula, resuspended in a physiological saline solution, and filtered through cheesecloth. The spore concentration (1×10^5 CFU/mL, colony-forming units) was determined using a Goryaev chamber (Germany). Prior to inoculation, instruments (scalpels, needles) were sterilised in a T-EDGE 10 autoclave (Israel) at 121°C for 20 minutes.

For inoculation, sterile 1 mL BD Plastipak syringes with 25G needles were used. The spore suspension was injected into the stems at the height of the third to fourth node, simulating the natural entry of the pathogen through mechanical damage. Following injection, the site of entry was disinfected using a sterile cotton swab soaked in 70% ethanol to prevent secondary infections. All procedures were carried out in a laminar flow cabinet in accordance with biosafety guidelines (CDC, 2020). The use of standardised protocols for culturing *Fusarium graminearum* (potato dextrose agar), accurate spore quantification (Goryaev chamber), and commercial DNA extraction kits was essential to ensure experimental reproducibility, minimise the risk of contamination, and obtain high-quality data for PCR analysis – critical for validating sensor-based diagnostic methods.

Each week throughout the study period, 20 plants (10 infected and 10 control) were sampled from each plot for PCR analysis. Leaf and stem tissues were first homogenised, and DNA was extracted using the DNeasy Plant Pro Kit (Germany). The quality of the extracted DNA was then assessed spectrophotometrically (Nanodrop 2000, USA) by measuring purity (A260/A280 = 1.8-2.0) and concentration Amplification was performed using a T100 thermal cycler (USA) following this protocol: initial denaturation at 95°C for 3 minutes; 35 cycles at 95°C for 30 seconds, at 58°C for 30 seconds, and at 72°C for 45 seconds; followed by a final extension at 72°C for 5 minutes. PCR products were visualised on 2% agarose gels (USA) stained with ethidium bromide under UV illumination, using electrophoresis at 100 V for 40 minutes. Sequencing was carried out by Macrogen DNA sequencing services (South Korea) to eliminate false positives. All samples with DNA purity within the A260/A280 range of 1.8-2.0 and sufficient concentration (>20 ng/μl) were included in the subsequent analysis. Samples falling outside this range or with low DNA quality were excluded from the study.

Sensor platforms and data collection. A combination of aerial and ground-based technologies was employed for data collection. A DJI Phantom 4 Multispectral unmanned aerial vehicle (China), equipped with *Red Green Blue* (RGB) cameras (2 MP resolution) and multispectral sensors (*Near InfraRed* (NIR), RedEdge, Blue; spectral range 450-860 nm, resolution 1.2 MP), was used for weekly imaging at an altitude of 50 metres, delivering a ground resolution of 2 cm per pixel. Thermal imaging was carried out using a FLIR Vue TZ20 thermal camera (spectral range 7.5-13.5 μm, accuracy ±5°C), integrated with the drone.

A total of 40 TEROS 12 sensors (USA) and 40 HOBO MX2301 sensors (USA) were installed across the experimental field, with one set (TEROS 12 + HOBO MX2301) placed in each of the 40 plots. In total, 80 sensors were used for simultaneous monitoring of soil moisture (±3%), air temperature, relative humidity, and solar radiation. Each TEROS 12 sensor was inserted 20 cm into the soil, while the HOBO MX2301 sensor was mounted 50 cm above the soil surface to capture microclimatic parameters in real time.

The concentration of *Fusarium graminearum* spores in the soil was determined microscopically using a Goryaev chamber. Soil samples were collected from a depth of 20 cm, homogenised in sterile saline solution, and applied to the chamber grid for counting colony-forming units (CFU) per millilitre.

Imaging was carried out weekly between 10:00 and 14:00 under sunny conditions to minimise the influence of variable light intensity. The spectral indices NDVI and NDRE were calculated using the following formulae:

$$NDVI\ \frac{(NIR-Red)}{(NIR+Red)}, \tag{1}$$

where *NIR* is the reflectance in the near-infrared range (780-900 nm), and *Red* is the reflectance in the red range (630-690 nm). NDVI values range from −1 to 1, with the following interpretations: >0.6 – dense, healthy vegetation; 0.2-0.5 – sparse or stressed vegetation; <0.1 – bare soil or dead biomass.

$$NDRE = \frac{(NIR-RedEdge)}{(NIR+RedEdge)}, \tag{2}$$

where *NIR* is the reflectance in the near-infrared range (780-900 nm), and *RedEdge* is the reflectance in the red range (690-750 nm). NDRE values correspond to: >0.4 – high chlorophyll content; 0.20.3 – moderate level; <0.1 – low or absent photosynthetic activity. NDVI was used to assess the overall physiological activity of the plants, whereas NDRE served to detect changes in chlorophyll content associated with *Fusarium* infection.

A visual assessment of infection severity was conducted by five agronomists from the National

Academy of Agrarian Sciences of Ukraine (aged 32-45, with over five years of professional experience), using a 0-5 point scale adapted from the criteria described in EPPO Standard PP 1/257 (n.d.), tailored to the characteristics of the Podolianka wheat variety and the conditions of Ukraine's forest-steppe zone. The scale comprised the following criteria: 0 – no visible symptoms; 3 – spotting on 50% of the leaf surface; 5 – necrosis and complete tissue death. This method served as a reference standard for comparison with automated diagnostic approaches. To evaluate logistical efficiency, a comparative analysis of time expenditure was performed by recording the duration of each diagnostic stage (imaging, data processing, interpretation) for both the automated and visual methods across a 1-hectare plot.

Data processing and statistical analysis. Data processing began with image calibration in Pix4D Mapper to eliminate spectral noise and correct geometric distortions. Disease classification was performed using the Random Forest algorithm (Breiman, 2001) from the "scikit-learn" library (Python 3.10). The training dataset consisted of 2,800 images (70% of the total), and the test dataset included 1,200 images (30%). Model parameters included 1,000 trees and the Gini impurity criterion for splitting. For statistical analysis, Pearson's correlation coefficient was used to assess the relationship between NDVI values and infection levels. ANOVA was applied to evaluate the effect of data collection timing, and a bootstrap analysis (1,000 iterations) was conducted to verify the representativeness of the results.

The use of the DJI Phantom 4 Multispectral drone was justified by its capability to detect spectral variations in the NIR range (860 nm), which is critical for early stress diagnosis. The Random Forest algorithm was selected due to its robustness to noise and its efficiency in handling large multispectral datasets. The study was conducted following the international guidelines of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Effectiveness of *Fusarium* detection using sensor platforms

The Random Forest algorithm achieved an average accuracy of 92% in detecting *Fusarium* infection at early stages (1-2 weeks post-infection). The analysis utilised data from the NIR (860 nm) and RedEdge (720 nm) spectral bands, which are sensitive to structural changes in chlorophyll and cellular integrity. The model's sensitivity, indicating its ability to identify infected plants, was 89%, while its specificity, reflecting the accuracy in excluding healthy specimens, reached 94%. For example, out of 200 infected plants, the system correctly classified 178, with 22 false negatives. In the control group of 800 healthy plants, only 48 were misclassified as infected.

The visual method, based on the 0-5 scoring scale developed by the National Academy of Agrarian Sciences of Ukraine, demonstrated significantly lower effectiveness. The average accuracy was 68%, with a sensitivity of 62% and a specificity of 73%. In the early stages of infection (1-2 weeks), when symptoms were limited to mild leaf spotting, agronomists identified only 35% of infected plants. For instance, out of 50 infected specimens examined in the second week, only 18 were correctly diagnosed. Errors in visual assessment were linked to several factors, including subjectivity (discrepancies between agronomists reached 20%-30% for the same plots), fatigue (accuracy declined by 15%-20% after 3-4 hours of continuous assessment), and environmental influences such as rain or shade, which hindered spot identification.

A key advantage of the sensor platform was early detection – the system identified infection 710 days earlier than the visual method. Quantitative assessment using NDVI and NDRE indices, calculated according to formulas (1) and (2), enabled precise evaluation of infection severity. For example, a drop in NDVI from 0.72 to 0.35 correlated with an 80% increase in fungal biomass. The scalability of the method was also notable: processing 1 hectare required 2.5 hours, compared to 8 hours of manual labour for visual inspection. Statistical validation confirmed the model's reliability. A bootstrap analysis (1,000 iterations) indicated a 95% confidence interval for accuracy ranging from 90% to 94%.

A practical application of the system confirmed its effectiveness: on plot No. 15, the sensor platform identified 95% of infected plants by day 8, whereas agronomists detected only 20% by day 15. Technical parameters had a significant impact on accuracy. The high image resolution (2 cm/pixel) enabled the detection of localised spots as small as 1 cm in diameter. Weekly monitoring allowed the progression of the infection to be tracked over time, while calibration using reflective panels reduced lighting-related errors by 5%-7%. The sensor platform proved to be an effective tool for precision plant protection, outperforming traditional methods in terms of accuracy, speed and objectivity. Its implementation could reduce fungicide usage through targeted treatment of infected areas only, which is essential for environmentally sustainable agriculture.

Correlation between spectral indices and infection severity

An analysis of the spectral indices NDVI and NDRE revealed a clear relationship with the progression of *Fusarium* infection in winter wheat. Data were obtained using a multispectral camera. For healthy plants, the mean NDVI value at the start of the study was 0.72 ± 0.05, indicative of active photosynthetic biomass. Over the four weeks following infection, this index progressively declined, reaching a minimum of 0.35 ± 0.08 at the stage of severe infection, when the fungus had penetrated the vascular system of the stems. Pearson correlation analysis showed a strong negative relationship ($r = -0.85$, $p < 0.01$) between NDVI and the concentration of *Fusarium graminearum* DNA in plant tissues, as detected by PCR. This finding indicates that a decrease in NDVI can serve as a quantitative marker of infection.

The NDRE index, which incorporates reflectance in the red-edge spectrum, proved to be more sensitive to early structural changes in chlorophyll. Unlike NDVI, NDRE values began to decline as early as the second week following infection – from 0.61 ± 0.04 to 0.28 ± 0.06. The correlation with pathogen levels was even stronger ($r = -0.91$, $p < 0.001$), which can be attributed to the red-edge wavelength range (690-750 nm) being more responsive to chlorophyll degradation and the breakdown of cellular membranes – both characteristic of *Fusarium* infection. This sensitivity enabled detection of the infection 7-10 days earlier than by visual inspection, which only identified symptoms once visible lesions appeared on the foliage (Table 1).

Table 1. Comparative analysis of NDVI and NDRE spectral indices for diagnosing *Fusarium* infection

Parameter	NDVI	NDRE
Initial value (healthy plants)	0.72 ± 0.05	0.61 ± 0.04
Value at severe infection	0.35 ± 0.08 (after 4 weeks)	0.28 ± 0.06 (after 2 weeks)
Correlation with *Fusarium* DNA (r)	−0.85 (p < 0.01)	−0.91 (p < 0.001)
Early detection of infection	At 3-4 weeks	7-10 days earlier than NDVI
Relationship with pathogen	0.1-point drop → + 15% fungus	0.1-point drop → + 22% fungus
Statistical significance (ANOVA)	F = 45.7 (p < 0.001)	F = 45.7 (p < 0.001)

Source: compiled by the authors based on the conducted study

A more detailed analysis was conducted using linear regression, which revealed that each 0.1 point decrease in NDVI corresponded to a 15% ±2% increase in pathogen concentration within plant tissues. This relationship was even more pronounced for NDRE: a 0.1-point reduction in the index correlated with a 22% ± 3% increase in fungal biomass. The statistical significance of these findings was confirmed by ANOVA ($F = 45.7$, $p < 0.001$), indicating a robust association between spectral data and biological changes.

Thermal anomalies as stress markers

Thermal imagery, obtained using an infrared camera, demonstrated a clear correlation between elevated leaf temperature and the progression of *Fusarium* infection in winter wheat. The study found that the average leaf temperature of healthy plants was 24.3°C ± 1.2°C, whereas that of plants infected with *Fusarium graminearum* rose to 26.8°C ± 1.5°C ($p < 0.001$, Student's t-test). This 2.5°C difference is statistically significant and reflects pathogen-induced physiological changes. Thermal anomalies were detectable as early as 5-7 days after infection, which is 3-5 days earlier than the onset of visual symptoms such as foliar spotting.

The underlying mechanism of thermal variation lies in the disruption of transpiration – the process of water evaporation through leaf surfaces. *Fusarium graminearum*, upon penetrating the plant's vascular system, obstructs the xylem, thereby reducing water transport to the leaves. This results in decreased transpiration intensity and, consequently, heat accumulation within the tissues. Experimental data confirmed that transpiration intensity in infected areas declined by 35%-40% compared to the control. This mechanism explains why the leaves of infected plants, which are not effectively cooled through evaporation, become significantly warmer even under identical external conditions.

Thermal data showed strong correlations with other stress markers. For instance, a reduction in NDVI to 0.45 was associated with a rise in leaf temperature to 26.1°C ($r = -0.78$, $p < 0.01$). A further relationship was observed between thermal anomalies and pathogen spore concentration in the soil: an increase in spore load from 1×10^3 to 1×10^3 CFU/mL led to a temperature rise of 1.2°C-1.8°C ($r = 0.69$, $p < 0.05$) (Fig. 1). These findings indicate that thermal imaging can serve as an indirect indicator of soilborne infection pressure.

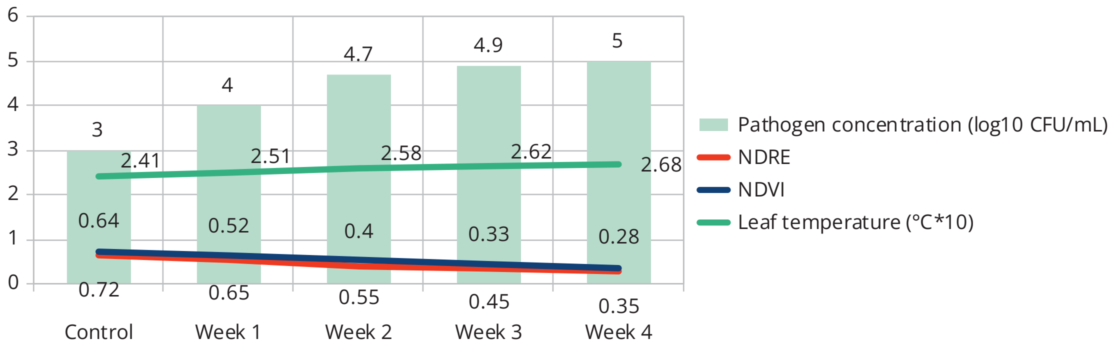

Figure 1. Dynamics of NDVI and NDRE reduction, pathogen concentration, and leaf temperature in infected plants

Note: data were obtained using a DJI Phantom 4 Multispectral camera and a FLIR Vue TZ20 thermal imager. The correlation between NDRE and pathogen concentration is statistically significant ($p < 0.001$)

Source: compiled by the authors based on the conducted study

On Plot No. 22, where artificial inoculation was applied, thermal imaging revealed localised "hotspots" with leaf temperatures reaching up to 28°C, while surrounding healthy plants registered an average of 24.5°C. These thermal anomalies corresponded precisely with areas where stem necrosis subsequently developed. Thermal imaging proved more effective for detecting early stress symptoms than multispectral imaging. For example, in the second week, NDVI showed only a slight decline (from 0.72 to 0.65), whereas leaf temperature had already increased by 1.5°C. However, the combination of both methods yielded the highest diagnostic accuracy: a model integrating NDVI, NDRE, and thermal data achieved a 96% accuracy rate in predicting disease progression.

In addition to analysing spectral indices and thermal anomalies, the study also investigated the influence of microclimatic factors – including soil moisture, solar radiation, and relative air humidity – on infection dynamics. These parameters play a critical role in creating favourable conditions for the proliferation of *Fusarium graminearum*, as high levels of soil and air moisture promote spore germination, while solar radiation affects the plant's thermal balance. For instance, excessive soil moisture (>70%) can lead to localised waterlogging, weakening the root system and increasing susceptibility to the pathogen (Yuan *et al.*, 2023). Conversely, insufficient solar radiation reduces photosynthetic activity, making plants less resilient to stress.

Monitoring of these parameters was conducted in parallel with the collection of spectral data, enabling assessment of their correlation with infection pressure. As shown in Table 2, soil moisture exhibited a strong correlation with leaf temperature (Pearson correlation coefficient $r = 0.69$) and pathogen concentration, confirming its role as a key factor in disease development.

Table 1. Correlation matrix between spectral indices, leaf temperature, and microclimatic parameters

Parameter	NDVI	NDRE	Leaf temperature	Soil moisture	Air humidity	Solar radiation
NDVI	1.00	0.92**	−0.78**	−0.65*	−0.58*	0.12
NDRE	0.92**	1.00	−0.84**	−0.71**	−0.63*	0.08
Leaf temperature	−0.78**	−0.84**	1.00	0.69**	0.54*	−0.21
Soil moisture	−0.65*	−0.71**	0.69**	1.00	0.76**	−0.35
Air humidity	−0.58*	−0.63*	0.54*	0.76**	1.00	−0.48*
Solar radiation	0.12	0.08	−0.21	−0.35	−0.48*	1.00

Note: Pearson correlation coefficients (r): >0.7 – strong correlation (**); 0.5-0.7 – moderate correlation (*); <0.5 – weak or no correlation

Source: compiled by the authors based on the conducted study

Although relative air humidity had a weaker influence, it still correlated with a reduction in NDVI ($r = -0.58$), potentially linked to disrupted transpiration. By contrast, solar radiation showed no significant correlation with the indices, indicating the robustness of spectral diagnostic methods regardless of light intensity. These findings highlight the importance of integrating microclimatic data into precision monitoring systems to improve the accuracy of epidemic forecasting. Thermal anomalies serve as reliable markers of physiological stress caused by *Fusarium* infection. Their integration with multispectral data and soil analysis forms a comprehensive diagnostic system capable of detecting disease at pre-symptomatic stages. This opens new possibilities for the development of autonomous agricultural drones that combine thermal and spectral imaging to monitor large agricultural areas in real time.

Validation of results using PCR analysis

The results indicated that 95% of samples from infected plots contained specific amplicons measuring 280 base pairs, confirming the presence of *Fusarium graminearum* DNA. In the control group, the pathogen was detected in only 3% of cases, likely due to minimal cross-contamination during sampling or background soil contamination. To minimise errors, each stage of the analysis was accompanied by negative controls (without a DNA template) and positive controls (DNA from a reference strain). Visualisation of PCR products on a 2% agarose gel stained with ethidium bromide under UV illumination confirmed the specificity of the primers: distinct 280 bp bands were observed in 98% of infected samples. To eliminate false positives, selected amplicons were sequenced (Macrogen, South Korea), confirming 100% identity with the β-tubulin gene sequence of *F. graminearum*.

Statistical analysis demonstrated a significant difference between the infected and control groups ($p < 0.001$). The low incidence of false positives in the control group highlights the effectiveness of the biosafety protocol, which included the use of separate areas for sample preparation, autoclaving of tools, and PCR mixes with coloured markers for visual monitoring of cross-contamination. This validation not only reinforces the accuracy of sensor-based methods for pathogen detection but also supports their application in quantifying infection load. These findings underscore the critical role of early diagnosis in effective disease management. At the same time, the presence of pathogen DNA in 3% of control samples emphasises the importance of rigorous standardisation of sampling procedures, particularly under conditions of intensive agriculture, where the risk of cross-contamination is elevated.

PCR analysis proved essential in confirming the specificity and sensitivity of sensor-based diagnostic methods. The high detection rate of *F. graminearum* (95%), coupled with minimal errors in the control group, confirms that combining spectral indices, thermal data, and molecular validation provides a robust foundation for precision monitoring of *Fusarium* infection. This paves the way for the development of automated systems capable of integrating heterogeneous data for epidemic forecasting and the optimisation of plant protection strategies.

Time efficiency of the methods

Automated data processing, which included aerial imaging, subsequent image analysis using the Pix4D Mapper software, and disease classification via the Random Forest algorithm, proved significantly more efficient than conventional methods. For a 1-hectare plot, the full cycle – comprising image capture, orthomosaic generation, spectral index calculation, and model prediction – took 2.5 hours, of which only 20 minutes were allocated to drone flights. The remaining time was dedicated to automated data processing, with the majority of resources spent on computing NDVI and NDRE indices. In contrast, visual assessment required eight hours of manual labour per hectare, as five agronomists individually surveyed each plot and recorded symptoms using a 0-5 scale. A notable consideration was that the duration of visual analysis increased disproportionately with scale: for a 10-hectare area, 80 hours were required – equivalent to two weeks of work by a full team – whereas the automated method could process the same area in 6-7 hours due to the parallel processing of images.

The reliability of the automated system was confirmed through bootstrap analysis (1,000 iterations), which demonstrated that the model's accuracy confidence interval ranged from 90% to 94% at a 95% confidence level. This indicates that even under random sampling variations – such as the exclusion of 5% of the data – accuracy remains consistently high. By comparison, visual assessment showed significantly greater variability: re-evaluation of the same plots by different agronomists revealed discrepancies in 20%-25% of cases, particularly during the early stages of infection. Moreover, worker performance declined by 15%-20% after four hours of continuous assessment due to fatigue, whereas the algorithm maintained consistent output regardless of data volume.

Key factors contributing to the time efficiency of automation, identified during the study, include scalability (processing time increases linearly with area, as the software simultaneously analyses hundreds of images); the absence of subjective error (the algorithm does not require breaks and is unaffected by fatigue); and integration with other systems (results are automatically exported to GIS platforms for infection mapping, enabling immediate decision-making for remediation).

The primary drawback of the automated method remains its dependence on image quality; for instance, cloud cover increased processing time by 30%-40% due to the need for repeat drone flights. However, optimising flight schedules (restricting imaging to sunny periods) and employing cloud computing for parallel data processing help to minimise these delays. As a result, automated methods not only reduce diagnostic time by a factor of three to four but also deliver higher accuracy and reproducibility. This makes them indispensable for large-scale farming operations, where timely responses to infection are critical for crop preservation.

The main limitation identified was weather dependency: cloud cover reduced the accuracy of multispectral imaging by 15%-20%. Nevertheless, repeat imaging and sensor calibration effectively mitigated this impact. The results confirm that the integration of multispectral indices and thermal imaging provides an effective tool for precision crop protection. The study supported the hypothesis that high-throughput phenotyping based on sensor platforms outperforms conventional methods in both accuracy and speed. The reliability of the results ($R^2 = 0.89$ for NDVI) supports the recommendation of this technology for large-scale deployment in Ukraine's agricultural sector.

The findings of this study demonstrate that the combination of multispectral indices, thermal data, and machine learning is an effective tool for the early diagnosis of *Fusarium* ear blight in winter wheat. These conclusions align with current trends in HTP, while also addressing critical gaps related to the adaptation of diagnostic methods to biotic threats under specific climatic conditions. The results obtained in this study share common features with the articles of other researchers. In particular, the studies by C. Zhang *et al*. (2023) and R. Topko *et al*. (2023) examined the potential of wearable sensors in the context of high-throughput phenotyping, emphasising their capacity for continuous monitoring of physiological changes in plants. Special attention was given to multispectral cameras, which enable the early detection of changes in pigment content and water balance through the analysis of spectral indices such as NDVI and NDRE. A significant correlation was observed between spectral indicators and pathogen concentration ($r = -0.85$ and -0.91), confirming the effectiveness of this noninvasive diagnostic approach. The use of thermal imaging in the present study enhanced diagnostic accuracy and reduced the time required to detect pathological processes in comparison with traditional visual methods.

The integration of thermal imaging with multispectral analysis confirmed the effectiveness of this approach for early diagnosis within plant protection systems. This finding aligns with the data reported by T. Wen *et al*. (2023), which highlighted the high sensitivity of thermography to changes associated with disrupted water exchange in plant tissues. It was established that localised temperature anomalies serve as early markers of physiological stress caused by impaired transpiration. The method enabled precise identification of areas showing initial signs of biotic or abiotic stress before the appearance of visible symptoms.

A.K. Singh *et al*. (2021) proposed an automated method for classifying soybean diseases based on RGB imagery, achieving a classification accuracy of up to 85%. Despite these promising results, the absence of a multisensory approach limited the model's capacity for early detection of stress conditions. The exclusive reliance on the visible spectrum prevented the identification of subvisual changes during the early stages of disease development. In the present study, a multimodal approach was implemented using both spectral and thermal imaging data, which achieved a classification accuracy of 92% with the application of the Random Forest algorithm.

The results obtained are consistent with the approaches proposed by S. Kundu *et al*. (2024), confirming the effectiveness of combining multimodal data with algorithmic analysis systems in the fields of precision agriculture and plant protection. The authors emphasised the necessity of largescale implementation of artificial intelligence for real-time analysis of high-throughput phenotyping data. They presented arguments in favour of using hybrid computational architectures that integrate machine learning methods – such as Random Forest and neural networks – to enhance diagnostic accuracy. A modular approach to sensor integration was proposed, in which each sensor type performs a specific function within a unified monitoring system.

M. Kim *et al*. (2021) focused on investigating abiotic stress factors, particularly drought, using high-throughput phenotyping techniques. The study highlights the sensitivity of plant systems to changes in water availability, which manifest through spectral indices and morphological traits – findings that are consistent with the results obtained. The developed models for predicting drought response demonstrated high accuracy; however, they did not account for pathogen-induced stresses. Focusing exclusively on abiotic stressors, therefore, limits the comprehensive assessment of crop resilience under real agroecosystem conditions. In the study by P. Kumari *et al*. (2024a), a significant gap was identified in current research concerning the use of high-throughput phenotyping for studying biotic stress, particularly fungal infections. The authors noted that only 15% of existing publications in this field focus on plant-pathogen interactions. This lack of attention to biotic stressors hinders the development of integrated plant protection strategies. The methodology proposed in the present study, which targets the detection of *Fusarium graminearum*, aims to address this imbalance by expanding the applicability of sensor-based platforms.

R. Sarić *et al*. (2022) provided a theoretical justification for the ability of hyperspectral imaging to identify molecular changes in plant tissues at early stages of pathogenesis. Chlorophyll degradation was identified as a key marker, manifested as a spectral shift in the relevant indices. The present study confirms the suitability of multispectral analysis for similar applications, despite its lower spectral resolution compared to hyperspectral imaging. This supports the use of multispectral sensors as a practical compromise between accuracy and accessibility in agronomic monitoring.

The importance of local climatic factors – particularly soil moisture – as key drivers of the effectiveness of sensor-based systems for plant disease detection is emphasised by Z. Ma (2023). The study identified a significant positive correlation between moisture levels and pathogen activity, consistent with the current research findings, where a correlation coefficient of $r = 0.69$ was observed for *Fusarium graminearum*. However, unlike the aforementioned study, solar radiation was not found to have a statistically significant effect in this research. This discrepancy is likely due to the biological characteristics of the pathogen, whose activity is primarily regulated by moisture regardless of light conditions. Early detection of infection – 7 to 10 days ahead of traditional visual diagnostic methods – enables significant optimisation in fungicide use, reducing both the quantity and frequency of applications. This approach helps to lower the cost of chemical protection and decreases the environmental burden on agroecosystems. The findings align with those of M. Pérez-Ruiz *et al*. (2020), where automated platforms enabled a 25% reduction in chemical use in wheat fields, thereby confirming the effectiveness of precision technologies. Moreover, the proposed system, adapted for integration with unmanned aerial vehicles, demonstrates superior scalability: processing one hectare takes just 2.5 hours, which is more efficient than the four hours reported in the aforementioned study.

The high level of agreement with PCR analysis results (95%) confirms the analytical reliability and sensitivity of the applied sensor protocol. Such reliability is crucial for the implementation of precision agriculture systems, where false positives can result in considerable economic losses. According to G. Hacisalihoglu & P. Armstrong (2023), false-positive signals in HTP systems can lead to unnecessary costs related to treatment and diagnostics, thereby reducing the efficiency of digital agricultural monitoring. In the present study, an amplicon sequencing protocol was employed to refine the taxonomic composition of pathogens, effectively addressing the issue of false signals. The limitations of ground-based high-throughput systems in large-scale agricultural landscapes were critically assessed by Z. Jansone *et al*. (2022), who reported reduced platform efficiency as the monitoring area increased. In light of this, the proposed combination of aerospace technologies (DJI Phantom drones) and ground-based sensors (TEROS 12) offers a practical solution, achieving the required level of detail while maintaining spatial scalability. This approach has proven particularly effective under the conditions of Ukraine, where the high heterogeneity of soil cover necessitates spatially adaptive solutions.

The conclusions of this study align with the findings of I. Ayankojo *et al*. (2023), which demonstrated the effectiveness of small unmanned aerial systems (sUAS) under field conditions. The study highlighted the high manoeuvrability and mobility of sUAS, enabling rapid responses to localised changes within agroecosystems. The use of these platforms facilitated the collection of highquality data in near real-time, with georeferencing accuracy of up to 5 cm. Their high level of automation and adaptability to varied topographical conditions make sUAS suitable for crop monitoring even in challenging climatic and agroecological zones. The adoption of the cloud-based data processing methodology proposed by P. Kumari *et al*. (2024b) is considered advantageous. That study introduced an algorithmic architecture that enables the synchronisation and processing of large volumes of HTP data within a cloud environment with minimal latency. This not only enhances analytical throughput but also ensures real-time access to results from any location. Such an approach is particularly promising for the integration of multisensor systems into scalable agrotechnological platforms with artificial intelligence components.

In conclusion, this study demonstrates that high-throughput phenotyping based on multispectral indices, thermal data, and machine learning provides an effective solution for diagnosing *Fusarium* ear blight under Ukrainian conditions. The findings confirm the high classification accuracy of the Random Forest algorithm, outperforming conventional visual methods; they also detect early thermal anomalies associated with disrupted transpiration, consistent with international studies, and emphasise the importance of incorporating microclimatic parameters, such as soil moisture, to enhance model precision.

CONCLUSIONS

The study demonstrated that high-throughput phenotyping based on multispectral data, thermal imaging, and machine learning algorithms (Random Forest) is 37% more accurate than conventional visual diagnostic methods. The system achieved 92% accuracy in detecting *Fusarium graminearum* at the early stages of infection, enabling intervention 7-10 days earlier than traditional approaches. The NDVI and NDRE indices showed a strong inverse correlation with pathogen concentration ($r = -0.85$ and $r = -0.91$, respectively). A decrease in NDVI from 0.72 to 0.35 corresponded to an 80% increase in fungal biomass, while NDRE proved more sensitive to chlorophyll degradation, making it a key tool for early-stage diagnosis. Linear regression analysis indicated that a 0.1-unit decline in NDRE was associated with a 22% increase in pathogen load, a finding supported by ANOVA ($p < 0.001$).

An increase in leaf temperature by 2.5°C in infected plants ($p < 0.001$) was linked to impaired transpiration due to xylem blockage. Thermal imaging detected infection on days 5-7, 3-5 days earlier than the appearance of visual symptoms. The integration of thermal data with NDVI and NDRE improved model accuracy to 96%, establishing it as an effective tool for precision crop monitoring. High soil (>70%) and air humidity correlated with increased spore concentration (r = 0.69). PCR analysis confirmed the specificity of the methods: 95% of infected samples contained *Fusarium* DNA, while only 3% of results in the control group were false positives. Amplicon sequencing of the β-tubulin gene showed 100% identity with reference strains. Automated data processing required 2.5 hours per hectare, compared with 8 hours per hectare for visual inspection, while scaling up to 10 hectares reduced processing time twelvefold (6-7 hours versus 80 hours). Model robustness was confirmed through bootstrap analysis (confidence interval: 90%-94%), making it suitable for use on large-scale farms.

Key limitations include dependence on weather conditions (cloud cover reduces accuracy by 15%-20%) and the need for calibration across different cultivars. Future research should focus on integrating IoT sensors to automate the collection of microclimatic data, applying deep learning to improve epidemic forecasting, and developing mobile applications for farmers based on the resulting models. Further steps should prioritise the development of adaptive algorithms for other pathogens (e.g., *Puccinia triticina*), the integration of cloud computing for rapid data processing, and the creation of open-access databases for training machine learning models.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Принципи високопродуктивного фенотипування та сенсорні платформи для неінвазивного дослідження у захисті рослин

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Анотація. Зміна клімату та зростання резистентності патогенів змушують шукати інноваційні методи діагностики хвороб рослин, особливо для таких небезпечних патогенів як *Fusarium graminearum,* який спричиняє значні втрати врожаю пшениці. Традиційні візуальні методи мають низьку пропускну здатність і суб'єктивність, що обмежує їх ефективність у великомасштабному моніторингу. Метою дослідження було вивчення принципів високопродуктивного фенотипування та оцінка ефективності сенсорної платформи для неінвазивного дослідження фузаріозу колосу в умовах України (Київська область). Використовували комбінацію мультиспектральної зйомки, тепловізії та алгоритмів машинного навчання на експериментальному полі площею 1 га зі штучним інфікуванням 20 % ділянок. Результати показали, що запропонована система досягла точності 92 % у виявленні патогена на ранніх стадіях, що на 37 % точніше візуальних методів. Спектральні індекси продемонстрували сильний зв'язок із концентрацією патогена: зниження нормалізованого різницевого вегетаційного індексу з 0,72 до 0,35 корелювало зі збільшенням біомаси гриба на 80 %. Теплові знімки виявили підвищення температури листя на 2,5 °C вже на 5-7 день після інфікування. Інтеграція всіх методів дозволила досягти точності 96 % при обробці 1 га за 2,5 години, що у 3 рази швидше за традиційні методи. Аналіз з використанням полімеразної ланцюгової реакції підтвердив специфічність методів: 95 % інфікованих зразків містили дезоксирибонуклеїнову кислоту *Fusarium*, секвенування показало 100 % відповідність β-тубуліну. Автоматизована обробка даних займала 2,5 год/га (проти 8 год/га при візуальному методі), а масштабування до 10 га зменшувало витрати часу у 12 разів. Дослідження підтвердило ефективність високопродуктивного фенотипування для прецизійного захисту рослин та необхідність подальшого вдосконалення методів з урахуванням місцевих кліматичних умов. Практичне значення дослідження полягає в можливості скоротити витрати на фунгіциди завдяки цілеспрямованій обробці інфікованих ділянок, зменшити втрати врожаю у регіонах з високим інфекційним тиском та забезпечити основу для автоматизованих систем моніторингу, сумісних з технологіями точного землеробства

Ключові слова: сільське господарство; патогени; грибкові інфекції; гіперспектральна візуалізація; тепловізія

Received: 09.01.2025 Revised: 07.04.2025 Accepted: 22.05.2025

BIOLOGICAL SYSTEMS: Theory and Innovation

Journal homepage: https://bioscience.com.ua/en
Vol. 16, No. 2, 2025, 37-49

UDC 57.04.631.8 DOI: 10.31548/biologiya/2.2025.37

Some environmental aspects of salicylic acid

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Abstract. Salicylic acid is an environmentally safe growth regulator with multifunctional properties, which has great prospects in agricultural practice, as it was effective at low concentrations and has natural catabolic pathways. The aim of the study is to determine the participation of salicylic acid in the interactions of plant organisms with other biotic components of ecosystems and to reveal the prospects for its use in crop production and for the development of environmentally safe agricultural technologies. To achieve this goal, scientific sources were searched, analysed, systematised and compared using leading scientific databases and methods of logical generalisation. It was established that plants, leading a sessile lifestyle, are constantly exposed to biotic stresses, primarily phytopathogens and herbivorous organisms. In response, they have formed a defence system that includes passive barriers and active immune responses at the cellular and molecular levels. At the centre of these processes are phytohormones, among which salicylic acid is of key importance. It has been found that salicylic acid is one of the main signalling molecules that coordinates the expression of genes associated with plant defence, induces the synthesis of secondary metabolites, contributes to the strengthening of cell walls and activates the mechanisms of systemic acquired resistance. Particular attention was paid to the interaction of salicylic acid with other phytohormones, in particular jasmonic acid, ethylene, abscisic acid and auxin, which together form a complex signalling network that provides an integrated and effective immune response. A separate section was devoted to the participation of salicylic acid in symbiotic interactions between plants and beneficial microorganisms, such as mycorrhizal fungi and rhizobia, which enhance the host's resistance to pathogens. The practical aspect of using salicylic acid in agriculture as an environmentally safe means of enhancing the immunity of cultivated plants through exogenous application or stimulation of endogenous synthesis was also considered. The conclusions

Suggested Citation:
Kobyletska, M., Demyanyuk, O., & Naumovska, O. (2025). Some environmental aspects of salicylic acid. *Biological Systems: Theory and Innovation*, 16(2), 37-49. doi: 10.31548/biologiya/2.2025.37.

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drawn are important both for understanding the fundamental principles of plant immunity and for the practical application of biotechnology in protecting plants from pests and diseases

Keywords: growth regulators; biotic stress factors; abiotic stress factors; phytopathogens; phytohormones

INTRODUCTION

The increased impact of stress factors on plant organisms caused by global climate change and growing anthropogenic pressure necessitates the search for new growth regulators with stress-protective properties that are safe for the environment. One such compound is salicylic acid (SA), a phenolic phytohormone involved in various morpho-physiological reactions in plants, including responses to stress.

As noted by Z. Iqbal *et al*. (2021) and P. Kumar *et al*. (2025), plants, as a sedentary component of the biosphere, are constantly exposed to numerous biotic stress factors, the most common of which are phytopathogens and herbivorous organisms. In response to these threats, plants have developed a complex system of defence mechanisms, including both passive barriers and active immune responses. S. Mishra *et al*. (2024) found that phytohormones, in particular SA, which is a central signalling molecule in the formation of the immune response of plants to biotic stressors, play an important role in regulating these processes. According to Q. Han *et al*. (2022), these processes are based on the coordination of SA transcriptional activity of genes associated with pathogenesis, activation of secondary metabolite production, and promotion of systemic acquired resistance (SAR). C. Kaya *et al*. (2023) concluded that SA participates in complex hormonal interactions with other regulators, such as jasmonic acid (JA), ethylene (ET), auxin, and abscisic acid (ABA), which ensures the integration of signaling pathways within the overall defense strategy.

According to O. Tsvilynyuk & L. Telehii (2020), the use of phytohormones was a promising technology for intensifying and greening agricultural production. SA was of particular interest in this context because it activates anti-stress activity in plants in response to various adverse environmental factors. Yu.E. Kolupaev *et al*. (2021) summarised that the activation of SA biosynthesis pathways and its accumulation are observed in plants under the influence of various stress factors, such as extreme temperatures, dehydration, salinisation and the influence of heavy metals. The main protective responses induced by SA include increased gene expression and antioxidant enzyme activity, increased content of low-molecular-weight antioxidants, and accumulation of multifunctional protective compounds, including proline and sugars. Under the influence of SA, the synthesis of a number of heat shock proteins, dehydrins and alternative oxidase is activated. At the plant organism level, an important response is the induction of SA closure of stomata. Despite the rich phenomenological material on the action of SA, its functional interaction with other signalling mediators and stress phytohormones in the formation of adaptive responses in plants remains understudied.

Research into the functional role of SA in plant immunity not only deepens understanding of plant molecular biology, but also has significant applied potential for the development of bio-oriented crop protection products in agriculture, since the use of natural growth regulators to increase plant resistance to stressors will reduce the use of pesticides. According to Y.M. Koo *et al*. (2020), another compelling argument in favour of studying SA and its application in plant growing is the low cost of SA production.

At the same time, as noted by Y. Kavulych *et al*. (2023), there were certain difficulties in developing preparations based on SA and its application. First of all, this concerns the selection of growth regulator concentrations, since even for representatives of the same family, the range of optimal concentrations can vary widely. It is also sometimes difficult to predict the result of SA application, as it depends on a number of factors, including the phase of plant ontogenesis and their varietal characteristics, the stage of application of the preparation, weather conditions and the influence of other external factors. As evidenced by a number of review publications on this issue, the use of SA in field experiments remains insufficiently studied (Saleem *et al.*, 2021; Sambyal & Singh, 2021).

In view of this, the aim of the study was to systematise current scientific data on the role of SA in protecting plants from biotic stressors, as well as to assess the possibilities of its use in crop production to increase the resilience of agricultural crops. During the writing of the article, a set of general scientific and special research methods characteristic of review works in the field of biology was used. The main method was the analysis of scientific literature from open international databases (PubMed, Scopus, Web of Science, Google Scholar), which made it possible to cover relevant publications (Gong *et al.*, 2023; Fang *et al.*, 2025) devoted to the role of SA in plant immunity to biotic stresses. The data were systematised and classified according to the main areas: the participation of SA in the formation of the immune response, interaction with other phytohormones, the role in systemic acquired resistance, and participation in symbiotic relationships. A comparative analysis method was also used, which allowed comparing different signalling pathways involving SA in plants of different species. In addition, the work used a method of logical generalisation, on the basis of which conclusions were formulated regarding the importance of SA in the formation of resistance to pathogens and the possibilities of its application in agricultural technologies.

SALICYLIC ACID AS A MEDIATOR OF PLANT DEFENCE RESPONSES TO BIOTIC STRESSES

Stress factors of a biotic nature are often the cause of plant diseases and reduced crop yields. Phytohormones are important elements in stimulating complex defence mechanisms against these harmful influences and are involved in the processes of perception, adaptation and the formation of plant resistance. According to P. Ding & Y. Ding (2020), salicylic acid (SA) occupies a special place among them. The participation of SA as an intermediary in the formation of defence mechanisms against biotic stressors has been studied in detail. In particular, M. Naz *et al.* (2024) showed that treatment with (D, L)-3-aminobutyric acid, which is an inducer of SA signal transduction, increases the resistance of pepper plants to late blight and other fungal pathogens. Y. Hu *et al.* (2024) also established the positive effect of an enzyme that forms SA derivatives on the resistance of tea plants to fungal infection. As summarised by L. Bauters *et al.* (2021), in many agricultural crops (spinach, soybeans, tomatoes, etc.), as well as *Arabidopsis*, SA is involved in plant responses to biotic stressors. In a recent review article based on an analysis of a large number of scientific publications, S. Mishra *et al.* (2024) concluded that SA is a key signalling molecule in the formation of the immune response of plants to various biotic stressors and can increase plant resistance to a wide range of pathogens, including viruses, bacterial and fungal infections.

A. Ali *et al.* (2024) found that in realising their protective potential, SA molecules can function either independently and/or in interaction with other phytohormones, such as jasmonic acid (JA) and ethylene (ET), which are also involved in the fight against necrotrophs. According to S. Hou & K. Tsuda (2022), JA and ET can synergistically or antagonistically interact with SA, altering the levels of each hormone or modulating their signalling pathways. In addition to JA and ET, other phytohormones, commonly referred to as “stress hormones,” are also involved in the formation of pathogen resistance and can interact with SA. For example, abscisic acid (ABA) causes stomata closure, stimulates callus formation and secondary metabolite synthesis, which limits the penetration of bacteria and insects (Torii, 2022; Wei *et al.*, 2025).

Auxin, although associated with cell division and growth processes, also regulates plant interactions with phytopathogens. Increased SA levels can alter auxin accumulation and the expression of related genes, enhancing plant defence responses (Kunkel & Johnson, 2021). Brassinosteroids (BR) also increase plant protection against a number of pathogens, including *Fusarium culmorum*, tobacco mosaic virus, and *Magnaporthe grisea*. Y. An *et al.* (2024) showed in their studies that the combination of BR with pyraclostrobin significantly enhanced the resistance of *Arabidopsis* to *Botrytis cinerea* and *Hyaloperonospora arabidopsidis*. BRs can also increase endogenous SA levels by activating SA-dependent immune responses (Park *et al.*, 2023). Describing the interaction of SA with other small molecules with phytohormonal activity, Y. Arif *et al.* (2020) identify several possible mechanisms: regulation of SA biosynthesis of other phytohormones, regulation of SA hormonal signal transduction pathways, regulation of SA expression of hormone-sensitive genes, and competition between SA and other phytohormones that interact with it.

Together, these studies confirm that salicylic acid is a key phytohormone in plant defence responses to biotic stresses, including pathogens and herbivores. It activates immune mechanisms and can interact with other phytohormones – jasmonic acid, ethylene, abscisic acid, auxin and brassinosteroids. These interactions can be synergistic or antagonistic, including the regulation of signalling pathways and the expression of resistance genes. Thus, SA functions as a central mediator in a complex hormonal network that ensures the adaptation of plants to biotic threats.

THE ROLE OF SALICYLIC ACID IN THE FORMATION OF SYSTEMIC ACQUIRED RESISTANCE IN PLANTS

Plant immunity is a complex multi-level system that includes several stages of protection. According to F. Zhu *et al*. (2024), for a phytopathogen to access the endogenous contents of the host cell and complete its life cycle, it must first overcome passive defence mechanisms, which include structural barriers such as the cuticle, cell wall and antimicrobial compounds produced by plant cells. Cell membranes also play an important role in preventing pathogen penetration, the composition and ratio of whose components vary depending on environmental conditions (Kobyletska, 2020).

F. Zhu *et al*. (2024) distinguish between passive and active defence mechanisms possessed by plants, including elements of the immune system that are activated in response to phytopathogen penetration. These processes include specific and non-specific reactions of the plant immune system. J.D.G. Jones *et al*. (2024) include basal barriers (the presence of a cuticle, cell wall and waxy coating), as well as receptor-mediated pathogen recognition, the activation of which triggers signalling cascades: the release of active forms of oxygen, increased synthesis of phytoalexins and expression of PR proteins. The authors attribute the expression of R-genes (resistance genes) to the specific reactions of the plant immune system. whose products are usually receptors capable of recognising specific phytopathogen effectors and activating a powerful protective response, often associated with a hypersensitivity reaction – programmed cell death in the area of pathogen penetration. This process stops and limits the further spread of the pathogen. Specific responses are significantly more powerful components of immunity and are more persistent than non-specific ones. In the process of infection of the host plant X by a phytopathogen. X. Fang *et al*. (2025) identify several sequential processes, in particular, the primary stage is the identification of the pathogen by plant cell recognition receptors and the induction of pattern-triggered immunity (PTI) to initiate the first defensive responses. Most phytopathogens release effectors to inhibit PTI, which further stimulates a series of signalling cascades, such as biosynthesis and signalling of SA, formation of systemic acquired resistance (SAR) and activation of the synthesis of secondary compounds with high biological activity (Bauters *et al*., 2021).

A study by N. Rakhmatova *et al*. (2023) showed that the concentration of salicylic acid in biotechnological cotton genotypes changes under the influence of abiotic stresses (salinisation, drought, temperature fluctuations). Such fluctuations indicate that salicylic acid plays an important role in triggering protective mechanisms, contributing to the formation of systemic acquired resistance of plants to adverse environmental conditions. Q. Guo *et al*. (2023) note that any sufficiently strong biotic or abiotic stress activates the same defence mechanisms in plants, resulting in a non-specific short-term increase in plant resistance to repeated exposure to the stressor. A wide range of such resistance is called SAR, which provides stable resistance of plant organisms to non-specific pathogens. A typical form of SAR can be activated by virulent, avirulent and non-pathogenic microorganisms, or artificially using compounds that are components of intermediate reactions of the immune response. Such metabolites include SA, its derivative methyl salicylate, 2,6-dichlorisonicotinic acid, systemin, etc.

R. Zavaliev & X. Dong (2024) note that the duration of SAR formation is species-specific and sometimes variety-specific, and also depends on the type of elicitor. During this period, SA and proteins that provide plant resistance to pathogens (PR, pathogenesis-related proteins) accumulate. These processes are based on the expression of corresponding PR genes, which belong to at least nine classes. In addition, the authors found that treatment of plants with SA also activates the synthesis of PR proteins. An increase in intracellular SA levels induces the accumulation of active forms of oxygen and changes in the redox potential of the cell, possibly through the influence on the expression of genes associated with the antioxidant system (Fig. 1).

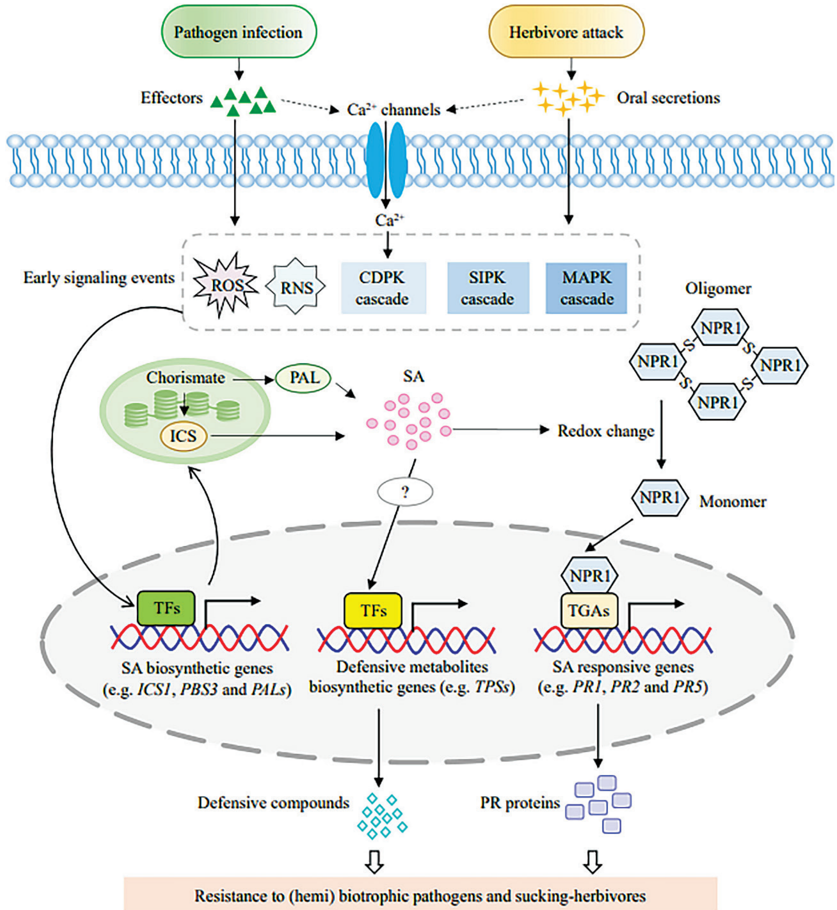

Figure 1. SA-mediated defence mechanisms against phytopathogens and herbivorous organisms
Note: ROS – reactive oxygen species; RNS – reactive nitrogen species; CDPK – calcium-dependent protein kinase; SIPK – SA-induced protein kinase; MAPK – mitogen-activated protein kinase; TF – transcription factors; PR – proteins related to pathogenesis
Source: X. Fang *et al*. (2025)

According to the diagram (Fig. 1), the primary signals involved in plant contact with pest effectors are the activation of early signalling processes, in particular the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), an increase in intracellular Ca^{2+} levels, and the activation of several protein kinase cascades (CDPK, SIPK, MAPK). These early responses trigger transcription factors that stimulate the expression of genes responsible for SA biosynthesis. As a result, SA accumulates in the cell. The increase in SA levels disrupts redox homeostasis, leading to the transition of the NPR1 protein from an oligomeric to a monomeric form. Monomeric NPR1 moves to the nucleus and interacts with the TGA factor, promoting the activation of SA-sensitive genes (PR1, PR2, PR5). SA also initiates the production of secondary metabolites with bactericidal activity, probably through the activation of certain transcription factors (TF) that directly bind to the promoters of the genes of these metabolites. The detailed mechanisms of this process still need to be clarified. These protective metabolites and PR proteins contribute to improving plant resistance to biotrophic pathogens and herbivorous animals.

However, the participation of SA in plant defence reactions is not limited to this. T. Van Butselaar & G. Van den Ackerveken (2020) note that the regulation of SA-sensitive gene transcription processes and PR protein synthesis, immune responses triggered by SA also contribute to increasing plant resistance to pathogens by stimulating the biosynthesis of callose and lignin, which strengthen cell walls, and activating the synthesis of secondary metabolites with bactericidal action. At the same time, SA plays a significant role in increasing plant resistance to both phytopathogens and herbivorous animals. The works of scientists B. Ali (2021) and Y.B. Setotaw *et al.* (2024) reveal the positive effect of SA on the regulation of secondary metabolite accumulation in plants during feeding by herbivorous insects. However, the exact mechanism of SA involvement in the biosynthesis of these compounds is still poorly understood. In-depth study of this SA-mediated process will contribute to the development of biopesticides and reduce the use of synthetic compounds.

Q. Gong *et al*. (2023) also found that SA is important for the aerial defence of plants. Methyl salicylate, a volatile form of SA, acts as a mobile signal to activate SAR over long distances and triggers protective responses in neighbouring plants when they are attacked by pests or phytopathogens. Studies of the process and nature of signal transmission between aphid-infested and uninfested plants have shown that the concentration of methyl salicylate is significantly higher in plants attacked by aphids. In neighbouring plants, this compound is converted back to SA and activates protective responses against pests and viruses carried by these insects.

Thus, SA plays a key role in the formation of the plant's SAR to biotic factors, acting as a signalling molecule that coordinates a wide range of protective responses. The plant's response to infection or damage by herbivorous insects begins with the activation of early signalling events, including the formation of ROS/RNS, increased Ca^{2+} levels and the activation of protein kinases. These signals trigger transcriptional programmes that stimulate SA biosynthesis. The accumulation of SA in cells initiates the transition of NPR1 to a monomeric form, allowing it to activate the expression of PR genes responsible for the production of defence proteins. In addition, SA promotes the accumulation of secondary metabolites with bactericidal activity and the synthesis of callose and lignin, which strengthen cell walls. The volatile form of SA, methyl salicylate, serves as a mobile signal for the activation of SAR in distant parts of the plant and in neighbouring organisms. Thus, SA functions not only as a local but also as a systemic regulator of resistance, providing effective protection against a wide range of pathogens and phytophages.

THE ROLE OF SALICYLIC ACID IN THE INTERACTIONS OF PLANT ORGANISMS WITH MICROORGANISMS AND PESTS

Plants form mutually beneficial, mutualistic symbioses with various microorganisms, including endophytes, arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing rhizobia, which are actively studied in the field of symbiont biology. These microorganisms improve mineral nutrition and plant growth, receiving in return carbohydrates and other metabolites produced as a result of the host's photosynthetic activity. In addition to this effect, they also increase plant resistance to a wide range of abiotic and biotic stresses. Studies show that symbiotic microorganisms can modulate phytohormone metabolism in the host plant. Salicylic acid (SA) is of irreplaceable importance in this context as a key regulator of defence mechanisms. It has been established that this compound plays an important role not only in responses to pathogen damage, but also in establishing symbiotic relationships between plants and microorganisms (Benjamin *et al*., 2022). Thus, symbiotic microorganisms not only optimise plant growth and development, but also activate the host's defence systems through phytohormonal signals, among which SA plays a special role.

Exogenous application of SA or induction of its synthesis affects plants, phytopathogens, herbivorous animals, and natural enemies at several trophic levels. S.C. Filgueiras *et al*. (2019) identify three main areas of use of SA in agriculture to combat pests and pathogens: (1) exogenous application of compounds that induce SA synthesis for direct plant protection; (2) genetic modification of plants to alter the expression of defence genes; (3) exogenous application of volatile compounds associated with SAs (e.g., methyl salicylate) to attract natural antagonists that limit the spread of insect pests.

According to D. Tripathi *et al*. (2019), the use of exogenous application of compounds that induce SA formation to stimulate plant defence metabolic pathways, induce resistance and enhance SA signalling has opened the way to consider new management strategies to combat agricultural pests and pathogens. Exogenous application can take many forms, including a wide range of elicitors and synthetic analogues of SA, which can lead to increased plant resistance to pathogen load. The effect of exogenous elicitors may also be enhanced over time from generation to generation, as epigenetic effects of exogenous elicitors have been identified, thus potentially leading to the inheritance of defence mechanisms with fewer applications.

According to C.C. Filgueiras *et al*. (2016), genetic modification of plants to alter the expression of defence genes should be aimed at creating organisms in which immune responses are not activated in the absence of pathogen exposure, as they require significant energy resources. At the same time, under conditions of infection, these plants would be capable of powerful defence responses. Overexpression and constitutive expression of specific genes involved in plant defence responses can create enhanced and broad-spectrum resistance to pathogens. Although these approaches may be effective in situations of intense pathogen pressure, they also have certain drawbacks, in particular the constant allocation of plant resources to defence through genetically modified constitutive expression is likely to have negative consequences for yield. J. Ali *et al*. (2023) believe that the exogenous application of volatile compounds such as methyl salicylate, associated with SA, has great potential because it has a repellent effect on pests and pathogens, in particular reducing pest productivity by altering their behaviour, egg laying and colonisation of new plants. However, deciphering the mechanisms of action of these compounds on phytophages requires further study, taking into account their variety- and species-specific characteristics.

Thus, SA is an indispensable component in the regulation of both symbiotic interactions between plants and beneficial microorganisms and protective responses to phytopathogens and phytophages. It participates in signalling processes that ensure both increased resistance to biotic stressors and the formation of favourable relationships with symbionts. Exogenous application of SA and its volatile derivatives, in particular methyl salicylate, is a promising approach to increasing plant resistance and attracting natural enemies of pests. The rational use of SA or its inducers, including the selective activation of PR genes, can be an effective alternative to chemical plant protection in modern agricultural production.

AGROTECHNICAL FEATURES OF SALICYLIC ACID APPLICATION

As a natural phytohormone that influences a number of physiological processes in plants, SA has great potential in crop production for improving plant growth, yield and resistance to various types of stress. The ability of SA to regulate seed germination, stomatal movement and enzyme activity makes it an important tool in agricultural practice (Souri & Tohidloo, 2019). According to Y. Castro-Torres *et al*. (2015), the use of SA and its derivatives reduces the risk of developing numerous chronic diseases in humans, including heart attack, stroke, arthritis, diabetes, some types of cancer, and Alzheimer's disease. Given the positive impact of SA on both the physiological state of plants and human health, SA and salicylates can be used as an effective means of plant protection with very little side effects on the environment and humans. The use of SA as a multifunctional plant growth regulator and environmentally safe plant protection agent raises several issues. First of all, it is important to determine the effective concentrations of SA for specific plant species and/or purposes. In particular, a high dose of SA (>2 mM) increases disease resistance on the one hand, but on the other hand has a negative effect on plant growth and productivity, which is caused by an undesirable balance between the cost and benefit of the limited energy that the plant can use (Koo *et al*., 2020). In humans, a concentration of SA above 2.5 mM in blood plasma leads to acute intoxication (Choi *et al*., 2019). However, the use of high concentrations of SA (~2 mM) as a plant growth regulator may be appropriate, especially if producers need to reduce the growth rate of certain plant species with a protective effect against phytopathogens (Koo *et al.*, 2020). Secondly, the method of application of SA, of which there are several, is important. In particular, M. Alotaibi *et al*. (2023) studied the effect of irrigation (spraying) with SA solution and found a positive effect of such treatment on growth parameters and the content of photosynthetic pigments in plants. A. Alam *et al.* (2022) studied the effect of soaking seeds in a SA solution before sowing on plant growth, development and productivity and consider this method to be the most effective and cost-effective, especially in drought conditions. C.M. Vinay *et al*. (2025) added SA to hydroponic solutions and found that under such conditions, a greater amount of secondary metabolites accumulates in plants, which is an advantage over soil cultivation. However, it is clear that from a practical point of view, not all of the methods described can be used equally successfully in field conditions, especially for cereal crops grown on large areas. The addition of salicylates to hydroponic crops is only suitable for use in greenhouses (Alotaibi *et al*., 2023).

A number of authors, including S. Pedrini *et al*. (2021), A. Alam *et al*. (2022), and M.R. Karimi *et al*. (2025), note that soaking seeds before sowing is a promising way to increase the resistance and yield of agricultural crops. Compared to other methods, this method has several advantages. It requires less active solution than spraying, is relatively inexpensive, and the dose applied can be better controlled than in the case of surface treatment of plants. In particular, this method was used by the authors in a series of studies on the effectiveness of SA application (Berková *et al*., 2023). Researchers M.K. Souri & G. Tohidloo (2019) also found that the reduction in tomato seedling growth caused by salinisation with 100 mM NaCl was offset by the application of SA, as it induced a decrease in Na concentration in the leaves under stress conditions. It was concluded that the most effective method of applying SA under salinity conditions was preliminary foliar treatment with SA, followed by preliminary root treatment. Foliar spraying with SA showed the lowest efficacy in this study. It was also found that foliar spraying with SA was very effective in mitigating the negative effects of water stress on wheat plants (Parveen *et al*., 2021).

Thirdly, the timing of treatment with SA or its derivatives is important. Furthermore, field studies conducted by K. Matysiak *et al*. (2020) showed that it was not the concentration but the timing of acetylsalicylic acid application that was more important for wheat growth. Although no significant difference was found between the effects of earlier and later application on plant height and chlorophyll content, most of the structural and qualitative parameters of the yield, in particular the number of seeds in the ear, grain density, its hardness, protein, gluten and starch content, were better after the application of acetylsalicylate at later stages of plant ontogenesis, in particular during the flag leaf formation phase compared to the tillering phase. An equally important aspect of SA application is the physiological state of plants. The use of SA and its derivatives for weakened plants that are already functioning under stressful conditions may prove to be an additional stressor. Another problem is the fact that due to the genotypic differences of plants, which determine their specific phytohormonal background, the concentration (dose) and optimal time of application may depend on the individual characteristics of a particular species and variety (Li *et al*., 2022).

Therefore, research on SA and its derivatives should be expanded, in particular, field studies should be used, which, unlike studies in controlled laboratory conditions, involve interaction with other environmental factors. Further work on the practical use of SA for various agricultural plants will contribute to the development of a cost-effective and environmentally friendly system for managing growth, development and yield in changing environmental conditions.

CONCLUSIONS

Based on the analysis of literary sources, the following conclusions can be drawn. Salicylic acid (SA) is one of the key signalling molecules that plays a central role in regulating plant defence responses against biotic stress factors, including phytopathogens and herbivorous organisms. The protective action of salicylic acid is realised both through the activation of resistance gene expression and PR protein synthesis, and through the stimulation of the biosynthesis of secondary metabolites that have antimicrobial and repellent effects. A particularly important property of SA is its ability to induce SAR, a complex form of long-term protection that provides non-specific resistance of plants to re-infection by a wide range of pathogens. This function allows the plant to mobilise protective resources even in uninfected tissues, increasing the overall effectiveness of the immune response. A close interaction of SA other phytohormones – jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), auxin, and brassinosteroids (BR) – has been established, which form a complex and highly coordinated signalling network that ensures the coordinated work of numerous physiological and biochemical processes during the formation of an integrated immune response. In addition to its direct protective function, SA is involved in symbiotic interactions between plants and beneficial microorganisms (mycorrhizal fungi, rhizobia), which further increases plant resistance to pathogens and improves their nutrition. Such mutually beneficial relationships not only enhance plant resistance to stress, but also improve nutrient uptake, which overall contributes to increased yields. The practical use of SA through exogenous application or stimulation of its endogenous synthesis is a promising and environmentally safe tool for boosting the immunity of cultivated plants and reducing dependence on chemical pesticides, which is relevant in the context of sustainable agriculture, environmental protection and food security.

Further research into the mechanisms of action of SA is important for the development of bio-oriented agricultural technologies and the improvement of integrated plant protection methods in the context of sustainable agriculture. Therefore, SA can be considered one of the most promising objects for research and practical application in modern agrobiotechnology, the use of which will increase plant resistance to stress factors of various nature and reduce the use of herbicides.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Деякі екологічні аспекти впливу саліцилової кислоти

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Анотація. Саліцилова кислота є екологічно безпечним регулятором росту з поліфункціональними властивостями, яка має великі перспективи у практиці ведення сільського господарства, оскільки використовується у низьких концентраціях і має природні шляхи катаболізму. Метою роботи було з'ясувати участь саліцилової кислоти у взаємодіях рослинних організмів з іншими біотичними компонентами екосистем та розкрити перспективи її використання у рослинництві та для розробки екологічно безпечних агротехнологій. Для досягнення мети було проведено пошук, аналіз, систематизацію та порівняння наукових джерел із використанням провідних наукових баз даних та методів логічного узагальнення. Встановлено, що рослини, ведучи прикріплений спосіб життя, постійно зазнають впливу біотичних стресів, насамперед фітопатогенів і травоїдних організмів. У відповідь вони сформували систему захисту, що включає пасивні бар'єри та активні імунні реакції на клітинному й молекулярному рівнях. У центрі цих процесів знаходяться фітогормони, серед яких саліцилова кислота має ключове значення. Було з'ясовано, що саліцилова кислота є однією з основних сигнальних молекул, що координує експресію генів, пов'язаних із захистом рослин, індукує синтез вторинних метаболітів, сприяє зміцненню клітинних стінок та активує механізми системної набутої стійкості. Особлива увага приділена взаємодії саліцилової кислоти з іншими фітогормонами, зокрема жасмоновою кислотою, етиленом, абсцизовою кислотою та ауксином, що разом утворюють складну сигнальну мережу, яка забезпечує інтегровану і ефективну імунну відповідь. Окремий розділ присвячено участі саліцилової кислоти у симбіотичних взаємодіях рослин з корисними мікроорганізмами, такими як мікоризні гриби та ризобії, що посилюють стійкість господаря до патогенів. Було також розглянуто практичний аспект використання саліцилової кислоти у сільському господарстві як екологічно безпечного засобу підвищення імунітету культурних рослин шляхом екзогенного застосування чи стимуляції ендогенного синтезу. Отримані узагальнення є важливими як для розуміння фундаментальних основ імунітету рослин, так і для практичного впровадження біотехнологій у захисті рослин від шкідників і хвороб

Ключові слова: регулятори росту; біотичні стресові чинники; абіотичні стресові чинники; фітопатогени; фітогормони

Received: 03.02.2025 Revised: 18.04.2025 Accepted: 22.05.2025

BIOLOGICAL SYSTEMS: Theory and Innovation

Journal homepage: https://bioscience.com.ua/en
Vol. 16, No. 2, 2025, 50-60

UDC 633.16:581.1-032.3:581.4 DOI: 10.31548/biologiya/2.2025.50

Regulation of morphometric parameters of winter barley (*Hordeum vulgare* L.) using graphene oxide under water stress

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Abstract. The problem of food security due to extreme climate change is critical in Europe and globally, and its solution is one of the sustainable development goals, as the amount of precipitation decreases annually, and the average annual temperature increases, which leads to the spread of droughts, lengthening rainless periods, and complicates the cultivation of crops. The purpose of the study was to investigate the effect of structured graphene oxide nanoparticles during pre-sowing treatment of winter barley seeds (*Hordeum vulgare* L.) varieties 'Dostoynyi' of morphometric parameters of plants in conditions of water deficiency. Chemical, physical, and physiological methods were applied. The effect of graphene oxide at concentrations of 5, 10, 20, and 50 micrograms/ml on the morphometric parameters of winter barley was studied 17 days after seed treatment under conditions of regular watering and water stress. No phytotoxic effect of graphene oxide on barley seedlings with regular watering was detected. Pre-sowing treatment of seeds with graphene oxide at a concentration of 5 micrograms/ml showed a maximum increase in root length (by 35%) and plant height (by 23%) with water deficiency compared to the control. At other concentrations studied, plant growth was also stimulated, but the effect was less pronounced. The weight of plants at optimal concentrations of graphene oxide increased slightly, and with water deficiency, the increase was minimal. Graphene oxide effectively improved the development

Suggested Citation:

Tkachenko, T., Didur, Ye., Melezhyk, O., & Prylutska, S. (2025). Regulation of morphometric parameters of winter barley (*Hordeum vulgare* L.) using graphene oxide under water stress. *Biological Systems: Theory and Innovation*, 16(2), 50-60. doi: 10.31548/biologiya/2.2025.50.

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of the barley root system in conditions of water scarcity, which is important for plant adaptation to drought. The practical significance of the study lies in the fact that the results obtained substantiate the feasibility of using nanoparticles as an effective agricultural technology to increase the stress resistance of agricultural crops to water scarcity

Keywords: grain crops; drought resistance; innovative agricultural technologies; nanomaterials; pre-sowing treatment

INTRODUCTION

Rapid climate change is a significant problem in contemporary agricultural production. Every year, the amount of precipitation decreases, the average annual temperature increases, and there is a sharp unevenness alternating dry and rainy periods, which leads to prolonged droughts and, accordingly, complicates the cultivation of agricultural crops. The water regime plays an extremely important role in the functioning and vital activity of all living organisms, and in crop production it is a key factor in yield – it regulates all the necessary physiological and biochemical functions, in particular, the transport of nutrients, photosynthesis, and respiration, etc.

Research by A. Filipovic (2020) on the dynamics of water in the soil-plant-air system showed the extreme complexity of this process and its dependence on a number of factors. The researcher found out the key mechanisms that allow plants to adapt to water stress, including through the increased development of the root system, which is an adaptive defence mechanism in conditions of lack of moisture. Experimental results show that even minor changes in the amount of soil moisture have a significant impact on transpiration and overall plant productivity. The study by T. Kompas *et al*. (2024) showed that water-deficient stress is the main cause of reduced vital activity and crop yields, since with a lack of moisture, photosynthetic, respiratory, and metabolic processes are disrupted, leading to plant death and crop losses. According to A. Fathi *et al.* (2024), water deficiency negatively affects plant growth and development, resulting in inhibited DNA synthesis, disrupted cell division, and stretching phases. Consequently, water stress significantly affects agroecosystems and causes risks in shaping global food security not only in Ukraine and Europe, but also in the world as a whole.

Ukraine, despite socio-political conditions and military operations on its territory, remains one of the leading grain exporters in the world. That is why the issue of stable agricultural production is of strategic importance both for the national economy and for the state's economy to achieve Sustainable Development Goal 2 (Goal 2 – overcoming hunger, achieving food security, improving nutrition, and promoting sustainable agricultural development). However, contemporary environmental challenges, such as increasing climate risks, reducing natural soil fertility, including due to pollution due to fighting, frequent and prolonged droughts, reducing acreage due to fighting, make it difficult for farmers to obtain high yields and significantly increase the risks of losses.

A.A. Nagdalian *et al*. (2023) noted that common barley (*Hordeum vulgare* L.) is one of the most important grain crops with a wide range of uses: from the food industry to the feed base. It is characterised by adaptability to different climatic conditions, resistance to diseases and the ability to form a crop even with a lack of moisture. However, even this crop suffers significant yield losses in conditions of water scarcity. It is relevant to find innovative agrotechnological approaches to regulating stress resistance mechanisms in agricultural crops during prolonged droughts to increase the quantity and quality of plant raw materials. Research by S.V. Prylutska *et al.* (2024) showed the prospects of using structured carbon nanoparticles in this area, which, due to their unique physicochemical and biological properties, in particular, nanoscale and hydrophobicity, can penetrate through biological membranes into cells, transport nutrients, serve as a source of bioorganic substances, and have antibacterial, antiviral, fungicidal, and insecticidal effects.

Y. Yang *et al.* (2022) proved that graphene oxide (GO) is a two-dimensional nanomaterial consisting of carbon atoms organised in a hexagonal grid, on the surface of which there are numerous oxygen-containing functional groups (hydroxyl, carboxyl, epoxy). The presence of these groups in the GO molecule provides high hydrophilicity, which is manifested in the ability to retain water in the soil, dispersion in water, forms a significant specific surface area and the ability to chemical modification. These properties interact effectively with plant cells. The findings of J. Shoham (2020) proved the positive effect and biological activity of graphene oxide on the growth, development, formation of the reaction to oxidative stress and water imbalance of plants. It was also important to note that nanomaterials contribute to the long-term storage of fertilisers in the soil, respectively, they provide plants with the necessary nutrients for a significant period of time. This approach allows reducing the load on soil ecosystems and the amount of fertilisers used in the long term, minimising contamination of the pedosphere with nutrients. According to L. Zhao *et al.* (2022a), graphene oxide does not accumulate in plant tissues during prolonged use and application to the soil, which eliminates the risks of nanoparticle toxicity and environmental stress. However, questions about long-term field trials, the effect of GO nanoparticles on the soil microbiome, and the economic feasibility of their use remain relevant and require comprehensive further research. In addition, B. Anegbe *et al*. (2024) showed that graphene oxide, due to the unique structural and surface characteristics of the molecule, in particular, large specific surface area, porosity, chemical and mechanical resistance, catalytic activity, can exhibit high efficiency in extracting and removing various environmental pollutants, in particular, pesticides and pharmaceuticals, etc.

Given the urgency of the problem of water stress and the prospects of using nanotechnologies to increase plant resistance to adverse conditions, there is a need to study the effectiveness of using graphene oxide in agricultural production. The purpose of the study was to investigate the effect of pre-sowing treatment of ordinary barley seeds with structured graphene oxide nanoparticles on the morphometric parameters of plants under water deficiency conditions.

MATERIALS AND METHODS

The study was conducted during April – June 2024 at the educational and scientific laboratory "Biochemistry and phytobiotechnology" of the Department of Physiology, Plant Biochemistry and Bioenergy of the National University of Life and Environmental Sciences of Ukraine. The object of research were the certified seeds of winter barley (*Hordeum vulgare* L.) of the cold-and drought-resistant variety 'Dostoynyi', bred by the breeding and genetic Institute of the National Academy of Agrarian Sciences of Ukraine in 2006. The variety was characterised by high productivity, yield, and resistance to powdery mildew, black and blister smut. Winter barley remains a key agricultural crop in Europe, as it is characterised by a higher yield compared to spring barley, as it optimally uses moisture during the seasonal period.

Synthesis and characterisation of structured graphene oxide nanoparticles. Samples of water-soluble dispersed graphene oxide (GO) nanoparticles at an initial concentration of 4 mg/mL were provided by Graphenea S.A. (San Sebastian, Spain). Highly stable aqueous solutions of GO nanoparticles were used in the work. The authors confirmed the structural organisation and stability of the samples using high-resolution microscopy and dynamic light scattering. The studies used an aqueous solution of GO in the concentration range: 5, 10, 20, and 50 mcg/ml, as these are pilot studies of the phytotoxic properties of nanoparticles.

Experimental conditions. Pre-sowing treatment of barley seeds was carried out in two stages: sterilisation and priming (Fig. 1). To do this, the seeds were soaked sequentially: 1) in a 3% solution of H_2O_2 for 25 minutes, after which they were washed three times with distilled water; 2) in a solution of H_3BO_3 (0.6 g/l) for 15 minutes, then washed again three times with distilled water; 3) in GO solution at the test concentrations (5, 10, 20, and 50 mcg/ml) for 5 hours. Barley seeds in the control group (without GO treatment) were soaked only in distilled water.

The treated barley seeds were planted in a universal soil mixture produced by Florida (Ukraine) consisting of turf soil, high-moor peat, low-moor peat, river sand, mineral fertilisers (N, P, K), microelements (Fe, Zn, B, Cu, Mo, Mn), pH 5.5-7.5, in 1-litre containers. Each control and experimental samples contained 10 seeds. The seeds were sprouted at a temperature of +20°C and 15/9 photoperiod for 16 days.

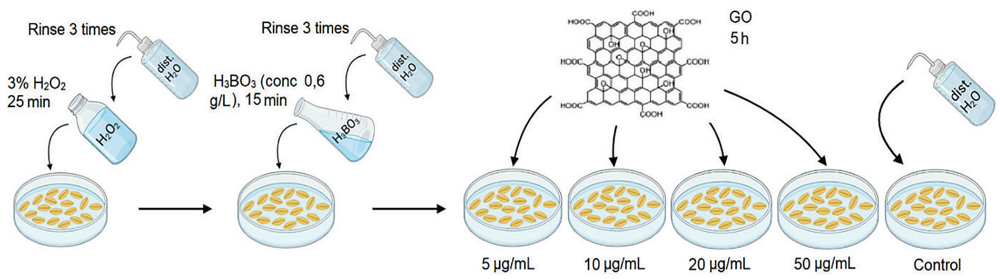

Figure 1. Scheme of seed treatment for winter barley *Hordeum vulgare* L
Source: created by the authors

The experiments included the following groups:

A) regular watering (5 variants): 1) control (without GO treatment) and experimental groups of plants treated: 2) 5 mcg/ml GO, 3) 10 mcg/ml GO, 4) 20 mcg/ml GO, 5) 50 mcg/ml GO.

B) water scarcity (5 variants): 1) control (without GO treatment) and experimental groups of plants treated: 2) 5 mcg/ml GO, 3) 10 mcg/ml GO, 4) 20 mcg/ml GO, 5) 50 mcg/ml GO.

Measurements were performed for at least three repetitions of each experiment variant.

Simulation of a drought under laboratory conditions. During the experiment, the drought for barley plants was simulated by the Tumanov wilting method, and the spraying of plants was stopped on day 2 after seed germination (Pykalo *et al.*, 2020). Watering of the samples was resumed 1 day before the end of the experiment. Spraying of control samples (without drought modelling) was carried out regularly – every second day. This method and duration were used in the work to model water scarcity, since it is universal for cereals, which was also confirmed by the reaction of barley due to the intensification of root length.

Morphological indicators in control (without processing GO) and experimental (processed GO) variants of plants with regular watering and water deficiency were evaluated on day 17 after seed germination, namely, in the vegetative phase of four leaf formation. Selection and preparation of plant material and determination of morphometric parameters (plant height, shoot and root length, plant weight) were carried out according to generally accepted methods. For each variant of the experiment, a minimum of 10 plants were selected in each sample, with at least three biological repeats per variant, which corresponds to the approaches for evaluating seedlings in contemporary studies on morphometry of barley and other crops (Mahmood *et al.*, 2022). To do this, the plants were carefully separated from the soil mixture by holding the root zone; the remaining substrate was removed by light washing in a tray with distilled water and short drying on filter paper until there was no free moisture on the surface. The height of the plant was determined as the distance from the root neck to the top of the highest leaf/shoot in a straightened but not stretched state; the length of the shoot – from the root neck to the top of the main shoot; the length of the root – from the root neck to the top of the longest root; all measurements were made with a ruler with an accuracy of 1 mm. The fresh weight of plants was determined immediately after linear measurements on analytical scales with an accuracy of 0.1 mg. Measurements were performed at the same time of day and under the same laboratory conditions to minimise the effect of daily fluctuations in turgor and evaporation on morphometric parameters (Ergun *et al.*, 2024).

Statistical processing of the study results were performed using ANOVA analysis of variance and Microsoft Excel 2010 and GraphPad Prism 7 software, the results were considered significant at the values $P < 0.05$. Experimental plant studies were in line with institutional, national, and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Water-deficient stress significantly affects the state of agroecosystems and the development of global food security in the world. Solving these complex agricultural production problems requires using the latest biotechnological approaches.

Nanoparticles, in particular graphene oxide, can be used as regulators of crop resistance to water stress in agricultural technologies. Its surface is modified with oxygen-containing functional groups, which provides high hydrophilicity and the ability to retain water in the soil and interact with plant cells. Nanoparticles mostly do not exhibit phytotoxic effects even at high concentrations, and optimal concentrations have a positive effect on the morphological and physiological parameters of plants (Zhao *et al.*, 2022a).

The study was conducted on winter barley plants (*Hordeum vulgare* L.) of the 'Dostoynyi' variety, which is characterised as a high-yielding and resistant to extreme environmental conditions and diseases variety. A preliminary study of the effect of graphene oxide nanoparticles on the morphometric parameters of plants of various species showed that at optimal concentrations of carbon nanomaterials (5-100 mcg/ml for seed treatment or 20-50 mg/l for foliar application), root growth was stimulated and aboveground mass was increased, yield and product quality were increased (Zhang *et al.*, 2021; Yang *et al.*, 2022; Prylutska *et al.*, 2024). Both growth regulators and graphene oxide affect plant growth depending on the dosage and concentration of the substance, so it is important to determine the optimal concentrations to achieve maximum agronomic effect. On day 17 of germination of common barley seeds treated in various concentrations, which were grown in artificial drought conditions, the main morphometric parameters were measured (Fig. 2).

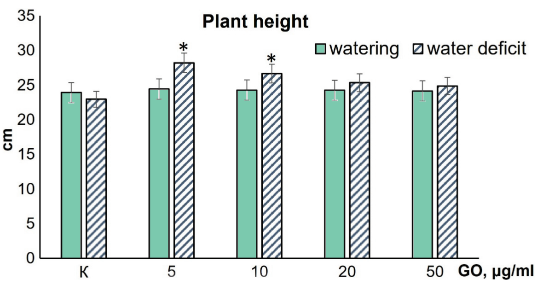

Figure 2. Plant height (cm) of winter barley (*Hordeum vulgare* L.) of the 'Dostoynyi' variety on day 17 after germination of the treated seeds with graphene oxide under conditions of regular watering and water deficiency ($M \pm m$, $n = 10$)
Note: *$p < 0.05$ compared to the control (untreated plants)
Source: created by the authors

Under conditions of regular irrigation, there were no statistically significant differences in the total length of barley plants (Fig. 2). But in the conditions of moisture deficiency in the GO seed treatment option of 5 mcg/ml, an increase in the total length was determined by 23%, and at a concentration of 10 mcg/ml – by 18%. In the remaining variants, no statistically significant length gain was observed. Higher concentrations also stimulated plant growth, but these indicators were insignificant and did not significantly differ from the control values.

A similar trend was observed in the increase in the stem length of barley plants (Fig. 3). In the case of using graphene oxide nanoparticles and regular irrigation of plants, a slight increase in stems was noted. The total length increased by 2-8% compared to the control, which is statistically insignificant. Moreover, even a small increase was dose-dependent – the greatest increase in stem length was observed at the GO concentration of 5 micrograms/ml, followed by a decrease in the indicator with an increase in the concentration of nanoparticles in solution.

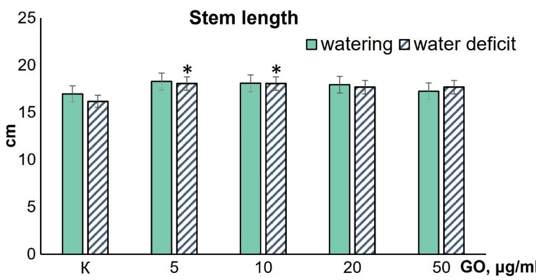

Figure 3. Plant stem length (cm) of winter barley (*Hordeum vulgare* L.) of the 'Dostoynyi' variety on day 17 after germination of the treated seeds with graphene oxide under conditions of regular watering and water deficiency ($M \pm m$, $n = 10$)
Note: *$p < 0.05$ compared to the control (untreated plants)
Source: created by the authors

Under drought simulation conditions, a pronounced effect of graphene oxide nanoparticles at concentrations of 5 and 10 micrograms/ml was observed – the stem length increased statistically significantly by 11-12% (Fig. 3). At higher concentrations (20 and 50 micrograms/ml of GO), the length increase was 9% compared to control plants. The effect of structured nanoparticles on seeds before sowing helps to better tolerate adverse conditions, including moisture deficiency, and promotes active growth of the aboveground part of plants. The dry period causes oxidative stress in the plant body, which, in turn, leads to the development of free radicals that damage cells and lead to plant death. Probably, nanoparticles contribute to the activation of the antioxidant system of the plant body, neutralising the resulting free radicals, allowing the plant to better tolerate stressful conditions and maintain vitality during the dry period. Thus, pre-sowing seed treatment contributes to the formation of stress resistance to drought and ensures the development of the stem part of the plant.

The development of the root mass of plants is the key to active growth and development of plants as a whole, but it is the root system that first of all experiences a lack of moisture and reacts to drought by reducing water exchange between plant parts. Therefore, the root length indicator is key in the development of plant resistance to arid conditions. In the experiment, under normal wetting conditions, there was no significant difference in the root length of the barley plant root system after treatment with GO nanoparticles (Fig. 4).

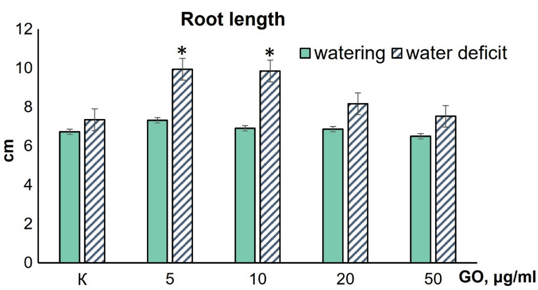

Figure 4. Plant root length (cm) of winter barley (*Hordeum vulgare* L.) of the 'Dostoynyi' variety on day 17 after germination of the treated seeds with graphene oxide under conditions of regular watering and water deficiency ($M \pm m$, $n = 10$)
Note: *$p < 0.05$ compared to the control (untreated plants)
Source: created by the authors

Figure 4 shows that under water stress, plants treated with graphene oxide at concentrations of 5 mcg/ml and 10 mcg/ml showed a significant increase in root length. These values are statistically significant compared to the control and amounted to 35% and 34%, respectively. Increases in graphene oxide concentrations did not significantly affect barley root growth, but were 2% (50 mcg/ml) and 10% (20 mcg/ml) higher than controls. A well-developed root system penetrates deeper layers of the soil, where capillary moisture is stored, which significantly improves the absorption of water and nutrients during the dry period. Thus, it can be argued that nanoparticles promote more intensive growth and development of the root system, have a positive effect on the development of stress resistance of barley plants to drought, ensuring the growth of the root system and, accordingly, providing a better water balance between the aboveground and underground parts of plants. A general indicator of plant stress resistance under extreme conditions, in particular the onset of dry periods during the growing season, is the vegetative mass of plants. According to this indicator, there are no statistically significant changes in common barley compared to the control both under normal conditions and under simulated drought conditions (Fig. 5).

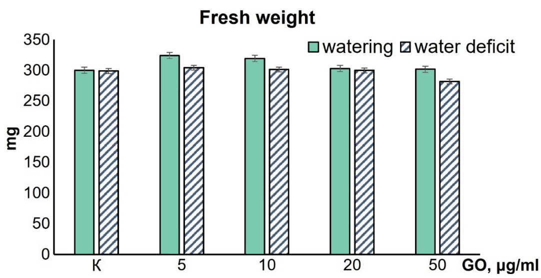

Figure 5. Fresh weight of plants (mg) of winter barley (*Hordeum vulgare* L.) of the 'Dostoynyi' variety on day 17 after germination of the treated seeds with graphene oxide under conditions of regular watering and water deficiency ($M \pm m$, $n = 10$)
Note: *$p < 0.05$ compared to the control (untreated plants)
Source: created by the authors

Figure 5 shows that under irrigation conditions, the increase in biomass of plants treated with graphene oxide at concentrations of 5 mcg/ml and 10 mcg/ml showed an 8 and 6% higher indicator compared to the control, while under conditions of moisture deficiency, this indicator increased only by 2 and 1%, which is a statistically insignificant value. Consequently, with water deficiency in plants treated with graphene oxide, a concentration-dependent effect on the main morphometric parameters was observed. When exposed to 5 micrograms/ml of graphene oxide, the length of the plant, stem, and root was 23%, 12%, and 35% longer, respectively, compared to untreated plants. And when exposed to 10 mcg/ml of graphene oxide, the mofrometric values of plant, stem, and root length were also higher compared to untreated plants by 18%, 11%, and 34%, respectively. At high concentrations (20 and 50 mcg/ml), graphene oxide did not affect the studied parameters.

Barley is the second most common grain crop in the world, widely used as raw materials for the food industry, kvass and brewing, production of animal feed (mixed feed), used in technical areas (biofuel production) and cosmetology. The study by A. Yiğit & F.-M. Chmielewski (2024) pointed out the urgent need to find ways to develop resistance to adverse factors in this crop, since further climate changes will lead to a significant decrease in its yield, which will affect a number of areas of agro- and food production. Thus, it can be argued that under water stress, graphene oxide nanoparticles at concentrations of 5 mcg/ml and 10 mcg/ml showed a significant stimulating effect on plant growth and the formation of their vegetative parts. Increasing the root mass by increasing the length of the roots probably allows plants to use soil moisture to maintain the main processes of vital activity, ensure the growth of aboveground parts of plants, and withstand the dry period without losing biomass.

At elevated concentrations of graphene oxide, no phytotoxic effect on plants was observed in the conducted experiments, namely, changes in the studied mofrometric parameters. The absence of phytotoxic effects in the studied range of graphene oxide concentrations is consistent with studies by other researchers. According to Y. Yang *et al*. (2022), graphene oxide at a concentration of 0.2 mg/mL stimulated seed germination of *Festuca arundinacea*, and at a concentration of 50 mg/mL – seed germination of *Spinacia oleracea* and *Allium cepa*, growth of the aboveground and underground parts of *Zea mays*. A. Buziashvili *et al*. (2024) showed that a concentration of 25 mg/mL accelerated germination and growth of *Solanum lycopursicum*. These data fully coincide with the results obtained in the experiment, which confirms the positive effect of graphene oxide nanoparticles on the growth processes during pre-sowing seed treatment. The absence of phytotoxicity at high concentrations is a direct consequence of the fact that increased concentrations of GO (more than 25 mg/mL) did not show a stimulating effect, but there was no negative effect on plant growth and development. In large quantities in solutions, nanoparticles can aggregate, forming micro- and macroscopic clusters of substances, the size of which does not allow them to enter plant cells and enter into metabolic processes. Thus, a significant number of nanoparticles remain in the soil or settle on the surface of the roots, unable to interact with intracellular structures and influence vital physiological and biochemical processes.

There is evidence of the negative effects of graphene oxide on plants. Research by S. Zhao *et al*. (2023) showed that seed treatment with high concentrations of graphene nanoparticles caused growth inhibition, changes in the anatomical structure of roots, and reduced nutrient uptake in alfalfa *(Medicago sativa* L.), and research by S. Zhao *et al*. (2022b) showed a decrease in the biomass growth of white clover shoots (*Trifolium repens* L.). The substance itself is not phytotoxic, but it can cause negative effects due to the obstruction of access of nutrients and soil moisture to plants. GO agglomerates formed at high concentrations settle on the roots, disrupting normal metabolic processes in the plant. That is why the direction of application of nanoparticles in agrobiotechnologies is promising and requires further detailed and comprehensive research.

In addition, there are a number of literature data on other areas and mechanisms of the influence of graphene oxide on the physiological and agrotechnical parameters of plants. According to M.T. Miranda *et al*. (2020), one of the protective responses in water scarcity and drought is osmotic adaptation of plants, in which the concentration of organic substances, in particular glucose, in roots and leaves increases, and root growth is accelerated compared to shoots. L. Zhao *et al*. (2022a) showed that graphene oxide promotes soybean growth (*Glycine max* L.) without phytotoxic effects and prevented the negative effects of drought on plants. In soybean plants treated with graphene oxide at a concentration of 100 mcg/ml compared to untreated plants, the water content in stems and leaves increased by 127 and 128%, respectively, the root parameters (total length, surface area, diameter and volume) increased by 33, 38, 34 and 35%, the activity of antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase increased by 29, 57, 28 and 66%, the levels of jasmonic, salicylic, and abscisic hormones increased by 32, 34, and 67%, and four drought-related genes were expressed: gmp5cs, gmgols, gmdreb1, and gmnced1. The study showed that graphene oxide directly increased plant defence enzymes, hormone content, and expression of drought-related genes, thereby improving soybean resistance to the negative effects of drought.

The study by Y. Zhang *et al*. (2024) showed that after processing buckwheat (*Fagopyrum tataricum* with graphene oxide in the concentration range of 25-200 mg/l increased its yield, increased the content of nutrients in the soil of the rhizosphere, growth and activity of roots, the content of photosynthetic pigments, the activity of antioxidant enzymes, the content of auxin. The action of graphene oxide in soybean seedlings increased the content of proline, soluble sugars and other osmotic substances, which contributes to the preservation of cell turgor and resistance to drought (Zhao *et al*., 2022a). Independent research by L. Zhao *et al*. (2022a) and A.A. Nagdalian *et al*. (2023) showed that after treatment of barley and soybean seeds with silicon nanoparticles, the root length increased by 33-45%, the plant height by 19-22%, and the seedling weight by 10-15% compared to the control under water deficiency conditions. Such data are quite consistent with the results obtained for winter barley varieties 'Dostoynyi', which allows assuming the constancy of the effect of nanoparticles on physiological processes in plants of different species.

N.E. Mahmoud & R.M. Abdelhameed (2021) showed that corn and millet were also treated with graphene nanoparticles to improve morphometric and biochemical parameters, and crop yields increased even on saline soils. T. Lopes *et al*. (2021) proved that graphene oxide and other carbon nanomaterials effectively regulate drought-induced oxidative stress in plants, stimulating root system development and improving morphometric parameters (root length, plant height, biomass). The study by S. Park *et al*. (2020) noted that the use of GO effectively increases the yield and resistance of barley and other crops to water scarcity, which is particularly relevant in the context of climate change. Moreover, exceeding the optimal doses of graphene oxide can cause growth inhibition, increased oxidative stress, and membrane damage, which indicates the need for clear control of compound concentrations. It is assumed that one of the possible mechanisms of protective action is the ability of nanoparticles to penetrate the seed shell, violate its integrity, which, in turn, facilitates the absorption of water molecules and, as a result, accelerates the germination of plants. In addition, due to oxygen-containing functional groups, graphene oxide retains water, and hydrophobic sites sp^2-structures promote the transfer of moisture to the seeds, stimulating the germination process of plants.

Considering the numerous positive results of the study of the influence of graphene oxide nanoparticles on the growth and biochemical processes of important agricultural crops, and the findings of researchers, it can be argued that the use of a solution of graphene oxide nanoparticles for pre-sowing treatment of seed material is an evidence-based effective way to increase plant yield, increase the level of their resistance to abiotic stresses, including water deficiency, which is a contemporary challenge of agricultural production in Ukraine.

CONCLUSIONS

Morphometric parameters of winter barley of the 'Dostoynyi' variety after priming seeds with graphene oxide in the concentration range of 5-50 mcg/ml under conditions of regular watering and water stress were studied on day 17 of plant vegetation. There was no negative effect of graphene oxide on barley plants both under conditions of regular watering and water deficiency. Under the influence of drought, there was an increase in the length of the root of plants by 9%. In barley plants sprouted under water stress and pre-treated with graphene oxide, an increase in the studied morphometric parameters was observed. The concentration-dependent effect of nanoparticles on plant growth and development was revealed. At optimally stimulating concentrations of graphene oxide of 5 and 10 mcg/ml, an increase in plant height by 23% and 18%, stem length by 12% and 11%, root length by 35% and 34%, respectively, was observed in relation to plants untreated with nanoparticles and sprouted under drought conditions. While at higher graphene oxide concentrations of 20 and 50 mcg/ml, the morphometric parameters corresponded to the control values of plants both under conditions of regular watering and water deficiency. The increase in biomass under the influence of nanoparticles was statistically insignificant.

The revealed stimulating effect of graphene oxide on common winter barley plants in arid conditions allows, based on the results obtained, considering nanoparticles as an innovative agricultural technology for adapting this grain crop to moisture deficiency and viewing nanoparticles as highly effective means of maintaining and preserving plant yield. The results obtained indicate the absence of phytotoxic effects of structured graphene oxide nanoparticles and, accordingly, their safety in agricultural production to increase the stress resistance of plants to water stress and long dry periods. However, it is important to elucidate the intracellular and biochemical mechanisms of plant resistance and establish the nature of adaptive responses to adverse growing conditions using structured nanomaterials, which requires further basic research.

ACKNOWLEDGEMENTS

The research was conducted within the framework of the fundamental project 110/1-ф-2023 "The regulation of stress resistance intracellular mechanisms of agricultural plants using carbon nanomaterials" (No. 0123U101993, 2023-2025).

FUNDING

This research was carried out with the financial support of the Ministry of Education and Science of Ukraine.

CONFLICT OF INTEREST

None.

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Регуляція морфометричних параметрів ячменю озимого (*Hordeum vulgare* L.) оксидом графену за водного стресу

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Анотація. Проблема продовольчої безпеки за екстремальних кліматичних змін є критичною у Європі і світі в цілому, а її вирішення є однією з цілей сталого розвитку, оскільки кількість опадів щорічно зменшується, а середньорічна температура зростає, що призводить до поширення посух, подовження бездощових періодів та ускладнює вирощування сільськогосподарських культур. Метою роботи було дослідити вплив структурованих наночастинок оксиду графену при передпосівній обробці насіння озимого ячменю (*Hordeum vulgare* L.) сорту 'Достойний' на морфометричні показники рослин за умов водного дефіциту. У роботі було використано хімічні, фізичні та фізіологічні методи. Вплив оксиду графену у концентраціях 5, 10, 20 і 50 мкг/мл на морфометричні показники озимого ячменю вивчали через 17 діб після обробки насіння за умов регулярного поливу та водного стресу. Виявлено відсутність фітотоксичної дії оксиду графену на проростки ячменю при регулярному поливі. За передпосівної обробки насіння оксидом графену у концентрації 5 мкг/мл спостерігалося максимальне збільшення довжини коренів (на 35 %) та висоти рослини (на 23 %) за водного дефіциту порівняно з контролем. За інших досліджуваних концентрацій також спостерігали стимулювання росту рослин, проте ефект був менш вираженим. Маса рослин за оптимальних концентрацій оксиду графену зростала незначно, а за водного дефіциту приріст був мінімальним. Оксид графену ефективно покращував розвиток кореневої системи ячменю за умов водного дефіциту, що є важливим в адаптації рослин до посухи. Практичне значення дослідження полягає в тому, що отримані результати обґрунтовують доцільність використання наночастинок як ефективної агротехнології для підвищення стресостійкості сільськогосподарських культур до водного дефіциту

Ключові слова: зернові культури; посухостійкість; інноваційні агротехнології; наноматеріали; передпосівна обробка

Received: 21.01.2025 Revised: 15.04.2025 Accepted: 22.05.2025

BIOLOGICAL SYSTEMS: Theory and Innovation

Journal homepage: https://bioscience.com.ua/en
Vol. 16, No. 2, 2025, 61-74

UDC 504.054

DOI: 10.31548/biologiya/2.2025.61

Screening of natural (^{238}U and ^{232}Th series, ^{40}K) and technogenic (^{137}Cs) radionuclides, as well as heavy metals in river sediments in Transcarpathia

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Abstract. Bottom sediments are an important indicator of the state of rivers, as they are capable of accumulating natural and technogenic substances, reflecting background geochemical features and anthropogenic load. The aim of the study was to analyse the content of natural and technogenic radionuclides and heavy metals in the rivers of Transcarpathia to identify patterns in their distribution and further assess bioaccumulation in the "bottom sediments – ichthyofauna" system. To achieve this goal, γ-spectrometric analysis was performed on the "ORTEC" complex and X-ray fluorescence determination of metals was performed using the "Elvax Pro" analyser. Samples were collected in 2023-2024 from eight points within the Uzh, Latorica, Borzhava, and Tysa rivers. The results showed spatial variability caused by a combination of geological, morphological and anthropogenic factors. The total activity of natural radionuclides (U- and Th- series) was found to be within the range of 340-534 Bq/kg, with the highest values found in the areas of Uzhhorod, Vylok, and Chop. The highest activity of the natural radionuclide K-40 (522-533 Bq/kg) was recorded in areas with the presence of potassium minerals and agricultural load – Pidpolozzia, Kolchyno and Chop. Cs-137 had low activity (2.9-7.0 Bq/kg), with a local increase in fine-grained silt. The geochemical index Th/U (0.63-1) showed the mineralogical diversity of sediments. Analysis of heavy metal content revealed spatial differences: Zn concentrations ranged from 1.4 to 89.9 mg/kg, Pb from 4.4 to 8.9 mg/kg, Cu from 3.9 to 9.7 mg/kg, Ni from 5.1 to 8.3 mg/kg, and Cr – from 6.3 to 13.0 mg/kg. The highest values of elements were found in urban areas (Uzhhorod, Chop), and the lowest – in mountainous areas with minimal anthropogenic impact. The concentrations of heavy metals did not exceed environmental standards, but local maxima indicated potential pollution. The practical significance of this study lies in the use of its results for

Suggested Citation:
Cherevko, Kh., Sukharev, S., & Svatiuk, N. (2025). Screening of natural (^{238}U and ^{232}Th series, ^{40}K) and technogenic (^{137}Cs) radionuclides, as well as heavy metals in river sediments in Transcarpathia. *Biological Systems: Theory and Innovation*, 16(2), 61-74. doi: 10.31548/biologiya/2.2025.61.

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environmental monitoring, predicting the migration of elements and assessing their bioaccumulation in aquatic food chains

Keywords: geochemical monitoring; background radiation; river sediments; toxic elements; river ecosystems

INTRODUCTION

Bottom sediments act as an indicator for the accumulation of hazardous substances in riverine ecosystems, as they have a high sorption capacity to accumulate both natural and technogenic substances. They can reflect the geochemical characteristics of a territory and the general environmental conditions, which makes it possible to assess the consequences of anthropogenic pressure on aquatic ecosystems. Analysing the composition of bottom sediments provides a generalised overview of the state of water bodies and helps to identify areas that require heightened attention within a monitoring framework. In the Transcarpathian region, such a study is particularly relevant because the rivers in the area flow through various landscapes and geological zones. This, in turn, allows for an analysis of pollutant concentration levels in different sections of the rivers. Special attention should be paid to radionuclides and heavy metals when assessing the state of the region's riverine ecosystems. A comprehensive analysis of these components makes it possible to characterise the ecological stability of riverine ecosystems and to identify potential sources of pollution or local foci of increased anthropogenic impact.

An analysis of modern scientific research confirms the relevance of studying the processes of radionuclide and heavy metal accumulation in bottom sediments across different geographical regions. These studies demonstrate that the activity of natural and technogenic radionuclides in bottom sediments depends on geomorphological, hydrological, and anthropogenic factors. In a study by R. Federico *et al.* (2020), the high mobility of Cs-137 in fine-grained sediments and its effective incorporation into trophic chains were noted, which allows for the assessment of potential risks of radionuclide transfer to organisms in riverine biocenoses. The work of T. Abere *et al.* (2025) showed that the activity of natural and technogenic radionuclides in bottom sediments varies significantly depending on the geomorphological and hydrological conditions of the rivers. The authors noted that the flow velocity, seasonal water level changes, and the nature of the bottom cover affect local radionuclide concentrations, which is especially important for assessing high-risk contamination zones. Ukrainian researchers O. Symkanych *et al.* (2024) emphasised the significance of combining geological and morphological factors with anthropogenic influence in forming the spatial distribution of radionuclides in the Transcarpathian region, while the work of N.I. Svatiuk *et al.* (2022) noted that systematic radioecological monitoring allows for the assessment of the intensity of human activity and its consequences for riverine ecosystems. Additionally, these studies demonstrate that a combined analysis of natural and technogenic radionuclides makes it possible to identify general trends in the impact of anthropogenic factors on the riverine ecosystem. The research by O. Hromyk *et al.* (2020) proved that the level of background radiation in bottom sediments depends on the geological characteristics of the area, and comprehensive radioecological monitoring (particularly concerning natural radionuclides) helps to identify potential sources of pollution. In the work of I. Cimbolakova *et al.* (2020), it was shown that heavy metals, particularly the mobile forms of Zn, Cu, Pb, and Ni, are characterised by high toxicity and the ability to be redistributed and accumulated in the biotic components of aquatic ecosystems, which determines their ecological danger. R.N. Khalef *et al.* (2022) and R. Osae *et al.* (2023) showed that solubility and the ability to form complex compounds determine the migration of heavy metals in the natural environment. These studies highlight the importance of a comprehensive approach to analysing heavy metal content, as the simultaneous determination of their concentrations and spatial distribution allows for the assessment of their impact on biota and the ecological stability of riverine ecosystems. At the same time, they demonstrate that the interaction of heavy metals with bottom sediments is determined not only by the chemical properties of the metals but also by the structural characteristics of the sediments, which affects their mobility and availability to living organisms.

The aim of the study was to conduct a screening analysis of the content of natural and technogenic radionuclides, as well as heavy metals in the bottom sediments of the main rivers of Transcarpathia (Uzh, Latorica, Borzhava, and Tysa), determining the characteristics of their distribution for monitoring the state of river ecosystems in the region and further assessing the bioaccumulation of pollutants in the "bottom sediments – ichthyofauna" system. To achieve this goal, the following tasks were set: selection and preparation of bottom sediment samples with subsequent determination of the specific activity of radionuclides using γ-spectrometry; determination of heavy metal concentrations by X-ray fluorescence analysis; assessment of the spatial distribution of the specific activity of radionuclides and the concentration of heavy metals in different sections of the rivers of Transcarpathia. The novelty of the work lies in the comprehensive screening of natural and technogenic radionuclides together with heavy metals in the bottom sediments of the region's rivers, and the identification of patterns in their spatial distribution.

MATERIALS AND METHODS

For the analysis, three bottom sediment samples were taken at each of the eight control points: Uzhhorod, Pidpolozzia, Kolchyno, Dovhe, Rakhiv, Vylok, Tiachiv, and Chop (within the main rivers of Transcarpathia: Uzh, Latorica, Borzhava, and Tysa) (Fig. 1). For each point, an average sample was prepared from the three collected probes. Some of the samples were collected in 2023, and the rest in 2024, during the spring-autumn period of both years, depending on the accessibility of the points and hydrological conditions. The results are presented as averages for the entire aforementioned period, which allowed for a general understanding of the spatial distribution of natural and technogenic radionuclides and heavy metals in the bottom sediments. Sampling, transportation, and storage were carried out according to standard methods in compliance with DSTU ISO 5667-12-2001 (2002) and DSTU ISO 5667-15:2007 (2011). Bottom sediment samples, weighing over 1500 g, were collected manually from a depth of approximately 5-15 cm. They were transported and stored in polyethylene bags with a label indicating the collection date, sample number, and information about the sampling location. The samples were dried at room temperature (18-25°C) until they reached a constant mass. This approach helped to avoid the loss of volatile components and preserve the total content of heavy metals. The dried samples were then ground by hand to a fine, homogeneous powder. After grinding, foreign residues (plant roots, stones, glass, etc.) were removed from the samples, and then the sample was sieved to a homogeneous mass with a fraction size of up to 100 µm.

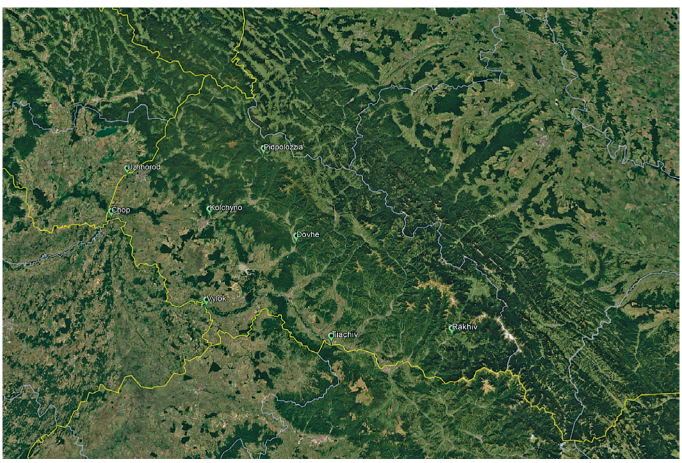

Figure 1. Location of bottom sediment sampling points in the Transcarpathian region
Source: developed by the authors based on Google Earth (n.d.)

Radiological studies were conducted at the Department of Photon-Nuclear Processes of the Institute of Electronic Physics of the National Academy of Sciences of Ukraine (Uzhhorod). The prepared silt samples, weighing 1-1.5 kg, were placed in a Marinelli beaker for radiospectroscopic analysis. The specific activity of gamma-active nuclides (U-238 (Pb-214, Bi-214, Ra-226), Th-232 (Pb-212, Bi-212, Ac-229, Tl-208), K-40, Cs-137) in the bottom sediments was determined using an "ORTEC" semiconductor γ-spectrometer system with a 150 cm³ HPGe-detector. During the measurements, the energy scale drift, resolution, and registration efficiency of the spectrometer were constantly monitored using standard Co-57 and Co-60 point gamma-active sources. The territory was mapped using the "QGIS 3.40.1-Bratislava" software, referenced to the coordinates of the bottom sediment sampling points.

The heavy metal content was determined using an "ElvaX Pro" X-ray fluorescence analyser (Ukraine), at the Transcarpathian Research Forensic Expert Service Centre of the Ministry of Internal Affairs of Ukraine. To assess the accuracy of this method, the Fe content was additionally determined in the samples using spectrocolorimetry. The results obtained by this method were used as a reference against which the relative concentrations of other elements, measured with the X-ray fluorescence analyser, were recalculated. The discrepancy between the methods did not exceed 10%, which is consistent with the stated error. The reproducibility of the results was confirmed by three measurements of one sample, with a difference of no more than 5-7%. The prepared samples were poured into a standard analytical cuvette of the instrument as a dense layer 3-5 mm thick, without additional binders or pressing. The analysis was performed in a vacuum mode, which improves the detection of light elements by reducing the absorption of X-ray radiation by atmospheric gases. Two operating modes were used: one for light elements and one for elements of medium and high atomic mass. In the first mode, the tube voltage was 12 kV, the current was 100 μA, and the pulse rate was stabilised at 300,000 pulses per second with a permissible deviation of ±10%. The second mode had the following settings: tube voltage – 35 kV, current – 200 μA, and the pulse registration rate was stabilised at 450,000 pulses per second with a permissible deviation of ±10%. The exposure time for each mode was 100-200 seconds. The presented heavy metal concentrations include a calculated error of ±10%, which accounts for the use of the non-pressing sample preparation method and the specifics of drying at room temperature. Furthermore, the stabilisation of the instrument's signal ensures the reproducibility of the measurements, which is confirmed by three independent analyses of the same sample. Spectral data processing was carried out using the instrument's integrated "Elvatech" software with an automatic peak identification and concentration calculation function (in percentages). Additional calculations were performed using Exel software to convert the percentage concentrations to mg/kg of dry mass. A conversion factor was used to convert the concentrations from percentage content to mg/kg, determined from the reference value for iron:

$$k = \frac{C_{Fe}}{\omega_{Fe}}, \quad (1)$$

where k – the conversion factor, C_{Fe} – the Fe concentration in mg/kg determined by spectrocolorimetry, ω_{Fe} – the Fe content in the sample expressed as a percentage.

The resulting coefficient characterises the correspondence between the percentage and mass expression of the iron concentration of each sample. For other elements, the concentration in mg/kg was calculated using the formula:

$$C_x = \omega_x \times k, \quad (2)$$

where C_x – the concentration of element x in the sample in mg/kg, ω_x – the content of element x in %, k – the conversion factor (mg/kg per 1%).

RESULTS AND DISCUSSION

The research data showed that the content of radionuclides and heavy metals in the bottom sediments of Transcarpathian rivers is characterised by significant spatial variability. The specific activities of natural (U-, Th-series and K-40) and technogenic (Cs-137) radionuclides showed a clear dependence on the geological features of the region. The obtained specific activity values indicate not only the influence of geological conditions (in particular, the type and mineralogical composition of rocks, granulometric composition of sediments), but also anthropogenic factors (such as the influence of urbanised areas, probable agricultural load). In the lowland areas of the region, elevated levels of natural and technogenic isotopes were recorded, while in mountainous areas, lower values were observed due to the predominance of coarse-grained fractions and high flow rates. Concentrations of heavy metals such as Zn, Pb, Cu, Ni and Cr reflect a combination of natural biochemical background and local sources of pollution. The analysis showed that fine-grained sediments in the lower reaches of rivers accumulate metals better due to the high absorption capacity of clay fractions and organic matter, while in fast-flowing mountainous parts of rivers with a predominance of coarse-grained material, metal accumulation is significantly lower. Data on the content of radionuclides K-40, Cs-137, natural series U-237 and Th-232 are given in Table 1.

Table 1. Results of the study of the specific activity of gamma-active radionuclides in bottom sediments of rivers in Transcarpathia

River sections	Specific activity of gamma-active radionuclides, Bq/kg										ΣA, Bq/kg	ΣTh232/ΣU237
	K40	Cs137	U237 Series			Th232 Series						
			Ra226	Pb214	Bi214	Pb212	Ac228	Ac229	Bi212	Tl208		
Uzh River												
Uzhhorod	494.4 ±49.4	5.1 ±0.5	133.6 ±13.4	79.4 ±7.9	84.0 ±8.4	52.9 ±5.3	70.2 ±7.0	44.1 ±4.4	53.9 ±5.4	15.8 ±1.6	533.9	0.8
Latorica River												
Pidpolozzia	522.5 ±52.3	4.3 ±0.4	55.0 ±5.5	100.3 ±10.0	104.6 ±10.5	48.2 ±4.8	61.2 ±6.1	39.5 ±3.9	39.3 ±3.9	15.7 ±1.6	463.7	0.8
Kolchyno	527.0 ±52.7	4.7 ±0.5	89.5 ±8.9	60.1 ±6.0	54.6 ±5.5	51.6 ±5.2	48.5 ±4.9	42.9 ±4.3	42.6 ±4.3	13.8 ±1.4	403.4	1.0
Borzhava River												
Dovhe	474.4 ±47.4	7.0 ±0.7	85.3 ±8.5	87.2 ±8.7	80.6 ±8.1	41.3 ±4.1	52.4 ±5.2	36.7 ±3.7	22.3 ±2.2	14.8 ±1.5	420.6	0.7
Tysa River												
Rakhiv	431.2 ±43.1	2.9 ±0.3	88.9 ±8.9	54.4 ±5.4	72.6 ±7.3	33.5 ±3.4	36.7 ±3.7	27.1 ±2.7	29.3 ±2.9	10.3 ±1.0	352.7	0.6
Tiachiv	418.4 ±41.8	3.4 ±0.3	61.8 ±6.2	6.0 ±6.3	68.0 ±6.8	32.1 ±3.2	33.9 ±3.4	31.7 ±3.2	39.9 ±4.0	10.0 ±1.0	340.4	0.8
Vylok	506.4 ±50.7	4.8 ±0.5	130.1 ±13.0	69.8 ±7.0	66.0 ±6.6	45.6 ±4.6	40.8 ±4.1	42.2 ±4.2	82.9 ±8.3	14.9 ±1.5	492.3	0.9
Chop	533.1 ±53.3	4.2 ±0.4	130.4 ±13.0	62.8 ±6.3	69.6 ±7.0	45.9 ±4.6	49.3 ±4.9	43.9 ±4.4	55.6 ±5.6	13.6 ±1.4	471.1	0.8
Average Values												
	488.6	4.6	96.8	72.1	75.0	43.9	49.1	38.5	45.7	13.6	434.8 ±43.5	0.8 ±0.1

Note: ΣA – total specific activity of all natural radionuclides studied, excluding K-40; ΣTh-232/ΣU-238 – the ratio of the total specific activity of isotopes from the thorium and uranium series, which was used as an indicator of the mineralogical properties of the bottom sediments

Source: developed by the authors

The results presented in Table 1 show a summary of the quantitative indicators of the main isotopes of the U- and Th-series, as well as the isotopes of natural K-40 and technogenic Cs-137 for different sections of the studied rivers in the Transcarpathian region. The data in the table show that the specific activities of natural radionuclides vary significantly depending on the section of the river. The highest values of the total specific activity of all studied natural radionuclides, except for K-40 (ΣA), were recorded in the area of Uzhhorod and near the village of Vylok, while the lowest values were recorded in Tiachiv and Rakhiv. K-40 showed higher activity in the lowland areas of the region. The specific activity of Cs-137, as a technogenic isotope, is generally low, but its level is elevated in deposits with a fine-grained fraction (e.g., Dovhe and Uzhhorod) (Table 1). Particular attention should be paid to the ratio of the total specific activity of isotopes of the thorium and uranium series (ΣTh232/ΣU237), which can be used as a marker of the mineralogical characteristics of deposits. The data in Table 1 allow to identify key spatial patterns, which are confirmed by the following figures in this section. The values of the total specific activity of natural radionuclides (U- and Th-series, except for K-40), illustrated in Figure 2, ranged from 340 to 534 Bq/kg.

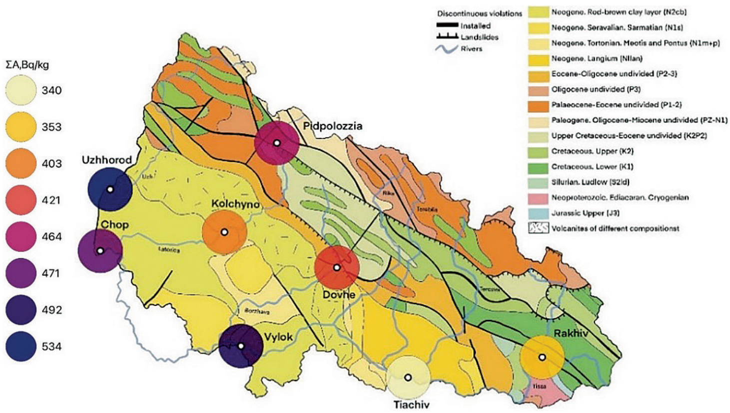

Figure 2. Visualisation of the spatial distribution of the total activity of natural radionuclides (ΣA) in bottom sediments of rivers in Transcarpathia, Bq/kg
Source: developed by the authors based on Geomap Land (n.d.)

Figure 2 shows that the maximum specific activities of natural radionuclides (U- and Th-series, except K-40) were recorded in Uzhhorod, Vylok and Chop. This is due to a combination of geological factors (the presence of sedimentary and volcanic rocks) and anthropogenic influences in urbanised areas. Similar patterns were described by M. Cabral-Lares *et al.* (2022), who found that in areas with active anthropogenic load and different geological formations, the accumulation of elements in bottom sediments increases. Also, in fine-grained silt sediments in the lowland part of the region, minerals and organic matter actively sorb uranium and thorium, which is consistent with the conclusions of E. Juranova *et al.* (2020). A similar effect is confirmed in the works of K.S. Patel *et al.* (2023), where fine fractions and organic matter were the main accumulators of U and Th for various aquatic and sedimentary ecosystems. Significantly lower values were found in mountainous areas (Tiachiv, Rakhiv), where coarse-grained fractions with a lower capacity to accumulate uranium and thorium prevail in fast-flowing streams. These results coincide with the conclusions of Y. Zou *et al.* (2025), who studied the mechanisms of accumulation of these elements in marine bottom sediments. Also, high flow velocity reduces the possibility of accumulating organic matter, which can be an active medium for uranium sorption. Figure 3 shows the specific activity of K-40 in selected bottom sediment samples.

Figure 3 shows that the specific activity of K-40 in the region ranged from 418 to 533 Bq/kg. The highest values of specific activity of K-40 were recorded in Chop, Pidpolozzia, and Kolchyno, which are located on alluvial terraces within volcanogenic-sedimentary and Neogene formations that are naturally enriched with potassium. This pattern is consistent with the geological structure of the area, as Chop is located in a zone of Neogene alluvial deposits on the flat part of Transcarpathia. The high K-40 content here can also be explained by the accumulation of fine-grained fractions that actively bind potassium, as well as the probable influence of agricultural load – in particular, the use of potassium-containing fertilisers in lowland areas of rivers with agricultural significance upstream. Kolchyno is located directly on the territory of the Vyhorlat-Hutin Ridge, which forms the inner part of the Eastern Carpathians and is characterised by the prevalence of rocks with a high content of potassium minerals. Similar conclusions are presented in the work of S. Gönczy *et al*. (2020), which analyses the geological conditions of the North-Eastern Carpathians and points to their high mineralogical diversity.

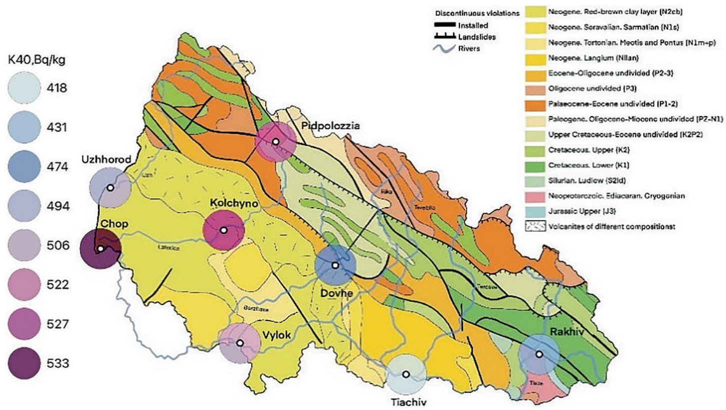

Figure 3. Visualisation of the spatial distribution of specific K-40 activity in bottom sediments of rivers in Transcarpathia, Bq/kg

Source: developed by the authors based on Geomap Land (n.d.)

The role of alluvial deposits and their ability to accumulate potassium is confirmed in a study by J.A. McIntosh *et al*. (2023), who found a significant content of potassium minerals in clay fractions. The level of specific activity of K-40 in Pidpolozzia is explained by the mineralogical composition of sedimentary rocks containing illite, muscovite and feldspars, which have a high potassium content and high adsorption capacity, which is consistent with the conclusions of V.V. Reinoso Carbonell *et al*. (2022). The lowest values are observed in Tiachiv, located in an area dominated by sandy-gravel material of mountain rivers with a relatively high flow velocity, due to which bottom sediments in this area are less capable of accumulating K-40. The same conclusions can be found in the study by H.E. Heldal *et al*. (2021). The data on the ratio of the total specific activity of the thorium series to the total specific activity of the uranium series (ΣTh-232/ΣU-238) in the studied areas of the Transcarpathian region are illustrated in Figure 4.

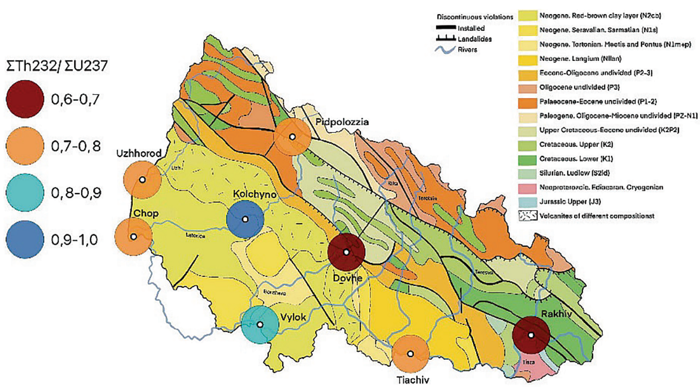

Figure 4. Visualisation of the ratio of total specific activity Th/U in bottom sediments of rivers in Transcarpathia

Source: developed by the authors based on Geomap Land (n.d.)

The Th/U ratio is an important indicator of the mineralogical diversity of bottom sediments in the region. Figure 4 shows that the lowest values (<0.7) were recorded in Dovhe and Rakhiv. In a study by L. Cao *et al*. (2021), this pattern is associated with the peculiarities of the geological structure, in particular the presence of Neogene and volcanogenic formations. In contrast, the highest values of the ratio (0.9-1.0) are characteristic of the southwestern part of the region, particularly in the Vylok and Kolchyno areas, where more consolidated Neogene sedimentary rocks dominate. At the same time, in the Uzhhorod and Chop areas, despite the presence of Neogene deposits, the ratio is lower (0.8), which can be explained by more active hydrodynamic processes. Figure 5 shows the distribution of technogenic Cs-137 in the bottom sediments of the rivers of Transcarpathia.

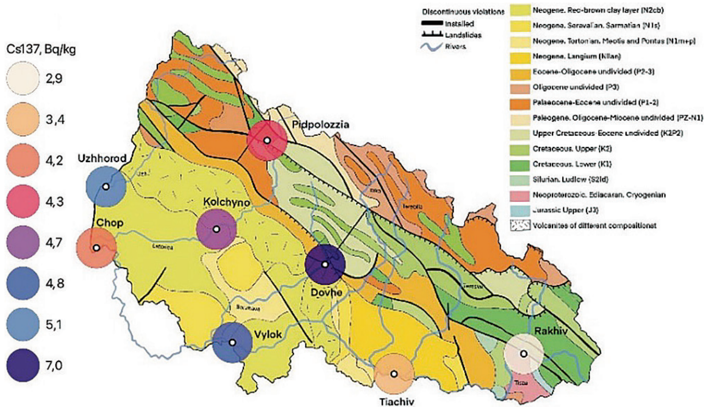

Figure 5. Visualisation of the spatial distribution of specific Cs-137 activity in river sediments in Transcarpathia, Bq/kg

Source: developed by the authors based on Geomap Land (n.d.)

As can be seen in Figure 5, the specific activity level of Cs-137 is generally low. The highest values were found in Dovhe, Uzhhorod, Vylok, and Kolchyno. Cesium is most effectively sorbed by clays and organic matter, especially in fine-grained silt. Similar patterns were noted by E.O. Akuo-ko *et al*. (2025) on the example of the Ghana coast: Cs-137 concentrations were highest in fine-grained and organically enriched sediments. In places with slow currents, such as in Dovhe, conditions are created for the accumulation and sedimentation of caesium. Similar results are presented in a study by G. Mouri (2020), which showed that Cs-137 was actively sorbed in areas with slow currents. Accordingly, the fast flow of mountain rivers does not contribute to sedimentation, and the organic content at these points is significantly lower. The concentrations of heavy metals in bottom sediments showed significant spatial variability. To convert the concentrations from percentage content to mg/kg, formulas 1 and 2 given in the "Materials and Methods" section were used. In particular, the conversion factor for Fe (formula 1) was first calculated, after which the concentrations of Zn, Pb, Cu, Ni and Cr in mg/kg were determined by multiplying the percentage content by this factor (formula 2). This allowed all data in Table 2 to be presented in mg/kg, ensuring the ac curacy of the assessment of the spatial variability of metals in different river sections.

Table 2. Concentration of heavy metals in bottom sediments of rivers in Transcarpathia, mg/kg

Point	Zn	Pb	Cu	Ni	Cr
Uzhhorod	15.3 ± 1.5	5.4 ± 0.5	5.9 ± 0.6	7.5 ± 0.8	11.4 ± 1.1
Pidpolozzia	1.4 ± 0.1	5.2 ± 0.5	4.3 ± 0.4	7.7 ± 0.8	10.4 ± 1.0
Kolchyno	26.1 ± 2.6	5.7 ± 0.6	6.9 ± 0.7	7.3 ± 0.7	13.0 ± 1.3
Dovhe	23.9 ± 2.4	6.2 ± 0.6	6.8 ± 0.7	6.9 ± 0.7	11.5 ± 1.1

Table 2. Continued

Point	Zn	Pb	Cu	Ni	Cr
Rakhiv	10.9 ± 1.1	4.4 ± 0.4	4.8 ± 0.5	7.2 ± 0.7	8.3 ± 0.8
Vylok	33.4 ± 3.3	6.4 ± 0.6	9.5 ± 1.0	8.2 ± 0.8	10.9 ± 1.1
Tiachiv	12.6 ± 1.3	–	3.9 ± 0.4	5.1 ± 0.5	6.3 ± 0.6
Chop	89.9 ± 9.0	8.9 ± 0.9	9.7 ± 1.0	8.3 ± 0.8	11.6 ± 1.2

Source: developed by the authors

In the work of S.A. Baliuk *et al*. (2023), the following limits are specified for the first suitability class: Zn ⩽ 100 mg/kg, Cu ⩽ 50 mg/kg, Pb ⩽ 50 mg/kg, Ni ⩽ 25 mg/kg, and Cr ⩽ 40 mg/kg. From Table 2, it is evident that the Zn value recorded in Chop (89.9 mg/kg) is close to the upper limit. This indicates the presence of a local anthropogenic impact, particularly from the significant transport infrastructure - border crossing points and railway lines. In Vylok and Kolchyno, the Zn value is three times lower than the limit, and in Pidpolozzia, it is significantly below even the natural background level. Similar spatial variations of zinc were observed by M. Sojka & J. Jaskuła (2022) in Polish rivers. Other locations show intermediate concentrations, which suggests a weak anthropogenic influence. Pb concentrations varied from 4.4 to 8.9 mg/kg. The maximum was recorded in Chop, where likely sources are road transport and domestic waste. In Kolchyno, Vylok, and Uzhhorod, concentrations are at a moderate level, indicating a combination of background concentrations and local anthropogenic impact. Rakhiv recorded the lowest concentration, while no Pb content was detected in Tiachiv. This confirms the results of V. Khomenchuk *et al*. (2022), where areas with a low population density and fewer sources of pollution had minimal Pb concentrations. Overall, all Pb values in the region do not exceed the aforementioned limits, though in combination with other metals, they could cause a synergistic toxic effect.

The maximum Cu values in Chop and Vylok are likely due to agricultural activity and natural processes. In Pidpolozzia and Rakhiv, concentrations are at a background level, while the lowest Cu concentration is in Tiachiv, which is characteristic of unpolluted zones and is related to the geochemical and landscape diversity of the river. These patterns are supported by the data from J. Muneer *et al*. (2022), who showed that the impact of anthropogenic sources on Cu accumulation is more noticeable in the lower reaches of rivers than in mountainous areas. Ni concentrations were in the range of 5.1-8.3 mg/kg, which is below the limit and likely also reflects the natural geochemical background, as noted in S.A. Baliuk *et al*. (2023). The lowest value was also in Tiachiv. The maximum Cr concentrations were observed in Kolchyno, linked to its geological origin (volcanic rocks at the foot of the Volcanic Ridge). Moderate levels were found in Dovhe, Chop, Uzhhorod, and Vylok, while the lowest were in Rakhiv and Tiachiv. Similar trends were described in the work of M. Sojka & J. Jaskuła (2022), where geological origin determined the baseline level of Cr in sediments.

The data obtained can be integrated into regional environmental monitoring and ecological management systems, as they allow for the creation of detailed maps of element accumulation across riverine ecosystems. This, in turn, enables the identification of areas with a potentially elevated anthropogenic impact and the assessment of the effectiveness of control measures. The data can also be used for the environmental certification of territories, including the delineation of specific areas with their natural background and potential ecological risks. The results of this screening study enable the identification of risk zones during emergencies (in case of accidental spills of toxic or radioactive elements into riverine ecosystems), which would increase the effectiveness of rapid response and, accordingly, reduce the negative impact on aquatic ecosystems and ensure public safety. The work of M. Hrechaniuk *et al*. (2023), which studied the activity of natural and technogenic radionuclides in the bottom sediments of Lake Bryt in the Chornobyl Exclusion Zone, is similar to the present study on Transcarpathian rivers. Like this study, the authors consider bottom sediments to be an indicator of the accumulation of natural (U-, Th-series, K-40) and technogenic (Cs-137) radionuclides and analyse the spatial variability of their distribution.

A common feature of both works is the use of γ-spectrometric analysis and a focus on the influence of geological and anthropogenic factors on the radioecological state of aquatic ecosystems.

It is important to note that bottom-dwelling organisms and ichthyofauna are particularly susceptible to the accumulation of radionuclides and heavy metals, as they use bottom sediments as a habitat or food source. A study by F. Jamil Emon *et al*. (2023) found that a high content of toxic elements in the lower reaches of rivers can lead to their bioaccumulation in fish tissues, which potentially reduces biodiversity and the stability of riverine ecosystems. Research by M. Shahjahan *et al*. (2022) shows that the accumulation of metals damages organs, alters respiratory and digestive functions, and affects the growth of living organisms. The results obtained in this study are important within the context of the "bottom sediments – ichthyofauna" system, as bottom sediments act as the primary depot for radionuclides and heavy metals, which fish can then accumulate in their tissues through food chains. Such patterns are confirmed by studies from B.M. Mitrović *et al*. (2025) and T. Brázová *et al*. (2025).

The data from this study have practical value for future monitoring: they allow for the determination of the bioaccumulation levels of heavy metals and radionuclides in ichthyofauna. The "bottom sediments – ichthyofauna" system can be used as an effective indicator of the ecological state of aquatic ecosystems. Thus, the study's results show that the content of radionuclides and heavy metals in the bottom sediments of Transcarpathian rivers has a clear spatial variability, which is due to a combination of natural and anthropogenic factors. In the lowland areas of the region with developed urbanisation, transport infrastructure, and agricultural activity, an increased accumulation of radionuclides and metals (especially Zn, Pb, Cu, and K-40) is observed. This is positively influenced by the high ability of fine-grained sediments and organic matter to sorb these elements. In mountainous areas with fast-flowing currents and a predominance of coarse-grained bottom sediments, the accumulation of radionuclides and metals is significantly lower. This is explained by the low adsorption capacity of the sediments and active hydrodynamic processes. Furthermore, the Th/U ratio indicates the mineralogical diversity of the deposits, while the specific activity of Cs-137 shows a minimal anthropogenic impact of technogenic origin. Overall, the results obtained make it possible to assess the nature of the spatial distribution of elements in bottom sediments, identify areas with a potentially high risk of metal and radionuclide accumulation, and can be integrated into a regional water body monitoring system. The data can also be used for the environmental certification of territories and for establishing risk zones in emergencies.

CONCLUSIONS

The conducted screening of the bottom sediments in Transcarpathian rivers indicates the presence of spatial variability in the content of natural (K-40, U- and Th-series) and technogenic (Cs-137) radionuclides, as well as heavy metals (Zn, Pb, Cu, Ni, Cr). This variability is caused by a combination of natural geochemical factors and local anthropogenic impacts. The total specific activity of the U- and Th-series is highest in low-lying urbanised areas (Uzhhorod, Vylok), while the maximum K-40 content is found in Chop, Kolchyno, and Pidpolozzia. This is linked to volcanogenic-sedimentary rocks, alluvial deposits, and the potential use of potassium-containing fertilisers. The Th/U ratio, within the range of 0.63-1, reflects the mineralogical heterogeneity of the bottom sediments and the geochemical differences between various river sections. Technogenic Cs-137 generally has low activity (2.9-7.0 Bq/kg), however, local increases were found in Dovhe and Kolchyno due to the accumulation capacity of fine-grained silt and organic matter.

Analysis of heavy metals shows elevated concentrations of Zn, Pb, and Cu in urbanised areas (Chop, Uzhhorod), which indicates local anthropogenic pollution from the presence of important transport infrastructure, such as border crossing points and railway lines. The lowest metal values were found in the mountainous part of the territory (Tiachiv, Rakhiv, Pidpolozzia). Overall, all heavy metal concentrations do not exceed the established environmental limits for bottom sediments, but local increases were recorded in the urbanised part of the region with transport infrastructure (in Chop). The results obtained provide an important basis for a further assessment of the bioaccumulation of pollutants by indigenous ichthyofauna, as well as for monitoring the state of the region's riverine ecosystems. Analysing concentrations in bottom sediments makes it possible to identify areas with an increased risk of accumulation, assess the impact on the biota, and create spatial distribution maps of elements to identify areas with increased anthropogenic pressure. The prospects for further research lie in assessing the bioaccumulation of radionuclides and heavy metals by local ichthyofauna and predicting the impact on the population structure of aquatic organisms. Additional studies could include long-term monitoring of changes in the geochemical state of bottom sediments, taking into account seasonal fluctuations in flow and element concentrations. This would allow for an assessment of the dynamics of element accumulation and migration depending on the season and for predicting the potential impact on riverine ecosystems.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Скринінг вмісту природних (^{238}U і ^{232}Th-рядів, ^{40}K) і техногенного (^{137}Cs) радіонуклідів, а також важких металів у донних відкладах річок Закарпаття

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Анотація. Донні відклади є важливим індикатором стану річок, оскільки здатні акумулювати природні та техногенні речовини, відображаючи фонові геохімічні особливості і антропогенне навантаження. Метою дослідження був аналіз вмісту природних і техногенного радіонуклідів та важких металів у річках Закарпаття для виявлення закономірностей їхнього розподілу та подальшої оцінки біоакумуляції у системі «донні відклади – іхтіофауна». Для досягнення мети було застосовано γ-спектрометричний аналіз на комплексі «ORTEC» та рентгенофлуорисцентне визначення металів за допомогою аналізатора «Elvax Pro». Зразки було відібрано у 2023-2024 рр. з восьми пунктів у межах річок Уж, Латориця, Боржава та Тиса. Результати показали просторову варіабельність, зумовлену поєднанням геологічних, морфологічних та антропогенних чинників. Сумарна активність природних радіонуклідів (U-і Th- рядів) виявлена в межах 340-534 Бк/кг, а максимальні значення були виявлені в межах пунктів Ужгород, Вилок, Чоп. Найвищу активність природного радіонукліду K-40 (522-533 Бк/кг) було зафіксовано в районах із присутністю калієвих мінералів та аграрним навантаженням – Підполоззя, Кольчино та Чоп. Cs-137 мав низьку активність (2,9-7,0 Бк/кг), з локальним підвищенням у дрібнозернистому намулі. Геохімічний індекс Th/U (0,63-1) показав мінералогічну різноманітність седиментів. Аналіз вмісту важких металів виявив просторові відмінності: концентрації Zn в межах 1,4-89,9 мг/кг, Pb - 4,4-8,9 мг/кг, Cu - 3,9-9,7 мг/кг, Ni - в межах 5,1-8,3 мг/кг, а Cr - від 6,3 до 13,0 мг/кг. Найвищі значення елементів виявлені в межах урбанізованих територій (Ужгород, Чоп), а найнижчі – у гірських районах із мінімальним антропогенним впливом. Концентрації важких металів не перевищували екологічні нормативи, проте локальні максимуми показали потенційне забруднення. Практичне значення цього дослідження полягає у використанні його результатів для екологічного моніторингу, прогнозування міграції елементів та оцінки їхньої біоакумуляції у водних трофічних ланцюгах

Ключові слова: геохімічний моніторинг; фонове радіаційне випромінювання; річкові седименти; токсичні елементи; річкові екосистеми

БІОЛОГІЧНІ СИСТЕМИ: Теорія та Інновації

Науковий журнал
Том 16, № 2. 2025

Заснований у 2010 р. Періодичність випуску: щоквартально

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

Відповідальний редактор:
Н. Шевченко

Підписано до друку 22 травня 2025 р.
Формат 70*100/16
Умов. друк. арк. 6,2
Наклад 100 прим.

Адреса редакції:
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 13, м. Київ, Україна
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BIOLOGICAL SYSTEMS: Theory and Innovation

Scientific Journal
Volume 16, No. 2. 2025

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:
N. Shevchenko

Signed for print of May 22, 2025
Format 70*100/16
Conventional printed pages 6.2
Circulation 100 copies

Editors office address:
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03041, 13 Heroiv Oborony Str., Kyiv, Ukraine
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