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Biogeochemical cycling of nickel in the hornbeam forest ecosystems of the Middle Dnipro Region

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Abstract. Heavy metals, particularly Ni, are priority pollutants of atmospheric air. Urban green spaces and forest ecosystems play a crucial role in reducing pollution levels. However, they themselves suffer from adverse impacts that induce phytotoxic effects, diminish the resilience of vegetation to pests, diseases, and other negative factors. This study aimed to assess the balance of the biogeochemical cycle of Ni in forest ecosystems to determine the consequences of pollution on vegetation within urbanised environments under varying anthropogenic pressures. Hornbeam forests from two territories of the natural reserve fund of Ukraine were selected as model ecosystems of broad-leaved forests in the Middle Dnipro Region: Holosiivskyi National Nature Park (NNP) and the Kaniv Nature Reserve. The study conducted on these model ecosystems, the methods of atomic absorption spectrophotometry and ICP-OES spectrometry were employed to ascertain the characteristics of Ni accumulation in soils. An evaluation of Ni stocks in the phytomass of hornbeam forests was performed, along with an analysis of the dynamics of metal compound accumulation in the forest litter. Furthermore, Ni's vertical migration rates were assessed using a lysimetric method, and the levels of metal compounds in the deposition process via atmospheric precipitation onto the hornbeam forests were determined. It has been established that the biogeochemical systems of Ni migration in the hornbeam forest of the Kaniv Nature Reserve are characterised by a balanced metal flow. The ecosystem of the hornbeam forest within the Holosiivskyi NNP exhibits an imbalance in the biogeochemical cycle of Ni. Consequently, in the functioning of the Ni biogeochemical cycle within the ecosystem of the Holosiivskyi NNP, the biological component of the "litter-soil-plant" system plays a crucial role, as Ni is actively absorbed by common hornbeam (*Carpinus betulus* L.) and accumulates in the phytomass. The ecosystem of the hornbeam forest of the Holosiivskyi NNP in the conditions of Kyiv is undergoing progressive Ni pollution, which is manifested in the active accumulation by vegetation, which must be taken into account when assessing the condition of existing green spaces and when designing new ones, choosing plants that are resistant to high concentrations of Ni

Keywords: soil; forest litter; leaf fall; atmospheric deposition; bioaccumulation; mineralisation; heavy metal migration

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INTRODUCTION

Urban air pollution is a global problem recognised as a leading factor affecting human health. In urban environments, green spaces and forest ecosystems play a crucial role not only in regulating the microclimate, shielding against noise pollution, and enhancing the aesthetic appeal of urban settings but also in reducing the level of air pollution. Heavy metals, particularly nickel (Ni), after entering a forest ecosystem, accumulate in its components, which can lead to phytotoxic effects and reduce the resistance of green spaces to negative environmental factors. The main mechanisms for reducing the amount of heavy metals in terrestrial ecosystems are migration processes with water flows. It is assumed that a sign of the stability of a forest ecosystem to pollution is the balance of metal input fluxes with fluxes of its removal from the ecosystem. Therefore, to understand the degree of nickel pollution and the consequences of its accumulation in urban green space ecosystems, it is necessary to assess the balance of its biogeochemical cycle under conditions of varying degrees of anthropogenic load.

According to A. Diener & P. Mudu (2021), air pollution worldwide is responsible for over 4 million premature deaths annually. A study by A. Francini *et al*. (2022) demonstrated the crucial role of urban green spaces in reducing air pollution from particulate matter (dust) and aerosols in urban environments. Scientists M. Khodadadi *et al*. (2024) concluded in their study that particulate matter is the primary pollutant with heavy metals, which are absorbed by tree leaves. Research by S. Maksimtsev *et al*. (2021) established that roadside protective forest belts reduce the concentration of dust and aerosols in the atmosphere, which in turn leads to the accumulation of heavy metals in the soil, forest litter, and plant phytomass. At the same time, litter is a key component of the forest ecosystem that regulates the migration processes and bioavailability of chemical elements for plants (Montemagno *et al*., 2022). Meanwhile, in the research of N. Ryzhenko *et al*. (2020), it was shown that the absorption of heavy metals by urban green spaces and suburban forest ecosystems affects the balance of the functioning of biogeochemical cycles of chemical elements and leads to changes in the functional state of green spaces, reducing their resistance to pollutants, including as a result of the appearance of phytotoxic effects. In particular, A. Diener & P. Mudu (2021) showed an increase in defoliation of evergreen plantings in urban park ecosystems during periods of maximum air pollution during the heating season.

Nickel is one of the priority pollutants in atmospheric air. Despite this, the number of studies on its accumulation and migration in forest ecosystems is rather limited. Anthropogenic emissions of nickel, in terms of potential toxicity, are more significant than natural sources of its entry into the environment. It has been established that about 90% of global anthropogenic nickel emissions come from the combustion of petroleum products, which determines its danger specifically for urban ecosystems and the health of urban populations (Begum *et al.,* 2022). It has been shown that nickel causes phytotoxic effects at a concentration of 135 mg/kg in sod-medium podzolic soils and 150 mg/kg for typical low-humus chernozems (Ryzhenko, 2018). Therefore, as a result of the absorption of heavy metals by plants from the atmosphere and soil, a significant increase in their concentration in the forest litter and their subsequent entry into the soil has been shown, which determines their availability for root absorption by plants and poses a potential threat to the normal functioning of forest ecosystems (Maksimtsev *et al.*, 2021; Montemagno *et al.*, 2022). While root uptake from the soil is the primary pathway for Ni entry into higher plants, the possibility of its foliar uptake by plant leaf biomass directly from the atmosphere has been confirmed (Oliveira *et al.*, 2024). Researchers E.F. Infante *et al.* (2021) note that the phenomenon of biomagnification is not typical for nickel in terrestrial ecosystems, and therefore the assessment of absolute values of its accumulation in plant phytomass cannot serve as a correct criterion for the degree of environmental pollution. Therefore, to understand the consequences of nickel entry into green spaces in urbanised environments, it is necessary to analyse the features of the functioning of its biogeochemical cycle under varying degrees of anthropogenic load to assess the balance of fluxes of input and loss of this chemical element and to establish the key ecosystem components that ensure its accumulation.

Hornbeam forests are a zonal type of broad-leaved forest ecosystem in the Middle Dnipro

Region that play an important nature conservation role. At the same time, the extremely high efficiency of air purification from heavy metals by common hornbeam plantations in linear protective forest belts of highways has been demonstrated (Maksimtsev *et al.*, 2021). Research by A. Splodytel *et al.* (2021) established a significant accumulation of Mn, Ti, and Pb by common hornbeam. It has been shown that the common hornbeam has a high potential for phytoremediation of soils contaminated with Pb and Zn (Opeña *et al.*, 2022).

Monospecific ecosystems of hornbeam forests are a convenient model for establishing the patterns of functioning of biogeochemical migration systems of heavy metals in broad-leaved forests and determining the criteria for their resistance to polymetallic pollution, and the assessment of the balance of nickel cycle fluxes of these ecosystems, which have undergone varying degrees of anthropogenic impact, has become the aim of a long-term and comprehensive study. To achieve which the following tasks were performed: to establish the features of nickel distribution in soil horizons and its content in groundwater of model forest ecosystems to assess accumulation and vertical migration; to determine the concentration of nickel in atmospheric precipitation and to estimate the annual volumes of its input fluxes into model forest ecosystems, to determine the features of the dynamics of forest litter formation and the content of nickel in its composition to assess its role in the processes of functioning of the biogeochemical migration system "leaf-litter-soil"; to analyse the dynamics of nickel accumulation in the phytomass of the main tree species of the hornbeam forest and to conduct a quantitative assessment of the fluxes of metal input in the composition of leaf fall into the forest litter and soil; and to assess the balance of biogeochemical migration systems of nickel in model forest ecosystems and to determine the ecological factors that determine the features of their functioning.

MATERIALS AND METHODS

To conduct a comparative assessment of the features of the Ni biogeochemical migration system, two model forest ecosystems of the Ukrainian nature reserve fund were selected: a hornbeam forest of the Holosiivskyi National Nature Park (NNP), which is a unique forest massif within the metropolis of Kyiv, and the Kaniv Nature Reserve, which is one of the oldest forest reserves in Ukraine and, accordingly, represents conditions that are as close to natural as possible. The model plots of the selected hornbeam forests in the nature reserves of the Middle Dnipro Region are similar according to the forest inventory data and are located on flat horizontal sections of the plateau (Table 1).

Table 1. Taxonomic description of model forest ecosystems and indicators of their biological productivity

Quartet/Plot	Area, ha	Stand characteristics	Layer	Age, years	Height, m	Diameter, cm	Yield class	Forest type	Density	Timber stock, m³/ha	Stock of phytomass of dominant tree species, t/ha				Annual phytomass increment, t/ha
											Trunk with bark	Branches	Leaves	Roots	
Holosiivskyi NNP															
26/9	0.8	10Ca. be.	1	70	25	28	I	D_2HO	0.70	320	282.1	45.5	9.3	98.4	2.76
Kaniv Nature Reserve															
15/2	7.0	10Ca. be	1	100	25	28	II	D_1HO	0.70	300	259.7	45.2	7.9	88.6	1.06

Source: developed by the authors based on Ya. Didukh *et al*. (2024)

According to calculations of biological productivity, the mass of timber in the trunks (with bark), branches, and roots of a fresh hornbeam forest D_2HO of the first yield class I, aged 70 years, which corresponds to the hornbeam forest of the Holosiivskyi NNP, is 426 t/ha (Didukh *et al.*, 2024). For the hornbeam forest of the Kaniv Nature Reserve, where the stand corresponds to a dry hornbeam forest D_1HO of the first yield class II, aged 100 years, this value is 393.5 t/ha. Of this, 66%, or

282.1 t/ha for the Holosiivskyi NNP and 259.7 t/ha for the Kaniv Nature Reserve, falls on the trunks with bark. Atmospheric precipitation was collected using rain gauges placed at ground level under the forest canopy, spaced 2 m from the tree trunk according to the method of R. Laskowski *et al.* (1995). The collectors were constructed from plastic containers and funnels with a diameter of 13 cm. The funnels were protected from the ingress of solid particles by a polypropylene mesh with a 5×5 mm mesh size. 4-8 rain collectors were placed on each experimental plot. Water sampling for analysis in the cold period of the year was carried out monthly, in case of heavy rains and in the summer months – within a day after the end of precipitation. To determine the concentration of heavy metals in water, a combined sample from all collectors of the corresponding experimental plot was used. If the volume of collected precipitation exceeded 1 L, the combined sample was divided into multiples of 1 L.

To assess vertical groundwater flow, stationary *zero-tension pan lysimeters* were used. These devices provide a means of measuring the movement of soil water and dissolved substances *in situ* (Schmidt & Henry, 2008; Makowski *et al.*, 2020). The lysimeters were fabricated from sanitary polypropylene without the use of metal components. Each lysimeter had an area of 0.1 m^2. Four lysimeters were installed at a depth of 10-12 cm in each plot to collect vertical soil water flux from the humus horizon. Soil water samples from the lysimeters were collected after intensive rainfall during the growing season or as they filled, once a month. In the case of a small volume of lysimeter water, samples from individual lysimeters were combined to obtain a combined sample volume of 1 L. Samples of atmospheric precipitation and lysimeter water were filtered through medium filtration "white tape" paper filters (pore diameter 7-20 μm) and concentrated by evaporating 1 L to 10 mL on a sand bath without bringing it to a boil, with the addition of 1 mL of 4 N HNO_3 ("special purity") per 1 L of sample (Nabivanets *et al.*, 1996).

The study was conducted following the Convention on Biological Diversity (1992). Sampling plots for soil sample collection were determined according to DSTU GOST 17.4.3.01:2019 (2019). Point samples were taken using the "envelope" method in three 3×3 m plots from two genetic horizons: the humus horizon at a depth of 5 cm and the eluvial horizon at a depth of 20-25 cm. A combined sample weighing at least 1 kg was prepared for each sampling plot. The combined soil samples were dried at a temperature of 95°C, ground in a porcelain mortar, and the volume was reduced by quartering according to DSTU ISO 11464:2007 (2007). To determine the concentration of heavy metals, *aqua regia* extraction was used by treating 0.5 g of each soil sample with 2 mL of HNO_3 and 6 mL of HCl for 2 hours according to DSTU ISO 11466-2001 (2001).

Samples of forest litter were collected monthly throughout 2018-2023 from 1×1 m plots. The collected material was dried at room temperature and weighed to determine the litter stock. A portion of the litter sample was used to determine the fractional composition, separating leaves, branches, bark, seeds, and undifferentiated fermented mass (dry rot). The mass of each fraction was determined separately. Samples of leaf phytomass of common hornbeam were collected manually from low-hanging branches, or where possible, from fallen trees and branches. Wood samples were collected from fallen trees or large skeletal branches with a diameter of more than 10 cm. Dried samples of phytomass and forest litter were ashed for 12 hours at 450°C. The resulting ash weight of 1 g was boiled in 15 mL of 4 M HNO_3 for 30 minutes. After cooling, the solution was filtered through a paper filter. The filter was washed twice with distilled water to a volume of 10 mL. The determination of heavy metal concentrations was carried out at the Department of Ecology and Zoology of the Educational and Scientific Centre "Institute of Biology and Medicine" using an atomic absorption spectrophotometer C115-M1 (SELMI, Ukraine). Analysis was performed by direct introduction of the liquid sample into an acetylene-air flame. A deuterium background corrector was used to compensate for non-selective absorption by the flame. Analytical data were recorded using a KAS-101 computer-analytical complex. Samples from 2020-2021 were also analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an iCAP 6000 ICP Spectrometer (Thermo Fisher Scientific Corporation, USA) at the Institute of Botany of the Leibniz University Hannover (Turcios *et al.*, 2021). The concentration of Ni in solid samples (soil, litter, plant material) was expressed in mg/kg of dry matter, in atmospheric precipitation water – in μg/L, and groundwater – in mg/L. Mean values were represented as the arithmetic mean (*X*), and their variability was assessed as the standard deviation (*SD*). The normality of the data distribution was established using the *Shapiro – Wilk's W-test* as the most sensitive according to Guidance for Data Quality Assessment (2000) and M. Conti *et al.* (2005).

As most measurements were limited in number (3-5 measurements), the non-parametric *Mann-Whitney U-test* was used for pairwise comparisons of sample means with a significance level of $p < 0.05$, as it is the most powerful for small samples according to Guidance for Comparing Background and Chemical Concentrations in Soil for CERCLA Sites (2002). The statistical significance of differences between multiple samples was determined using the One-Way ANOVA (ANOVA Calculator, n.d.). In conducting the research, the authors adhered to ethical norms of ecological design and experimental planning in urban green landscapes (Pataki *et al.*, 2021). Thus, using the aforementioned methods, a comprehensive analysis was conducted on the distribution of Ni concentrations in the main components of model ecosystems of hornbeam forests in the Middle Dnipro Region – atmospheric precipitation, soil genetic horizons, groundwater, leaf litter and leaf fall, wood, and leaf phytomass of common hornbeam. Annual volumes of atmospheric precipitation, the amount of soil infiltration, the dynamics of forest litter stocks, and the phytomass of common hornbeam were determined. This allowed for the determination of Ni stocks in the specified ecosystem components and the assessment of the magnitude of Ni migration fluxes between them.

RESULTS AND DISCUSSION

Nickel concentrations in the genetic horizons of the soils in the studied model ecosystems did not differ significantly: in the Holosiivskyi NNP, the humus layer contained an average of 11.2 ± 1.1 mg/kg, and in the eluvial layer – 9.6 ± 3.3 mg/kg; in the Kaniv Nature Reserve, the average nickel concentration in the humus layer was 8.3 ± 2.1 mg/kg, and in the eluvial layer it was slightly higher – 10.5 ± 3.9 mg/kg, however, the difference was statistically insignificant (*Mann–Whitney*, $p = 0.129$). Thus, the uniform distribution of nickel concentration across the soil profile may indicate its vertical migration in the soils of both studied ecosystems. A similar situation was described for the vertical distribution of Ni in forest ecosystems of the parks "Levice" and "Želiezovce", in south-western Slovakia (Pivková *et al.*, 2022). Nickel concentrations in the soils of the studied ecosystems did not exceed the MPC for hazardous substances in soils (Resolution of the Cabinet of Ministers of Ukraine No. 1325, 2021). Also, no exceedances of background nickel concentration values for arable lands of the forest-steppe zone were found, which is on average 26 mg/kg (range 10-80 mg/kg) (Chornyy, 2018) and were lower than the total nickel content in the soils of the Holosiivskyi forest, which was 20 mg/kg (Samchuk *et al.,* 2019).

Given the absence of significant differences in soil nickel concentrations between the studied hornbeam forest ecosystems, estimates of metal stocks in genetic horizons were found to be similar. Specifically, in the humus layer of Holosiivskyi NNP and Kaniv Nature Reserve, metal stocks were 6.73 ± 0.60 kg×ha^{-1} and 4.95 ± 1.30 kg×ha^{-1}, respectively. In the eluvial horizon, due to its greater thickness, the mass of accumulated metal was 21.6 ± 7.3 kg×ha^{-1} and 23.6 ± 6.4 kg×ha^{-1}, respectively. Lysimetric analysis of groundwater allowed for the estimation of the magnitude of vertical migration fluxes of heavy metals to the eluvial horizon of the soils of the studied ecosystems. The total annual soil infiltration for the hornbeam forest of Holosiivskyi NNP was 13.5 L/m^2 (135 m^3×ha^{-1}), and for the Kaniv Nature Reserve – 29.3 L/m^2 (293 m^3×ha^{-1}). In the conditions of the Holosiivskyi NNP ecosystem, the maximum nickel concentration in groundwater was characteristic of July and was 4.81 ± 0.59 mg/L. In the Kaniv Nature Reserve, its maximum concentration was observed in November-December and corresponded to the range of 2.28-2.83 mg/L, which is 1.7-2.1 times lower.

Despite differences in maximum concentration, the average annual concentration of Ni in lysimeter waters of the studied ecosystems was not statistically different and was 1.83 ± 0.22 mg/L for the Holosiivskyi NNP ecosystem and 1.74 ± 0.21 mg/L for the Kaniv Nature Reserve. Similar concentrations of Ni in groundwater were shown in a review by R. Michopoulos (2021).

Specifically, in groundwater in brown forest soils at a depth of 35 cm, the concentration of Ni reached 4 mg/L, while in podzolic soils, the concentration of Ni was 2 mg/L, which the author explains by the higher content of organic matter in podzolic soils. The calculated value of the vertical migration flux of Ni with groundwater to the eluvial horizon in the conditions of the Kaniv Nature Reserve ecosystem during the year was 51.1 ± 6.2 g×ha^{-1}×year^{-1}, which is 2.9 times higher than the level established for the Holosiivskyi NNP ecosystem, where it was 17.6 ± 1.9 g×ha^{-1}×year^{-1}, reflecting the larger volume of soil infiltration in the Kaniv Nature Reserve.

It is well-known that wet deposition via atmospheric precipitation is a primary pathway for heavy metals into terrestrial ecosystems (Hůnová *et al.*, 2023). Moreover, transboundary transport of air masses and wet deposition processes determine the input of most chemical elements into European forest ecosystems (Schlutow *et al.*, 2021). The average concentration of Ni in atmospheric precipitation within the studied ecosystems did not differ significantly and was 4.5 ± 0.9 μg/L in the Holosiivskyi NNP ecosystem and 4.1 ± 0.4 μg/L in the Kaniv Nature Reserve ecosystem (*Mann-Whitney,* p = 0.684). The established range clearly corresponds to the average Ni concentration in atmospheric precipitation for background regions of central Greece, which was 4.58 μg/L (Michopoulos, 2021), but exceeded the value shown by N. Ezaldin (2023) for Iraq (Kurdistan) – 1.96 μg/L, which may reflect the overall level of industrial development in the region. It was established that the concentration of Ni in atmospheric precipitation under the canopy of the Holosiivskyi NNP was highest at the beginning of the growing season in May-June, after which there was a gradual decrease in its concentration (Fig. 1).

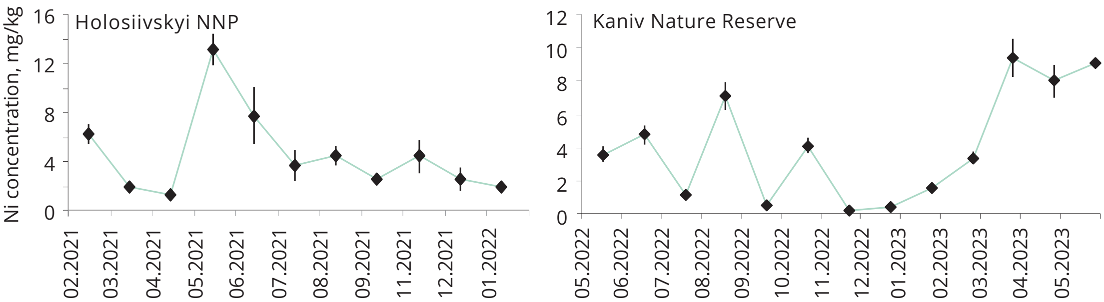

Figure 1. Annual dynamics of Ni concentration in atmospheric precipitation in hornbeam forest ecosystems of Holosiivskyi NNP and Kaniv Nature Reserve

Source: developed by the authors

In the Kaniv Nature Reserve, the concentration of Ni exhibited significant variability with a tendency to increase during the spring period. Such seasonal dynamics may reflect processes of metal leaching from young leaves, as the main accumulation of Ni occurs during its unfolding and growth through root uptake from the soil, as shown by the studies of A. Paul *et al.* (2021) and R. Michopoulos (2021).

An assessment of the annual input of Ni via atmospheric precipitation for both ecosystems revealed similar values: according to the Holosiivskyi NNP ecosystem, 29.1 ± 4.1 g×ha^{-1}×year^{-1} was deposited during 2021, and for the Kaniv Nature Reserve ecosystem, it was 38.8 ± 4.1 g×ha^{-1} ×year^{-1}. Thus, on the scale of large forest areas, the volumes of wet deposition of metals with atmospheric precipitation are more influenced by the regional transport of pollutants with air masses than by local emissions from individual pollution sources near the model forest ecosystem, which is confirmed by the results of studies by P. Michopoulos (2021). Analysis of the seasonal dynamics of heavy metal concentrations in the wood of common hornbeam throughout the year showed no statistically significant differences.

The average concentration of Ni in the wood of common hornbeam from the Holosiivskyi NNP was 2.63 ± 0.25 mg/kg, while in the Kaniv Nature Reserve, it was 3 times lower at 0.88 ± 0.26 mg/kg. To estimate the stocks of accumulated Ni in woody biomass, it was conditionally assumed that its concentration did not significantly differ in the wood of trunks, branches, and roots (some wood samples were obtained from large skeletal branches). For the Holosiivskyi NNP ecosystem, it was established that the wood of common hornbeam contained high stocks of Ni, estimated at 11.2 ± 1.25 kg/ha, while in the Kaniv Nature Reserve they were significantly lower and amounted to 0.35 ± 0.02 kg/ha. The dynamics of Ni concentration in the leaf biomass of common hornbeam during the growing season showed significant differences in the studied model forest ecosystems (Fig. 2).

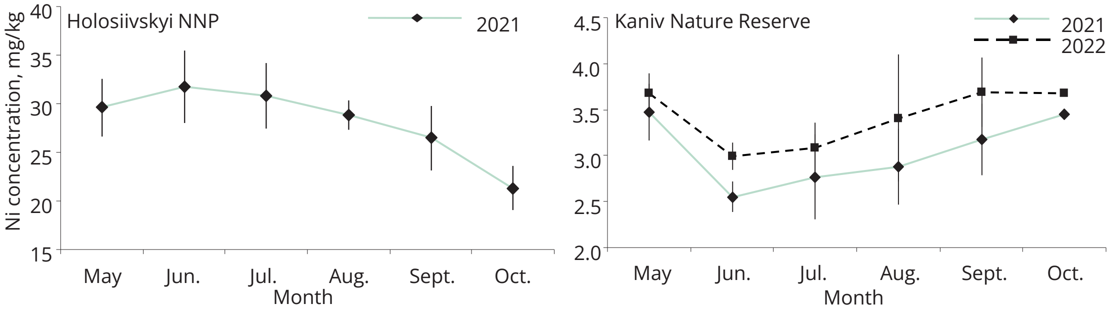

Figure 2. Dynamics of Ni concentration in the leaf biomass of common hornbeam during the growing season in hornbeam forest ecosystems
Source: developed by the authors

In the Holosiivskyi NNP, the maximum accumulation of the metal was characteristic of May-June, when the concentration was 29.6-31.7 mg/kg. Subsequently, there was a gradual decrease in the metal concentration in the leaves, reaching a minimum before leaf fall in October at 21.3 ± 2.28 mg/kg (1.5 times less compared to June). Such accumulation of the metal at the beginning of the growing season indicates its predominant intake from the soil during the process of root feeding of plants, as shown by other researchers (Paul *et al.*, 2021; Tisserand *et al.*, 2024). The subsequent decrease in metal concentration may be associated with both an increase in leaf biomass and dilution of the initial amount of metal ("*dilution effect*"), as well as leaching of the metal from the leaf biomass by atmospheric precipitation (Tőzsér *et al.*, 2023).

Conversely, in the Kaniv Nature Reserve, the concentration of Ni in common hornbeam leaves did not change significantly throughout the growing season (ANOVA, $p = 0.089$), although it was slightly higher in May (3.5-3.7 mg/kg) than in June (2.6-2.9 mg/kg). Subsequently, up until the leaf fall, there was a tendency for Ni concentration to increase. A similar constancy of Ni concentration throughout the growing season was described for *Phyllanthus rufuschaneyi*, where the metal concentration differed by only 0.2% in young and old leaves, indicating the formation of a metal stock in the early stages of leaf growth (Tisserand *et al.*, 2024). To estimate the amounts of leaf litter input, the results of the analysis of the seasonal dynamics of the component composition of the forest litter in the studied hornbeam forests were used, assuming that the mass of fallen leaves does not change significantly within a month after its entry into the litter. Calculations of the amount of leaf litter that entered the litter of both ecosystems during the year showed close values: for the Holosiivskyi NNP – 3,200 kg×ha^{-1}, for the Kaniv Nature Reserve – 2,500-3,600 kg×ha^{-1} (Table 2).

Table 2. Calculated values of Ni input into the forest litter via leaf fall in hornbeam forest ecosystems of Holosiivskyi NNP and Kaniv Nature Reserve

Month/ Year	Mass of leaf fraction in the litter, kg×ha^{-1}		Leaf fall input, kg×ha^{-1}	Amount of Ni input into forest litter via leaf fall, g×ha^{-1}	
	X	SD		X	SD
Holosiivskyi NNP					
07.21	400	50	-	-	
08.21	1,500	40	1,100	31.7	01.7

Table 2. Continued

Month/ Year	Mass of leaf fraction in the litter, kg×ha^{-1}		Leaf fall input, kg×ha^{-1}	Amount of Ni input into forest litter via leaf fall, g×ha^{-1}	
	X	SD		X	SD
09.21	1,900	150	400	10.6	1.3
10.21	3,200	140	1,300	27.7	3.0
11.21	3,300	200	100	2.1	0.2
12.21	3,600	200	300	6.4	0.7
Total			3,200	78.5	6.8
Kaniv Nature Reserve					
09.21	320	50	-	-	-
10.21	480	60	1,550	5.3	0.6
11.21	570	40	950	3.3	0.4
Total			2,500	8.6	1.0
06.22	30	10	-	-	-
07.22	40	10	120	0.4	0.02
08.22	80	20	310	1.1	0.1
09.22	140	10	650	2.4	0.4
10.22	260	20	1,190	4.4	0.4
11.22	340	40	830	3.0	0.3
12.22	390	30	500	1.8	0.2
Total			3,600	13.1	1.5

Source: developed by the authors

To calculate the flow of Ni in fallen leaves, the concentration in the leaves for the corresponding month and the mass of leaf litter that accumulated during that time in the forest litter were considered. It was established that the beginning of leaf fall and the formation of a new annual litter in hornbeam forests in the Middle Dnipro Region begins in July-August and ends in November-December. Despite changes in the concentration of Ni in the biomass of common hornbeam during the growing season, the main factor determining its input into the litter is the amount of leaf fall per month. As a result, from August to December 2021, 78.5 ± 6.8 g×ha^{-1} of Ni entered the litter of the hornbeam forest in Holosiivskyi NNP, while in the Kaniv Nature Reserve, this value in 2021 was 6-9 times lower and amounted to 8.6 ± 1.0 g×ha^{-1}, and in 2022 – 13.1 ± 1.5 g×ha^{-1}.

The concentration of Ni in the forest litter of the Holosiivskyi NNP during 2018-2019 and 2020-2021 ranged from 5.7 to 17.6 mg/kg; in the Kaniv Nature Reserve, it was 4.2-5.4 times lower, at 1.3-3.2 mg/kg. The seasonal dynamics of Ni concentration in the forest litter with the onset of leaf fall is characterised by an increase, with maximum metal accumulation occurring in November (Fig. 3).

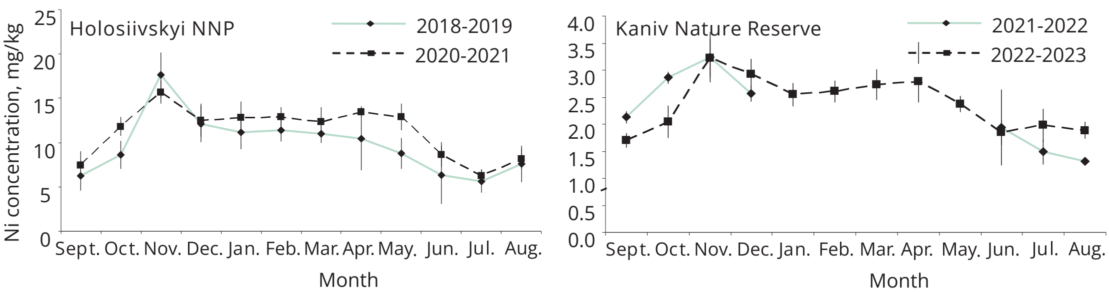

Figure 3. Annual dynamics of Ni concentration in the forest litter of the hornbeam forests in Holosiivskyi NNP and Kaniv Nature Reserve

Source: developed by the authors

In the Holosiivskyi NNP, the maximum concentration of Ni in November 2018 and 2020 was 17.6 ± 0.1 mg/kg and 15.7 ± 0.1 mg/kg, respectively. Meanwhile, the leaf biomass of common hornbeam in October before leaf fall contained 21.3 ± 2.3 mg/kg of this chemical element. In the ecosystem of the Kaniv Nature Reserve, the maximum concentration of Ni in the litter in November 2021 was 3.2 ± 0.5 mg/kg, and in 2022, it was 3.2 ± 0.4 mg/kg, which is 4.9-5.4 times less than in the Holosiivskyi NNP. The leaves of common hornbeam in October before falling contained 3.5-3.7 mg/kg of this chemical element. Thus, leaf fall serves as an important source of Ni input into the forest litter of the studied hornbeam forests. A similar phenomenon has been described by many other researchers, who have shown that leaf fall is the main flux that enriches the soil with Ni and Zn (Michopoulos, 2021; Bani *et al.*, 2024; Tisserand *et al.*, 2024).

An assessment of the dynamics of accumulated Ni in the forest litter of model forest ecosystems showed that it corresponds to the annual cycle of concentration changes – during the period of active formation of the forest litter, the stocks of the metal accumulate, reaching a maximum in November (Fig. 4).

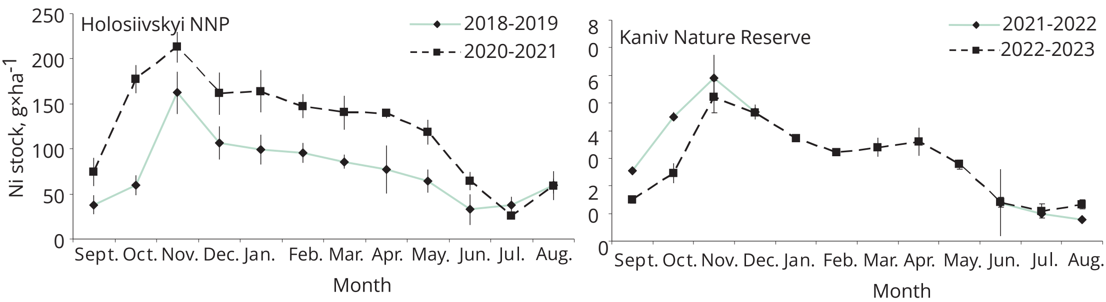

Figure 4. Annual dynamics of Ni in the forest litter of the hornbeam forests in Holosiivskyi NNP and Kaniv Nature Reserve
Source: developed by the authors

In the Holosiivskyi NNP ecosystem, the mass of accumulated Ni in the forest litter was significantly higher, 1.3-7.5 times greater, than the amount of the metal in the forest litter of the Kaniv Nature Reserve ecosystem. Moreover, in 2020-2021, the stocks of Ni in the forest litter of the hornbeam forest of the Holosiivskyi NNP were 1.3-3.0 times higher due to larger volumes of formed litter. In November 2018, in the Holosiivskyi NNP, the amount of Ni in the litter was 162 ± 40 g×ha^{-1}, and in November 2020, it was 213 ± 10 g×ha^{-1}. By December 2018, in the hornbeam forest of the Holosiivskyi NNP, the amount of Ni in the litter had decreased by 34% from the maximum volume in November, and in December 2020, this decrease in Ni stocks was 24%. In the ecosystem of the Kaniv Nature Reserve, in November 2021, the mass of accumulated metal in the litter was 59 ± 83 g×ha^{-1}, and in November 2022, it was 52 ± 6 g×ha^{-1}. Just one month later, in December 2021, there was a decrease in Ni stocks to 47 ± 3 g×ha^{-1} (by 21%), and in December 2022, to 47 ± 7 g×ha^{-1} (by 20%). Subsequently, from December to July-August, there was a gradual decrease in the amount of the chemical element until reaching a minimum at a level of 25-33 g×ha^{-1} in the Holosiivskyi NNP and 8-11 g×ha^{-1} in the Kaniv Nature Reserve, which was 79-88% of the metal accumulated in autumn.

To assess heavy metal fluxes in the model ecosystems, a 13-month period was chosen for which a complete dataset is available on metal stocks in the forest litter, volumes of metal input from atmospheric precipitation, lysimetric measurements, and estimates of the mass of fallen leaves in the forest litter. For the hornbeam forest ecosystem of the Holosiivskyi NNP, the assessment was conducted for the period from January 2021 to January 2022, and the ecosystem of the Kaniv Nature Reserve – from June 2022 to July 2023.

Over the year, the total accumulation of Ni in the forest litter of the hornbeam forest in the Holosiivskyi NNP was 115 ± 17 g×ha^{-1}×year^{-1}, which corresponds to the calculated value of the total input of the metal into the ecosystem through leaf fall and wet deposition processes from the atmosphere – 108 ± 10 g×ha^{-1}×year^{-1} (Table 3).

Table 3. Balance of Ni inputs and outputs in the biogeochemical cycle of the hornbeam forest ecosystem of the Holosiivskyi NNP

Month/Year	Ni stock, $g \times ha^{-1}$		Accumulation of Ni in the forest litter/ Output from the forest litter, $g \times ha^{-1}$		Total Ni input (leaf fall + atmospheric precipitation), $g \times ha^{-1}$		Ni input from leaf fall, $g \times ha^{-1}$		Ni input from atmospheric precipitation, $g \times ha^{-1}$		Soil Ni infiltration, $g \times ha^{-1}$	
	X	SD	X	SD	X	X	SD	SD	X	SD	X	SD
01.2021	164.0	6.5	-	-	-	-	-	-	-	-	-	-
02.2021	147.4	14.3	-16.7	10.4	6.5	-	-	-	6.51	0.82	2.05	0.25
03.2021	140.2	16.2	-7.1	15.3	0.5	-	-	-	0.50	0.05	-	-
04.2021	139.3	10.5	-0.9	13.4	0.6	-	-	-	0.59	0.13	-	-
05.2021	118.5	7.9	-20.8	9.2	9.8	-	-	-	9.83	0.98	4.48	0.55
06.2021	64.4	1.9	-54.1	4.9	1.8	0.5	-	-	1.78	0.55	2.70	0.33
07.2021	25.3	0.2	-39.1	1.1	2.3	0.8	-	-	2.25	0.81	6.11	0.75
08.2021	58.8	0.9	33.4	0.5	34.7	2.2	31.7	1.7	2.97	0.52	0.10	0.01
09.2021	58.8	3.0	0.1	2.0	11.1	1.4	10.6	1.3	0.55	0.08	-	-
10.2021	87.2	1.3	28.4	2.1	27.7	3.0	27.7	3.0	-	-	-	-
11.2021	107.0	5.9	19.7	3.6	3.5	0.7	2.1	0.2	1.41	0.45	-	-
12.2021	140.5	12.2	33.5	9.1	8.2	1.3	6.4	0.7	1.76	0.66	2.19	0.27
01.2022	101.8	10.8	-38.7	11.5	1.0	-	-	-	0.98	0.07	-	-
Total accumulation, $g \times ha^{-1} \times year^{-1}$			115.1	17.3	107.7	9.9	78.5	6.8	29.1	4.1	-	-
Total output, $g \times ha^{-1} \times year^{-1}$			-177.4	26.7	-	-	-	-	-	-	17.63	1.90
Ni accumulation in annual tree growth, $g \times ha^{-1} \times year^{-1}$			7.3	0.8								
Ni stocks in tree biomass, g/ha			11,218	1,153								
Ni stocks in 0-5 cm soil layer, g/ha			6,725	637								
Ni stocks in 5-20 cm soil layer, g/ha			21,647	7,347								

Source: developed by the authors

Meanwhile, the main contribution to Ni input was provided by leaf fall, which during the formation of new annual litter during November-December 2021 accounted for 73% of the total amount of the metal. Atmospheric precipitation accounted for 27% of the total amount of the metal – 29 ± 4 $g \times ha^{-1} \times year^{-1}$.

The total annual losses of Ni from the forest litter of the hornbeam forest in Holosiivskyi NNP were 177 ± 27 $g \times ha^{-1} \times year^{-1}$, which is 1.5 times higher than the amount of accumulated metal – 115 ± 17 $g \times ha^{-1} \times year^{-1}$. The imbalance between the accumulation and output of Ni from the forest litter in Holosiivskyi NNP is 62 $g \times ha^{-1} \times year^{-1}$. An analysis of the balance of Ni losses from the forest litter and its input over individual months showed that the forest litter loses more Ni for most of the year than it receives from atmospheric precipitation. At the same time, during the period of active formation of new annual litter in August-October, there is a correspondence between the input of Ni with leaf fall (70 $g \times ha^{-1} \times year^{-1}$) and an increase in its accumulation levels in the forest litter. At the same time, the losses of Ni from the Holosiivskyi NNP ecosystem with vertical groundwater flow per year were only 18 ± 2 g×ha^{-1}×year^{-1}, indicating the absence of active removal of the metal from the hornbeam forest ecosystem of Holosiivskyi NNP.

An assessment of Ni accumulation in the annual growth of common hornbeams revealed significant amounts of the accumulated metal – 7 ± 1 g×ha^{-1}×year^{-1} is fixed annually in the woody biomass of common hornbeams. In the leaf biomass, a significantly larger amount of Ni accumulates annually – 79 ± 7 g×ha^{-1}×year^{-1}, which, after leaf fall, returns to the soil during the decomposition of litter and becomes available for absorption by the root system of common hornbeam. At the same time, there is no active leaching of Ni by groundwater, as evidenced by its higher concentration in the humus layer (11.2 mg/kg) compared to the eluvial layer (9.6 mg/kg). Thus, in the ecosystem of the hornbeam forest of the Holosiivskyi NNP, the main part of the Ni biogeochemical cycle is provided by the "littersoil-plant" system, when Ni is extracted from the soil during root uptake and actively accumulates in the biomass of common hornbeam. The main part of the metal from fallen leaves (about 91%) returns to the soil, from where it is again absorbed by plant root systems, while the other part is fixed in the annual growth of common hornbeam wood. Approximately 91% of the metal from fallen leaves returns to the soil, where it is again absorbed by plant root systems, while the remaining portion is fixed in the annual growth of common hornbeam wood.

The leaching of Ni during vertical migration through the soil profile is negligible, indicating an accumulation of the metal in the ecosystem. A similar situation has been described for soils in Albania, where it was shown that plants absorb Ni from deep soil layers and redistribute it to the upper soil layer, leading to the enrichment of the litter with Ni due to the input of leaf fall (Bani *et al.*, 2024). Such a biogeochemical cycle is sensitive to excessive metal input from external sources, which can lead to the accumulation of Ni in the ecosystem. In particular, a comparison of the amount of Ni entering the hornbeam forest ecosystem of the Holosiivskyi NNP from atmospheric precipitation (29 ± 4 g×ha^{-1}×year^{-1}) and the amounts leached from the soil (18 ± 2 g×ha^{-1} ×year^{-1}) indicates a significant imbalance in the biogeochemical cycle of this chemical element. Over the year, the total accumulation of Ni in the forest litter of the hornbeam forest of the Kaniv Nature Reserve was 48 ± 4 g×ha^{-1}×year^{-1} (Table 4).

Table 4. Balance of Ni inputs and outputs in the biogeochemical cycle of the hornbeam forest ecosystem of the Kaniv Nature Reserve

Month/Year	Ni stock, g×ha^{-1}		Accumulation of Ni in the forest litter/ Output from the forest litter, g×ha^{-1}		Total Ni input (leaf fall + atmospheric precipitation), g×ha^{-1}		Ni input from leaf fall, g×ha^{-1}		Ni input from atmospheric precipitation, g×ha^{-1}		Soil Ni infiltration, g×ha^{-1}	
	X	SD	X	SD	X	X	SD	SD	X	SD	X	SD
06.2022	13.82	3.17	-	-	-	-	-	-	-	-	-	-
07.2022	9.82	4.67	-4.00	3.92	4.77	0.55	0.37	0.02	4.40	0.54	1.06	0.13
08.2022	7.85	1.52	-1.97	3.10	3.54	0.39	1.06	0.08	2.48	0.30	-	-
09.2022	14.91	2.18	7.05	1.85	3.74	0.61	2.40	0.45	1.35	0.16	15.72	1.92
10.2022	24.57	5.26	9.66	3.72	7.06	0.77	4.37	0.45	2.69	0.33	-	-
11.2022	52.25	5.83	27.69	5.55	3.49	0.36	3.05	0.31	0.44	0.05	15.00	1.83
12.2022	46.51	7.06	-5.75	6.45	5.55	0.64	1.84	0.19	3.72	0.45	11.28	1.38
01.2023	37.02	6.44	-9.49	6.75	0.14	0.02	-	-	0.14	0.02	-	-
02.2023	32.11	5.16	-4.91	5.80	0.27	0.03	-	-	0.27	0.03	-	-
03.2023	33.95	4.52	1.84	4.84	0.49	0.06	-	-	0.49	0.06	-	-
04.2023	35.88	6.02	1.92	5.27	1.18	0.14	-	-	1.18	0.14	-	-
05.2023	27.72	4.39	-8.16	5.21	10.03	1.22	-	-	10.03	1.22	8.08	0.99

Table 4. Continued

Month/Year	Ni stock, $g \times ha^{-1}$		Accumulation of Ni in the forest litter/ Output from the forest litter, $g \times ha^{-1}$		Total Ni input (leaf fall + atmospheric precipitation), $g \times ha^{-1}$		Ni input from leaf fall, $g \times ha^{-1}$		Ni input from atmospheric precipitation, $g \times ha^{-1}$		Soil Ni infiltration, $g \times ha^{-1}$	
	X	SD	X	SD	X	X	SD	SD	X	SD	X	SD
06.2023	14.22	3.57	-13.49	3.98	6.33	0.77	-	-	6.33	0.77	-	-
07.2023	10.93	4.02	-3.30	3.80	5.25	0.05	-	-	5.25	0.00	-	-
Total accumulation, $g \times ha^{-1} \times year^{-1}$			48.16	4.25	51.83	3.35	13.08	1.49	38.75	4.09	-	-
Total output, $g \times ha^{-1} \times year^{-1}$			-51.06	4.88	-	-	-	-	-	-	51.14	6.24
Ni accumulation in annual tree growth, $g \times ha^{-1} \times year^{-1}$			0.94	0.07								
Ni stocks in tree biomass, g/ha			347.2	24.3								
Ni stocks in 0-5 cm soil layer, g/ha			4,953	1,340								
Ni stocks in 5-20 cm soil layer, g/ha			23,637	6,409								

Source: developed by the authors

The estimated total annual loss of Ni from the litter was found to be 51 ± 5 $g \times ha^{-1} \times year^{-1}$, which is not statistically significantly different (*Mann–Whitney*, $p = 0.109$) from the total accumulation of the metal (the difference is only 3 $g \times ha^{-1} \times year^{-1}$). This indicates a balanced state of the nickel biogeochemical cycle in the hornbeam forest of the Kaniv Nature Reserve, where the amount of metal entering the litter is balanced by the processes of its output from the ecosystem.

The calculated total input of Ni from leaf fall and wet deposition from atmospheric precipitation is a similar value – 52 ± 13 $g \times ha^{-1} \times year^{-1}$. Unlike the Holosiivskyi NNP ecosystem, in the conditions of the Kaniv Nature Reserve, 75% of the total input of Ni into the hornbeam forest is determined by processes of wet deposition with atmospheric precipitation – 39 ± 4 $g \times ha^{-1} \times year^{-1}$, and 25% – by the amount of metal that entered as part of fallen leaves (13 ± 2 $g \times ha^{-1} \times year^{-1}$). In the conditions of the Holosiivskyi NNP ecosystem, wet deposition as part of atmospheric precipitation gives a similar value of metal input into the ecosystem – 29 ± 4 $g \times ha^{-1} \times year^{-1}$. A comparison of the total Ni input into the Kaniv Nature Reserve ecosystem (52 ± 3 $g \times ha^{-1} \times year^{-1}$), groundwater runoff ($51 \pm 6$ $g \times ha^{-1} \times year^{-1}$), and the total amount of Ni output from the litter (52 ± 5 $g \times ha^{-1} \times year^{-1}$) indicates the presence of a balance between the input and loss of metal in the ecosystem.

However, unlike the Holosiivskyi NNP ecosystem, in the Kaniv Nature Reserve, an increase in metal concentration to 10.5 ± 3.9 mg/kg is observed in the eluvial soil horizon at a depth of 20 cm, which may indicate its active vertical migration through the soil profile and leaching from the upper humus horizon. In particular, the volumes of groundwater runoff of Ni over the year exceed 1.3 times the amount of metal entering from atmospheric precipitation, which is 51 ± 6 $g \times ha^{-1} \times year^{-1}$ and 39 ± 4 $g \times ha^{-1} \times year^{-1}$, respectively. Therefore, the main pathway for Ni input into the Kaniv Nature Reserve ecosystem, unlike the Holosiivskyi NNP ecosystem, is atmospheric precipitation, which accounts for 75% of the total metal input. The amount of Ni entering with atmospheric precipitation corresponds to the amount of metal that was removed during leaching by vertical groundwater flow to the eluvial layer. The fixation of Ni in the annual growth of common hornbeam wood is insignificant and amounts to 0.94 ± 0.07 $g \times ha^{-1} \times year^{-1}$.

A review of recent scientific publications has revealed a lack of comprehensive studies on the biogeochemical cycle of Ni in European forest ecosystems. Most publications are dedicated to studying the accumulation of Ni by plants in model experiments to use them for phytoremediation of contaminated soils (Rosatto *et al.*, 2021; Samreen *et al.*, 2021; Reis *et al.*, 2024). In natural ecosystems, research focuses on studying the features of Ni accumulation in individual components of forest ecosystems – biomass (McQueen *et al.*, 2023), litter (Zhao *et al.*, 2024) and soil (Brandão *et al.*, 2022). In his review, P. Michopoulos (2021) attempted to estimate the magnitude of Ni fluxes in European forest ecosystems, but a complete assessment of the balance of the metal's biogeochemical cycle was not carried out. The most complete analysis of the Ni biogeochemical cycle was conducted in the studies of A. Paul *et al.* (2021) and R. Tisserand *et al.* (2024), that dedicated to studying the functioning of specific tropical ecosystems of Ni hyperaccumulator plants used for industrial extraction of metallic nickel from Ni-rich soils. Therefore, the results of such studies can only be partially used to compare the processes of Ni migration in European broad-leaved forest ecosystems.

As a result of the analysis of the main Ni fluxes in model hornbeam forest ecosystems of the Middle Dnipro Region, significant differences in the functioning of the metal's biogeochemical cycle were revealed. In the Holosiivskyi NNP ecosystem, the main pathway for metal input is leaf fall, which accounts for 73% of the metal volume. At the same time, in the Kaniv Nature Reserve, leaf fall accounts for only 25% of the total metal input (Fig. 5).

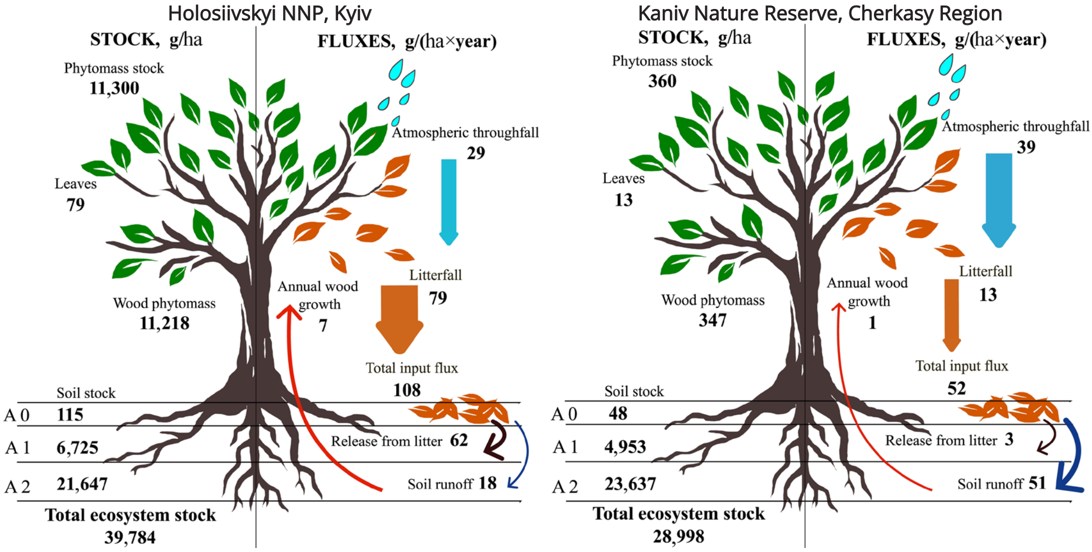

Figure 5. The biogeochemical cycle of Ni in the hornbeam forest ecosystems of the Holosiivskyi NNP and the Kaniv Nature Reserve

Source: developed by the authors

Similar values were indicated in the study by R. Tisserand *et al.* (2024), where leaf fall from the tropical nickel hyperaccumulator tree *P. rufuschaneyi* provided 74% of the total metal flux in the ecosystem formed on nickel-enriched soils. Despite the fact that the gross concentration of nickel in the soils of the hornbeam forests studied by authors does not differ significantly, in the Holosiivskyi NNP there is active accumulation of nickel by biomass, as a result of which the estimated nickel stocks in the leaf biomass of common hornbeam from Holosiivskyi NNP are 6 times greater (79 g/ha) than in the Kaniv Nature Reserve (13 g/ha). The differences in metal stocks in the wood were even more significant.

In the common hornbeam wood of Holosiivskyi NNP, 11.3 kg/ha of Ni is concentrated, which is 31 times more than in the common hornbeam wood of the Kaniv Nature Reserve – 0.35 kg/ha, which respectively accounts for 28% and 1.2% of the total metal stocks in the ecosystem. In tropical forest ecosystems formed on nickel-rich ultramafic serpentine rocks, the main part of this metal is concentrated in the plant biomass. This phenomenon was revealed in the study by A. Paul *et al.* (2024) when 90.3% of the total nickel stocks of the ecosystem were concentrated in the biomass of the tropical nickel hyperaccumulator tree *Pycnandra acuminata*. As a result of Ni fixation in the biomass of common hornbeam, the volumes of leaching by vertical groundwater flow in Holosiivskyi NNP are 2.8 times smaller, where the value of this component of the metal flow is 18 g×ha^{-1}×year^{-1}. In the Kaniv Nature Reserve, leaching to the eluvial soil layer is 51 g×ha^{-1}×year^{-1} and corresponds to the amount of annual Ni input with atmospheric precipitation. As a result, the forest litter of the hornbeam forest of the Kaniv Nature Reserve contains 2.4 times less nickel than the litter of the Holosiivskyi NNP ecosystem. Estimates of the magnitude of soil Ni infiltration, presented in the study by P. Michopoulos (2021), showed similar values. In particular, in a spruce forest in central Germany, the Ni flux was estimated at 11.5 g×ha^{-1}×year^{-1}. In a mixed stand of Norway spruce, birch, and aspen in Finland, the annual leaching of nickel was 6.2 g×ha^{-1}×year^{-1}. In the northeast of France, quite high Ni fluxes in the soil solution were found, amounting to 43 g×ha^{-1}×year^{-1} (Michopoulos, 2021).

Thus, the presented study of model hornbeam forest ecosystems in the Middle Dnipro Region has shown that forest ecosystems in urban conditions (Holosiivskyi NNP) and in natural conditions (Kaniv Nature Reserve) do not significantly differ in the concentration of Ni in soils, groundwater, and atmospheric precipitation, which can lead to a false understanding of the level of anthropogenic load on such ecosystems. However, an analysis of Ni accumulation levels in common hornbeam biomass showed significant differences, when the Ni stocks in hornbeam wood in Holosiivskyi NNP exceeded the metal stocks in Kaniv Nature Reserve by more than 30 times. The Ni stocks in the leaf biomass of hornbeam in Holosiivskyi NNP were 6 times higher, and the stocks in the forest litter were 1.3-7.5 times higher. Accordingly, a significant amount of nickel accumulated in the biomass of common hornbeam in Holosiivskyi NNP led to significant changes in the functioning of the nickel biogeochemical cycle, compared to the conditions of the Kaniv Nature Reserve, when the input of nickel from atmospheric precipitation and leaf fall was not balanced by the loss in the process of leaching by vertical groundwater flow, which led to the retention and accumulation of the metal in the ecosystem. The authors' results confirm the features of the nickel biogeochemical cycle described by other authors in conditions of natural geochemical anomalies of polymetallic deposits when the main part of the nickel biogeochemical cycle is provided by the "litter-soil-plant" system. The main part of the metal (over 90%) returns to the soil as part of leaf fall, from where it is again absorbed by plant root systems. The constant additional input of nickel as part of atmospheric precipitation and the slow leaching of the metal with groundwater creates a danger of continued accumulation of nickel in the biomass to levels that can cause phytotoxic effects in woody plants in urban environments. The imbalance of the nickel biogeochemical cycle in Holosiivskyi NNP indicates the presence of anthropogenic pollution of the ecosystem with this metal.

CONCLUSIONS

A comprehensive, multi-year assessment of the balance of Ni biogeochemical migration fluxes in model hornbeam forest ecosystems of Holosiivskyi NNP and Kaniv Nature Reserve has established that the main input of Ni into the Holosiivskyi NNP hornbeam forest ecosystem was through leaf fall, which, during the formation of a new annual litter layer, provided 73% of the total amount of the metal – 79 ± 7 g×ha^{-1}×year^{-1}. Atmospheric precipitation accounted for 27% – 29 ± 4 g×ha^{-1}×year^{-1}. Losses of Ni from the Holosiivskyi NNP ecosystem with vertical groundwater flow per year amounted to 18 ± 2 g×ha^{-1}×year^{-1}, indicating an imbalance in the Ni biogeochemical cycle. The slow removal of the metal from the ecosystem by groundwater runoff leads to the accumulation of Ni in the soil – with a higher concentration in the humus layer compared to the eluvial layer. As a result of active root uptake of Ni by plants, it accumulates in the biomass – 7 ± 1 g×ha^{-1}×year^{-1} is accumulated annually in the woody biomass of common hornbeam, and 79 ± 7 g×ha^{-1}×year^{-1} in the leaf biomass. It is shown that after leaf fall, Ni returns to the soil and becomes available for absorption by the root system of plants. In the hornbeam forest ecosystem of Holosiivskyi NNP, the main part of the Ni biogeochemical cycle is provided by the "litter-soil-plant" system. In the hornbeam forest ecosystem of Kaniv Nature Reserve, 75% of the total Ni input is provided by the process of wet deposition with atmospheric precipitation – 39 ± 4 g×ha^{-1}×year^{-1}. Leaf fall accounts for 25% of this chemical element – 13 ± 2 g×ha^{-1}×year^{-1}. A comparison of the total Ni input into the Kaniv Nature Reserve ecosystem (52 ± 3 g×ha^{-1}×year^{-1}), groundwater runoff to the eluvial soil layer (51 ± 6 g×ha^{-1}×year^{-1}) and the total amount of Ni output from the litter (52 ± 5 g×ha^{-1}×year^{-1}) indicates the presence of a balance between the input and loss of the metal in the hornbeam forest ecosystem of Kaniv Nature Reserve, and the main migration processes are provided by the "atmosphere-litter-soil" system.

The conducted research reveals the presence of complex mechanisms of heavy metal migration in forest ecosystems. To determine the functional state of urban green spaces and forest ecosystems, it is necessary to assess the degree of balance of heavy metal biogeochemical cycles, which will allow for the prediction of risks of toxicant accumulation in component parts with the aim of developing measures to increase resistance to pollution. This must be considered when designing new plantings resistant to heavy metal pollution.

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CONFLICT OF INTEREST

None.

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Біогеохімічний цикл нікелю в умовах екосистем грабових дібров Середнього Придніпров'я

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Анотація. Важкі метали, зокрема Ni, є пріоритетними забруднювачами атмосферного повітря. Міські зелені насадження та лісові екосистеми зменшують рівень забруднення, проте самі зазнають негативного впливу, що викликає фітотоксичні ефекти, зменшує стійкість зелених насаджень до шкідників, хвороб та інших негативних чинників. Метою дослідження було провести оцінку збалансованості біогеохімічного циклу Ni в лісових екосистемах для визначення наслідків забруднення зелених насаджень в умовах урбанізованого середовища за різниці антропогенного навантаження. Як модельні екосистеми широколистяних лісів Середнього Придніпров'я було обрано грабові діброви двох територій природно-заповідного фонду України: Національний природний парк (НПП) «Голосіївський» та Канівський природний заповідник. У дослідженні, проведеному на цих модельних екосистемах, були використані методи атомно-абсорбційної спектрофотометрії та ICP-OES спектрометрії для з›ясування особливостей накопичення Ni в ґрунтах, проведено оцінку запасів у фітомасі деревної породи грабових дібров та динаміку накопичення сполук металу у лісовій підстилці, оцінено величини потоків вертикальної міграції Ni лізиметричним методом, визначено рівні надходження сполук металу в процесі осадження у складі атмосферних опадів на територію грабових дібров. Встановлено, що біогеохімічні системи міграції Ni грабової діброви Канівського природного заповідника характеризуються балансом потоків металу. Екосистема грабової діброви НПП «Голосіївський» характеризується незбалансованістю біогеохімічного циклу Ni. Як наслідок, у забезпеченні функціонуванні біогеохімічного циклу Ni в екосистемі НПП «Голосіївський» основну роль відіграє біологічна складова системи «підстилка-ґрунт-рослина», коли Ni активно поглинається рослинами грабу звичайного (*Carpinus betulus* L.) та накопичується у фітомасі. Екосистема грабової діброви НПП «Голосіївський» в умовах міста Києва зазнає прогресуючого забруднення Ni, що проявляється у активному накопиченні рослинністю, що необхідно враховувати при оцінках стану існуючих зелених насаджень та при проєктуванні нових, обираючи рослини, які є стійкими до високих концентрацій Ni

Ключові слова: ґрунт; лісова підстилка; листяний опад; атмосферне осадження; біоакумуляція; мінералізація; міграція важких металів

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The impact of metal oxide-based nanofertilisers on the physicochemical properties of agricultural plants

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Abstract. This study aimed to conduct a comprehensive analysis of existing research on the effects of metal oxide-based nanofertilisers, such as zinc oxide (ZnO), iron oxide (Fe_2O_3), and titanium dioxide (TiO_2), on plant development and growth. The research methodology was based on the analysis of scientific articles examining the influence of nanofertilisers composed of ZnO, Fe_2O_3, and TiO_2 on the physicochemical properties of crops such as wheat and soya beans. The study employed methods such as the analysis, generalisation, and comparison of various research findings. It explored multiple aspects of using these nanofertilisers, including their effects on improving the growth, development, and health of agricultural crops, particularly wheat and soya beans, the determination of optimal concentrations, and potential risks that may impact productivity and environmental safety. Given the challenges associated with enhancing agricultural efficiency, reducing environmental impact, and ensuring stable yields, the research aimed to identify optimal concentrations of nanofertilisers that could maximise positive effects on plants without the risk of adverse consequences. The literature review included an analysis of laboratory experiment results involving the use of various concentrations of nanofertilisers at different stages of plant development. Key parameters examined in these experiments, such as chlorophyll content in leaves, antioxidant enzyme activity, water balance, and root growth and development, were discussed. The review suggested that low to moderate concentrations of nanofertilisers have a positive effect on photosynthetic activity, root system development, and the overall condition of plants. However, it was emphasised that high concentrations of nanofertilisers can inhibit plant growth and lead to negative consequences. These findings highlighted the importance of precise dosing and careful monitoring when applying nanofertilisers to achieve optimal results. The research demonstrated the

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significant potential of metal oxide-based nanofertilisers in agriculture to enhance crop resilience to environmental stress factors and improve their productivity

Keywords: mineral modification; nutrient bioavailability; agricultural productivity; antioxidant activity; effects on the root system

INTRODUCTION

Sustainable agricultural development is a crucial issue in the context of population growth and increasing pressure on natural resources. Traditional methods of enhancing crop yields, such as the use of synthetic chemical fertilisers, have contributed to increased production but have had adverse environmental effects. Excessive use of such fertilisers has led to soil degradation, water pollution, and disruption of ecological balance. This has reduced soil fertility, its water retention capacity, and caused eutrophication of water bodies and a decline in biodiversity. Consequently, the need has arisen for environmentally safe and economically viable alternatives to improve the efficiency of agricultural systems. One such solution has been the use of metal oxide-based nanofertilisers, which enhance the nutritional status of plants and improve their resilience to stress. Nanotechnology offers the potential to increase nutrient availability to plants through the unique properties of nanoparticles, facilitating more efficient nutrient delivery and stimulating physiological processes.

Nanotechnology, including nanofertilisers, offers significant advantages over traditional plant nutrition methods and introduces new possibilities in agronomy. Metal oxide nanoparticles, such as ZnO, Fe_2O_3 and TiO_2, due to their unique properties, have demonstrated effectiveness in agronomy. These nanoparticles possess a larger specific surface area, which promotes better adsorption and nutrient uptake by the root system, reducing the need for large volumes of conventional fertilisers and minimising negative environmental impacts. Nanoparticles interact with plant cells at the molecular level, allowing for more efficient element transport. For instance, ZnO influences chlorophyll synthesis, increasing the number of photosynthetic pigments and improving photosynthetic activity (Gorobets *et al.*, 2023). Fe_2O_3 nanoparticles act as cofactors for enzymes critical to protein synthesis, respiration, and nitrogen fixation, which positively affect plant growth and resilience. TiO_2 nanoparticle fertilisers stimulate root growth and enhance the plant's ability to absorb water and nutrients, improving drought tolerance and resilience to other stressful conditions. However, the use of nanofertilisers requires careful monitoring to assess potential risks and their long-term impact on ecosystems.

Iron (Fe) is an essential micronutrient for plants and plays a key role in various biochemical processes, including electron transport in respiration and photosynthesis, nitrate synthesis, and nitrogen fixation, all of which are vital for amino acid and protein formation, as well as plant hormone synthesis (Prasad *et al.*, 2017). Iron oxide nanoparticles, such as Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite), possess unique properties, including superparamagnetism and a large specific surface area. Superparamagnetism enables them to retain magnetic properties only in the presence of an external magnetic field, while the large specific surface area enhances iron absorption by plants. The biocompatibility of these nanoparticles ensures their safety and effectiveness for long-term use. Fe_3O_4 and γ-Fe_2O_3 nanoparticles are utilised not only in agronomy but also in advanced technologies, such as magnetic resonance imaging, due to their ability to improve nutrient delivery to plants, increasing their bioavailability and absorption.

The number of studies on the impact of metal oxide-based nanofertilisers on the growth and development of agricultural crops has significantly increased compared to previous decades when the focus was primarily on the use of traditional mineral fertilisers and chemicals to boost yields. This shift is due to growing interest in nanotechnology and its potential to address issues related to resource efficiency and environmental sustainability in the agricultural sector. Research on zinc, iron, and copper nanoparticles, in particular, has shown that their application can substantially increase crop yields, improve product quality, and reduce the need for conventional chemical fertilisers. For instance, zinc oxide nanoparticles can enhance the physicochemical properties of plants, increasing their photosynthetic activity and resilience to drought and soil salinity, owing to their ability to efficiently deliver nutrients. Moreover, nanofertilisers help reduce toxic emissions, contributing to environmental sustainability (Tombuloglu *et al.*, 2020). Despite their positive potential, the widespread use of nanofertilisers presents challenges, including potential risks to human health and the environment. High concentrations of nanoparticles may induce toxic effects on plants, delaying their growth and development due to accumulation in tissues and disruption of physiological processes (Yew *et al.*, 2020). There are also concerns about the accumulation of nanoparticles in soils and their possible entry into the food chain, which could have unpredictable consequences for ecosystems.

Therefore, it is crucial to conduct further research to understand the mechanisms of action, long-term effects, and potential risks of nanofertilisers to ensure their safe and effective use. This study aimed to perform a comprehensive analysis of research on the effects of metal oxide-based nanofertilisers, such as zinc oxide (ZnO), iron oxide (Fe_2O_3), and titanium dioxide (TiO_2), on plant growth and development, as well as their impact on plant physiological processes. The objectives included examining the influence of various sources on plant growth, analysing potential toxic effects, and assessing the long-term implications of their use.

MATERIALS AND METHODS

The literature search for this study was conducted using scientific databases such as Scopus and Web of Science, which provided access to a wide range of academic studies on the specified topic. The keywords used during the search included terms such as "magnetite nanoparticles", "wheat growth", "nanofertilisers", "plant physiology", and "plant nanoparticle uptake". Additional search queries were employed to refine the results, focusing on nanoparticle size and concentration, such as "8-10 nm magnetite nanoparticles" and "nanoparticle impact on wheat cultivation". Over 150 relevant scientific articles were identified. Based on a thorough analysis of the content and adherence to inclusion criteria, 35 articles were selected for detailed investigation. The inclusion criteria comprised publications released between 2010 and 2023, studies with a clear description of experimental conditions, the use of metal nanoparticles, particularly magnetite, and a detailed analysis of their effects on plants. Special attention was given to studies that included physiological measurements such as plant height, root length, chlorophyll content, and the plant's ability to absorb nanoparticles.

In contrast, studies lacking sufficient experimental data or providing an incomplete description of the methodology were excluded. Research involving other types of nanoparticles unrelated to metals, or those with insufficient replication or limited analytical methods, was also disregarded (Joshi *et al.*, 2022). For detailed examination and further analysis, 24 of the most relevant sources were selected, which are listed in the References section. These studies focused on key physicochemical properties of magnetite nanoparticles, such as their size and shape, which significantly influenced their effectiveness. In particular, the selected studies concentrated on the impact of magnetite nanoparticles on the growth and development of wheat, especially in terms of increasing plant height, root system length, enhancing chlorophyll content, and improving nutrient uptake. These studies formed the basis for subsequent conclusions regarding the effectiveness of using magnetite nanoparticles as fertilisers in this research. Based on the referenced scientific studies, various methods of introducing magnetite nanoparticles into hydroponic systems were thoroughly examined. By analysing the scientific literature, the impact of nanoparticles on the physiological and morphological characteristics of plants was assessed, enabling the identification of key patterns and the determination of the most effective nanoparticle concentration for enhancing plant growth.

RESULTS

A key focus of the research was to investigate the impact of nanoparticles on the uptake and translocation of elements within plant tissues. This is crucial because these processes directly influence the effectiveness of nanofertilisers as pesticides. Understanding how nanoparticles enter plant tissues is vital as it determines their efficacy as fertilisers. Nanoparticles can enter plant tissues through various pathways, including the root system and leaves. They may pass through root hairs and intercellular spaces, entering root tissues. Their size and surface properties play a significant role in this process (Telizhenko, 2020).

For instance, nanoparticles with a large specific surface area can easily adhere to root tissues, facilitating uptake. Once absorbed by root cells, nanoparticles are then transported throughout the plant to the stem and leaves via the xylem and phloem. In the xylem, which transports water and dissolved substances from the roots to other parts of the plant, nanoparticles can move along with water and solutes. In the phloem, which is responsible for transporting organic compounds from the leaves to other parts of the plant, nanoparticles are transported with essential nutrients. The efficacy of nanofertilisers depends on their ability to be absorbed and translocated. Nanoparticles that are successfully taken up and transported have a positive impact on the physical and chemical properties of the plant – they can increase the content of micronutrients and other nutrients and improve overall plant productivity (Kornarzyński *et al*., 2020). For example, iron nanoparticles can enhance root growth and improve the uptake of water and nutrients. Copper nanoparticles positively influence photosynthesis, increasing chlorophyll levels and biomass. However, the effectiveness of nanofertilisers can be hindered by various factors, including agronomic conditions, nanoparticle concentration, size, and shape. J. Li *et al*. (2016) investigated the effects of copper nanoparticles and showed that excessive concentrations can cause toxic effects such as oxidative stress, DNA damage, mitochondrial dysfunction, and apoptosis. High doses of nanoparticles were also found to affect the structure of cell membranes, altering their permeability and disrupting cellular homeostasis. This highlights the importance of determining optimal conditions for using nanofertilisers to maximise their benefits and minimise negative side effects.

Results of the study demonstrated that the root system development of plants treated with metal oxide-based nanofertilisers was significantly improved. Specifically, the use of iron oxide (Fe_2O_3) nanoparticles at concentrations of 10 mg/L and 20 mg/L resulted in a significant increase in root length and biomass. This was confirmed by experimental data showing an increase in root length and a significant increase in total root mass compared to the control group. Additionally, concentrations of zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles in the range of 5-15 mg/L were investigated. Treatment with these nanoparticles was found to promote root elongation and increased biomass. Experimental data showed that plants treated with ZnO and MgO nanoparticles had significantly longer roots and higher root biomass compared to control samples, indicating the effectiveness of these nanofertilisers in stimulating root development. The positive impact of metal oxide nanoparticles on root development can be attributed to their ability to enhance the uptake of minerals and water, which are essential for plant growth. Figure 1 illustrates the processes of nanoparticle uptake by plants, their transport through the root system, and their impact on different parts of the plant. The figure demonstrates how nanoparticles penetrate roots, are transported through the vascular system, and influence the growth and development of the above-ground plant parts. The mechanisms of nanoparticle interactions with plant tissues highlighted in the figure underscore the crucial role of the root system in their uptake and distribution.

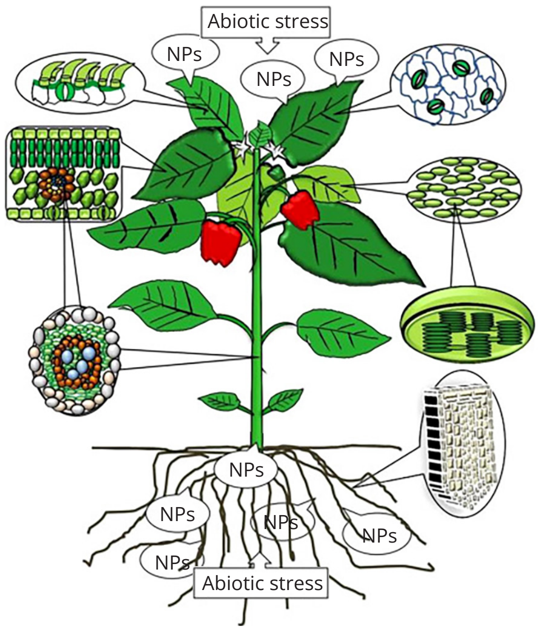

Figure 1. Schematic representation of nanoparticle uptake by a plant

Note: arrows indicate the direction of nanoparticle movement through plant tissues

Source: D.K. Tripathi *et al*. (2020)

A study conducted by N. Joshi *et al*. (2022) revealed a significant impact of biocompatible Fe_3O_4 and MnO_2 nanoparticles on tuber formation in potatoes (*Solanum tuberosum L.*). The experiment utilised nanoparticles at various concentrations (0.5; 1; 2 g/L), and the results demonstrated a substantial increase in tuber number by 25% and tuber size by 30%. This was achieved through the enhancement of physiological processes in plants, such as increased cell growth activity and improved nutrient uptake. The impact of nanoparticles also included the stimulation of new shoot formation and increased photosynthetic efficiency, collectively leading to higher potato yields. This confirms the importance of biocompatibility in nanomaterials for agriculture, as biocompatible nanoparticles can interact with biological systems without causing toxic effects, promoting plant growth and development.

K. Kornarzyński *et al*. (2020) investigated the effects of combining Fe_3O_4 nanoparticles with a constant magnetic field in the 100-200 mT range on seed germination and the levels of key nutrients, such as iron, phosphorus, and potassium, in plants. The results confirmed that this combination improved seed germination, leading to increased crop quality and yield. The constant magnetic field, together with Fe_3O_4 nanoparticles, facilitated increased concentrations of iron and phosphorus, which are critical for plant growth and development. Iron and phosphorus play key roles in plant metabolic processes, such as photosynthesis and respiration, as well as in the formation of new cells. These findings underscore the importance of integrating magnetic fields and nanoparticles to achieve better agronomic outcomes and improve agricultural practices. J.A. Teixeira da Silva & J. Dobránszki (2016) investigated the effects of 150 mT magnetic fields on plant physiological processes and overall growth and development. The results indicated that magnetic fields can stimulate plant growth by enhancing various physiological processes such as photosynthesis and respiration. Specifically, magnetic fields improve the transport of water and nutrients within plant tissues, leading to increased overall productivity. The application of magnetic fields opens up new avenues for modern agriculture, providing new opportunities to increase crop yield and quality. These findings suggest that magnetic fields can be used as an additional agronomic tool to improve crop cultivation efficiency. H. Zhu *et al*. (2008) studied the uptake, transport, and accumulation of iron oxide nanoparticles in pumpkin (*Cucurbita pepo*) plants. They demonstrated that iron oxide nanoparticles are effectively absorbed through the root system, transported via the xylem, and accumulate in leaves, stems, and fruits of plants. This confirms their potential to improve plant agronomic characteristics, such as increasing overall stress tolerance and enhancing nutrient uptake. Iron oxide nanoparticles, due to their efficient transport and accumulation properties, can enhance plant growth and development. H. Wang *et al*. (2011) studied the uptake and accumulation of magnetite (Fe_3O_4) nanoparticles in perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. The results showed that magnetite nanoparticles can improve plant growth, enhance photosynthetic activity, and strengthen antioxidant defence mechanisms. These effects contribute to an overall increase in plant tolerance to stress factors such as drought or diseases.

R. Rahmatzadeh *et al*. (2020) focused on the impact of Fe_3O_4 magnetic nanoparticles on tomatoes (*Solanum lycopersicum*) under cadmium stress conditions. The results indicate that magnetic nanoparticles can mitigate the negative effects of cadmium contamination, which is crucial for maintaining plant health and productivity in heavy metal-polluted environments. The application of Fe_3O_4 nanoparticles improved tomato growth, enhancing their tolerance to stressors such as cadmium contamination. This may be due to the ability of nanoparticles to neutralise the toxic effects of cadmium or to increase the antioxidant activity of plants, thus reducing oxidative damage and improving overall plant health. These findings support the potential of magnetic nanoparticles as an effective tool for enhancing plant tolerance to heavy metals and improving their overall productivity.

Figure 2 visually illustrates the key stages of magnetic nanoparticle uptake by plants and their impact on physiological processes. Initially, magnetite nanoparticles, due to their size and morphological characteristics, penetrate the plant root system through apoplastic or symplastic transport. This process occurs via root hairs, allowing the nanoparticles to effectively integrate

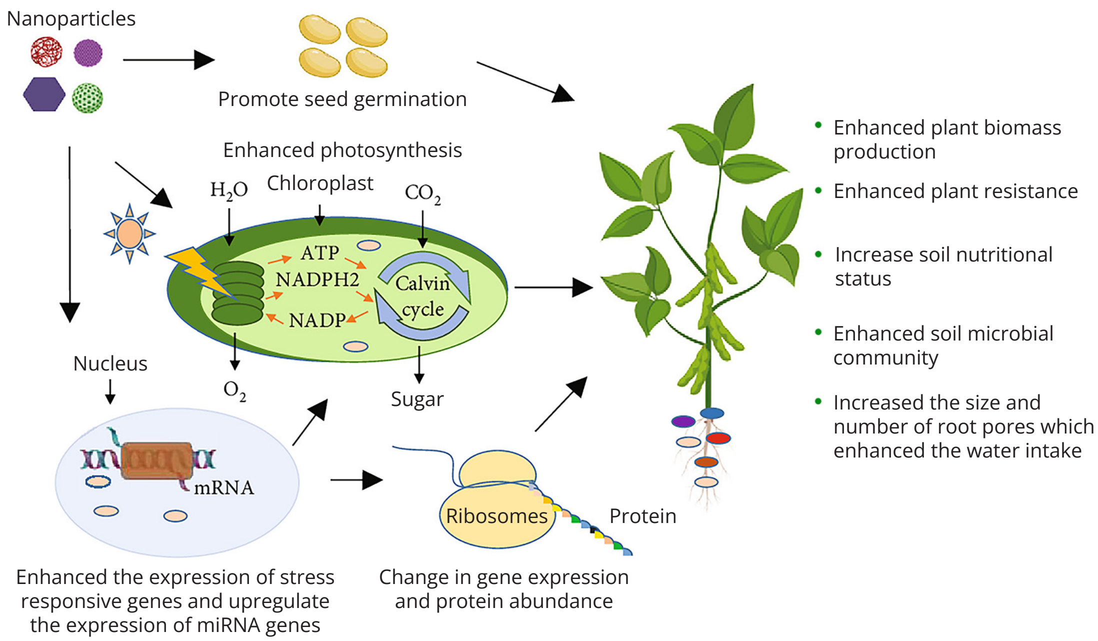

Figure 2. Positive impact of nanoparticles on plant growth and development
Source: S. Shahid *et al.* (2021)

into the plant tissues.

Studies investigating the effects of nanoparticles and magnetic fields on plants demonstrate significant potential for their use in agriculture to enhance plant growth and development, although caution is warranted due to potential negative effects. Numerous studies have revealed common trends confirming the positive impact of nanoparticles and magnetic fields on plants. Specifically, the study by N. Joshi *et al.* (2022) demonstrated that biocompatible Fe_3O_4 and MnO_2 nanoparticles significantly optimised tuber formation in potatoes, increasing tuber number by 25% and size by 30%. This highlights their great potential for improving root crop productivity. K. Kornarzyński *et al.* (2020) confirmed that the combination of Fe_3O_4 nanoparticles and an induced magnetic field not only improved seed germination but also increased the concentration of essential macronutrients such as iron and phosphorus, contributing to optimised plant growth and nutritional quality. This demonstrated the versatility of the positive impact of nanoparticles on plant physiological processes. Studies by H. Zhu *et al.* (2008) and H. Wang *et al.* (2011) indicated the effective uptake of magnetite and iron oxide nanoparticles by plants through the root system, their transport via the xylem, and their accumulation in various plant parts. H. Zhu *et al.* (2008). showed that iron oxide nanoparticles were absorbed by pumpkins and transported to leaves, stems, and fruits, confirming their ability to improve agronomic characteristics.

H. Wang *et al.* (2011) added that magnetite nanoparticles also enhanced plant growth, photosynthetic activity, and antioxidant defence, indicating their efficiency in transport and accumulation in plants. These combined results emphasised the importance of improved nanoparticle uptake and transport for achieving optimal agronomic results. J.A. Teixeira da Silva & J. Dobránszki (2016) demonstrated the positive impact of magnetic fields on plant growth and productivity. Scientists indicated that magnetic fields stimulated plant growth and development, contributing to improved physiological processes and overall productivity. Overall trends emerging from the studies confirmed that the use of nanoparticles and magnetic fields could positively influence plant growth, development, and productivity. The uptake and transport of nanoparticles, coupled with the stimulatory effect of magnetic fields, provided improvements in plant physiological and agronomic characteristics.

However, as shown in Table 1, various researchers concluded that to achieve optimal results, it is necessary to consider the concentration of nanoparticles and the strength of magnetic fields to avoid potential negative effects.

Specifically, M.H. Ghafariyan *et al*. (2013) noted that excessive concentrations of magnetite nanoparticles reduced chlorophyll content, negatively affecting plant photosynthetic activity. Other studies, such as that of M.F. Iannone *et al*. (2016), confirmed that optimal concentrations of magnetite nanoparticles improved antioxidant activity and contributed to increased tolerance to heavy metal-induced stress. As shown in Table 1, various authors have also investigated the effects of other metal nanoparticles. For example, M. Delfani *et al*. (2014) studied the effects of zinc and magnesium nanoparticles on plant root systems, and the results indicated improved root growth and biomass at optimal concentrations. Thus, these common trends highlighted the importance of further research and optimisation of technologies to maximise the positive impact of nanoparticles and magnetic fields on plant agronomic characteristics. Table 1 presents key studies, authors, and their findings regarding the impact of nanoparticles on the physiological processes of plants, allowing for a comparison of various approaches and concentrations to achieve the maximum positive effect.

Table 1. Research on the impact of nanoparticles on plant physiological processes

Research	The material under consideration	Plant species	Main findings	Conclusion
J. Li *et al.* (2016)	Copper nanoparticles	-	Excessive concentrations may cause toxic effects or impact cell membranes	It is important to determine optimal conditions for the use of nanofertilisers
N. Joshi *et al.* (2022)	Fe_3O_4 and MnO_2 nanoparticles	Potato (*Solanum tuberosum L.)*	Increase in the number and size of tubers, improvement of physiological processes	Biocompatible nanoparticles enhance tuber formation and yield
V.S. Telizhenko (2020)	Magnetite nanoparticles	Tomatoes (*Solanum lycopersicum*)	Increase in plant height and stem diameter	Magnetite nanoparticles improve the growth and morphological parameters of tomatoes
H. Wang *et al.* (2011)	Magnetite nanoparticles	Perennial ryegrass (*Lolium perenne*) and pumpkins Zucchini (*Cucurbita mixta*)	Improvement in growth, photosynthetic activity, and antioxidant defence	Magnetite nanoparticles enhance plant resistance to stress factors
H. Zhu *et al.* (2008)	Iron oxide nanoparticles	Pumpkins (*Cucurbita pepo*)	Absorption through the root system, transport to leaves and fruits	Iron oxide nanoparticles improve the agronomic characteristics of plants
R. Rahmatzadeh *et al.* (2020)	Magnetic Fe_3O_4 nanoparticles	Tomatoes (*Solanum lycopersicum*)	Reduction of the negative impact of cadmium stress, improvement in growth and stress resistance	Magnetic nanoparticles enhance plant resistance to heavy metal pollution

Source: compiled by the authors

Research into the effects of nanoparticles on plants has revealed a significant potential for their application in agriculture but has also highlighted the need to consider potential negative consequences. The use of nanotechnology, particularly magnetic and metallic nanoparticles, has had a marked impact on plant growth and development, enhancing stress tolerance and optimising physiological processes. However, research has shown that the concentration of these nanoparticles can have detrimental effects on plants if not properly controlled, underscoring the importance of precise dosing and monitoring during application.

Researchers have found that excessive concentrations of nanoparticles can negatively impact plants, causing disruptions to physiological processes and growth retardation. For instance, a study by M.H. Ghafariyan *et al*. (2013) demonstrated that high concentrations of magnetite (Fe_3O_4) significantly reduced the photosynthetic activity of soya beans (*Glycine max*). The primary cause of this was a decrease in chlorophyll levels in plant leaves, which directly affected photosynthetic efficiency. The experiment revealed that high concentrations of magnetite delayed seed germination and significantly reduced plant growth during the early stages of development. This negatively impacted final physiological parameters, such as plant biomass and nutrient uptake capacity. Specifically, plants treated with excessive nanoparticle concentrations exhibited impaired nitrogen and phosphorus uptake, indicating reduced efficiency at excessive application rates.

The research highlights the importance of precisely determining the optimal concentration of nanoparticles to avoid negative consequences and maximise positive effects; R. Rahmatzadeh *et al*. (2020) reported on the impact of magnetite (Fe_3O_4) magnetic nanoparticles and investigated the effect of magnetite on tomatoes (*Solanum lycopersicum*) subjected to cadmium stress. Results showed that at moderate concentrations, magnetite-treated plants were more resistant to cadmium toxicity, maintained higher chlorophyll concentrations, and had lower biomass losses. However, it was also emphasised that prolonged use of nanoparticles can lead to potential toxic effects, such as metal accumulation in plant tissues and metabolic changes. This indicates that for the effective use of nanoparticles in agriculture, their concentration must be carefully controlled following the principle of moderation. These observations highlight the need for further research into the mechanisms of nanoparticle action on plants to develop safer and more efficient technologies.

Research on metal nanoparticles has also shown that their effects depend not only on concentration but also on particle size and morphology. For instance, smaller magnetite nanoparticles (8-10 nm in size) penetrate plant tissues more effectively and interact more actively with cellular structures. However, to ensure the most effective and safe use of nanoparticles, several factors must be considered, including concentration, particle size, and duration of application. These factors can influence final agronomic performance and, if ignored, can lead to adverse outcomes. Notably, the findings of H. Zhu *et al*. (2008) confirmed that at appropriately selected concentrations, magnetic nanoparticles can mitigate the negative effects of toxic metals, such as cadmium, on plants. Excessive doses, on the other hand, led to accumulation in tissues, reduced product quality, and impacted environmental safety. These aspects should be given special attention when planning agricultural practices involving nanoparticles. It is also important to consider the impact of external environmental conditions, such as temperature, humidity, and soil type, on the efficacy of nanofertilisers. Further research can provide a better understanding of the mechanisms of interaction between nanoparticles and plant tissues and their long-term effects on crop productivity.

DISCUSSION

Research into the effects of metal oxide-based nanofertilisers on the physicochemical properties of agricultural plants is opening up new avenues for enhancing agronomic practices and ensuring sustainable agriculture. In the last 5 years, nanofertilisers have attracted significant attention due to their ability to improve plant growth and development, increase yield, and ensure more efficient use of soil resources. Studies conducted by F. Nudelman & N.A. Sommerdijk (2012) have demonstrated a significant impact of metal oxide nanoparticles, particularly iron oxide and copper oxide, on the growth, development, and physiological and biochemical characteristics of agricultural plants. One of the key advantages of using nanofertilisers is their ability to penetrate plant tissues due to their small size, which significantly enhances their efficacy compared to traditional fertilisers. In particular, nanofertilisers not only stimulate plant growth, increasing root length and overall biomass but also activate plant defence mechanisms, notably increasing antioxidant protection. This can significantly enhance crop tolerance to various stress conditions such as drought, high temperatures, soil salinity, and other environmental factors. Experiments investigating iron oxide nanoparticles have shown a significant impact on root development, which may be attributed to improved access to water and nutrients in the soil. This effect can be critically important in areas with limited water supply or under drought conditions, as demonstrated in studies. These studies indicate that the treatment of plants with iron oxide nanoparticles leads to a significant increase in root length, plant height, and biomass.

This effect can be attributed to improved water and nutrient exchange in plant tissues, facilitated by the ability of nanoparticles to penetrate plant tissues and stimulate root development. Increased root mass and length are critical factors influencing a plant's ability to absorb water and nutrients, especially under conditions of limited water supply or in soils with low nutrient content. Research findings by I. Afzal *et al*. (2021), who investigated the impact of a magnetic field and iron nanoparticles on the physiological and biochemical processes in sunflower plants, support these observations. Their results demonstrated that the combined effect of a magnetic field and iron nanoparticles significantly enhances plant growth and development, increasing yield. This is achieved through the activation of metabolic processes, improved protein synthesis, increased levels of antioxidant enzymes such as superoxide dismutase and catalase, and enhanced water retention in plant cells. Similar results were obtained in a study by M.F. Iannone *et al*. (2016), who investigated the effects of magnetite nanoparticles on wheat development. Their experiments showed that treating wheat with magnetite nanoparticles increased biomass and improved overall plant health. However, the authors also highlighted the risk of oxidative stress caused by increased levels of lipid peroxidation in cells. Oxidative stress can lead to damage to cell membranes, proteins, and DNA, negatively impacting plant growth and development. Therefore, despite the positive effects of nanoparticles on wheat growth, the possibility of negative side effects, such as increased levels of oxidative stress, must be considered. This may require additional measures to mitigate this stress, such as the combined use of antioxidant compounds.

Copper oxide has demonstrated a high efficacy in increasing plant biomass, which is likely due to enhanced photosynthetic processes within plant tissues. Copper oxide nanoparticles can influence several key aspects of photosynthesis, including increasing chlorophyll content, which is critical for efficient light absorption and, consequently, organic matter synthesis. Increased chlorophyll content can improve overall photosynthetic efficiency, which in turn promotes biomass growth. M. Delfani *et al*. (2014) provide further evidence for this, showing that iron and magnesium-based nanofertilisers can significantly impact plant physiological responses, particularly chlorophyll levels. Their experiments demonstrated that plants treated with iron and magnesium nanoparticles had significantly higher chlorophyll levels in their leaves, directly affecting their photosynthetic potential. This, in turn, led to increased biomass, indicating the ability of nanoparticles to optimise photosynthetic processes and improve overall plant productivity (Konate *et al*., 2017). The results also align with the study, investigated the impact of copper nanoparticles on plant physiological characteristics. In research employed non-toxic methods for synthesising copper nanoparticles, using ascorbic acid and polyvinylpyrrolidone, which enabled the creation of stable and highly dispersed nanoparticles. They demonstrated that these nanoparticles can significantly increase chlorophyll levels in plants, aligning with the findings regarding the positive impact of copper oxide on photosynthetic processes. An important aspect of the study is the approach to nanoparticle synthesis that ensures stability and effectiveness in agricultural applications, while simultaneously minimising potential toxic effects (Li *et al*., 2016).

An analysis of research into metal oxide-based nanofertilisers, particularly iron and copper oxides, reveals significant potential for enhancing the physicochemical properties of agricultural plants (Binhi, 2020). These findings open new avenues for further research and the practical application of nanoparticles in agriculture but also highlight the need for a cautious approach to their use (Anand *et al*., 2020). One of the primary advantages of nanofertilisers is their ability to significantly improve plant growth and development: studies by N. Joshi *et al*. (2022) and K. Kornarzyński *et al*. (2020) have demonstrated that combining magnetite nanoparticles with a magnetic field positively impacts crop yield and physiological parameters. These results indicate the potential of nanoparticles in developing new agronomic technologies that can significantly enhance agricultural productivity (Sear, 2021). Improving physiological parameters such as root growth and overall biomass is crucial for enhancing crop productivity and ensuring tolerance to environmental stresses. Research by H. Zhu *et al*. (2008) and H. Wang *et al*. (2011) has shown that magnetite and iron oxide nanoparticles are not only effectively absorbed by plants but also transported to various plant parts. This confirms their potential for improving agronomic properties, such as increased water and nutrient uptake.

Consequently, nanoparticles can more efficiently utilise soil resources and improve plant growth conditions, as demonstrated by J.A. Teixeira da Silva & J. Dobránszki (2016), where the positive impact of magnetic fields on plant growth and productivity opens up new horizons in agricultural technologies. The integration of magnetic fields with nanoparticles represents an innovative approach to the development of agricultural technologies (Osman *et al.*, 2023). This combination has the potential to become a significant tool for enhancing plant resilience to various stress conditions. Research findings demonstrate the significant potential of using metal oxide nanoparticles, such as magnetite and iron oxide, to enhance agricultural crop productivity. The combination of these nanoparticles and magnetic fields has proven effective in stimulating plant growth, development, and improving physiological properties, leading to increased yields and enhanced stress tolerance. This approach opens up new possibilities for developing agricultural technologies aimed at more efficient resource utilisation and increased agricultural productivity.

CONCLUSIONS

Research into metal oxide-based nanofertilisers, such as iron and copper oxides, has revealed significant potential for improving the physicochemical properties of agricultural plants. The findings underscore the importance of optimising the use of nanoparticles in agriculture to maximise benefits while minimising the risks of adverse side effects. Firstly, the authors' research confirmed that nanoparticles can positively impact plant growth and development. This is possible due to their small size, allowing nanofertilisers to penetrate plant tissues and significantly enhance their efficacy. A crucial aspect was that iron oxide nanoparticles stimulated root development in plants. This is particularly important under conditions of limited water supply or in soils with low nutrient content.

Research has shown that the application of a magnetic field combined with iron oxide nanoparticles significantly enhanced plant growth and development. Results demonstrated a 15-30% increase in yield, indicating the effectiveness of nanoparticles in providing plants with nutrients, even under stress conditions. Iron oxide nanoparticles penetrate the root system, improving water and nutrient uptake, which is particularly beneficial for crops grown on depleted or unstable soils. This made plants more resilient to stress and increased their productivity, highlighting the potential of iron oxide-based nanofertilisers to improve agronomic practices and sustainable development in challenging growing conditions.

Studies have shown that copper oxide can increase photosynthetic activity in plants by 20-40%, promoting higher chlorophyll concentrations and improved light absorption. This leads to a 15-25% increase in plant biomass. However, high concentrations of copper oxide can induce oxidative stress, inhibiting plant growth and development. Therefore, optimising the concentration of nanoparticles is key to maximising positive effects and minimising negative consequences. Future research prospects include developing optimal nanoparticle concentrations and integrating them with technologies such as drip irrigation systems and bioengineering, which could enhance plant efficiency and resilience to stress conditions. Although nanofertilisers have significant potential, further research is needed to better understand their long-term impacts and ensure their effective use in the agricultural sector.

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CONFLICT OF INTEREST

None.

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Вплив нанодобрив на основі оксидів металів на фізико-хімічні властивості сільськогосподарських рослин

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Анотація. Метою роботи був всебічний аналіз наявних досліджень впливу нанодобрив на основі оксидів металів, таких як оксид цинку (ZnO), оксид заліза (Fe_2O_3) та діоксид титану (TiO_2), на розвиток та вплив рослин. Методологія дослідження базувалась на аналізі наукових статей, що досліджують вплив нанодобрив на основі оксидів металів (ZnO, Fe_2O_3 і TiO_2) на фізико-хімічні властивості таких культур, як пшениця та соя, із використанням таких методів, як аналіз різних досліджень, їх узагальнення та порівняння. В дослідженні розглядалися різні аспекти використання цих нанодобрив, їх вплив на покращення росту, розвитку та здоров'я сільськогосподарських культур, зокрема пшениці та сої, визначення оптимальних концентрацій, а також потенційні ризики, які можуть вплинути на продуктивність та екологічну безпеку. Враховуючи виклики, пов'язані з підвищенням ефективності сільського господарства, зменшенням впливу на навколишнє середовище та забезпеченням стабільної врожайності, дослідження було спрямоване на визначення оптимальних концентрацій нанодобрив, які могли б максимізувати позитивний вплив на рослини без ризику негативних наслідків. Огляд літератури включав аналіз результатів лабораторних експериментів з використанням різних концентрацій нанодобрив на різних стадіях розвитку рослин. Обговорювалися ключові параметри, що вивчалися в цих експериментах, такі як вміст хлорофілу в листках, активність антиоксидантних ферментів, водний баланс, ріст і розвиток коренів. Огляд літератури свідчив, що використання низьких і середніх концентрацій нанодобрив позитивно впливає на фотосинтетичну активність, розвиток кореневої системи та загальний стан рослин. Було підкреслено, що високі концентрації нанодобрив можуть пригнічувати ріст рослин і мати негативні наслідки. Дані висновки важливі для точного дозування та ретельного моніторингу при застосуванні нанодобрив для досягнення оптимальних результатів. Дослідження продемонструвало значний потенціал використання нанодобрив на основі оксидів металів у сільському господарстві для підвищення стійкості культур до стресових факторів навколишнього середовища та покращення їх продуктивності

Ключові слова: модифікація мінералів; біодоступність поживних речовин; аграрна продуктивність; антиоксидантна активність; дія на кореневу систему

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Effect of fullerene C_{60} on morphometric parameters of microgreen peas (*Pisum sativum*) under water deficit conditions

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Abstract. The relevance of this study was to investigate the impact of abiotic stress, specifically fullerene C_{60} and drought, on the morphometric characteristics of pea microgreens. Drought negatively affects the growth and development of agricultural plants, leading to reduced yields. Carbon nanoparticles, particularly fullerene C_{60}, due to their unique physical, chemical, and biological properties, may serve as modulators of resistance to stressful conditions such as drought, enhancing the physiological and biochemical processes at both the cellular and whole plant levels. This research aimed to investigate the effect of fullerene C_{60} on the morphometric parameters of microgreen peas (*Pisum sativum*) of the ECO variety under water deficit conditions. Structured water-soluble carbon nanoparticles of fullerene C_{60} were employed. C_{60} molecules were transferred from an organic solution into the aqueous phase, followed by ultrasonic treatment. The morphometric indicators evaluated in the microgreen peas included shoot height, shoot diameter, number of leaves, leaf weight, plant weight, and root length.

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The experiment was conducted on the 14th day after treating pea seeds with an aqueous solution of fullerene C_{60} at concentrations of 0.1, 0.2, 0.5, and 1.0 µg/mL with planting in substrates of both linen mats and a soil mixture. Chemical, physical, and physiological methods were employed. Fullerene C_{60} at the given concentration range (0.1-1.0 µg/mL) did not exhibit negative phytotoxic effects on pea microgreens. Moreover, the drought-induced physiological state of the microgreens was restored by pre treatment of the seeds with the fullerene C_{60}. The results demonstrated the absence of phytotoxic effects and confirmed the protective effects of fullerene C_{60} against drought stress in pea microgreens, suggesting the potential of using structured carbon nanoparticles in agrobiotechnologies to regulate stress resistance mechanisms in crops

Keywords: agricultural plants; carbon; nanoparticles; drought; young sprouts

INTRODUCTION

Pea microgreens are a promising crop for cultivation in vertical farms, urban greenhouses, and home gardens, due to their high nutritional value and rapid growth cycle. The study of morphometric indicators enables researchers to assess the overall health of plants and their ability to adapt to various growing conditions. This is particularly important in urban agriculture, where efficient use of space and resources is a key factor. Plants, as living organisms, respond to changes in climatic conditions, and drought is one of the most significant factors affecting their development and functioning. Authors K.-J. Dietz *et al*. (2021) demonstrated that water deficit in the soil can lead to water stress in plants, resulting in stunted growth, reduced yields, leaf loss, and even plant death. Researchers K. Abbass *et al*. (2022) found that drought can also pose a threat to biodiversity, as vulnerable plant species may not be able to adapt to rapid climate change. Additionally, water deficit can deteriorate soil quality and cause erosion, which in turn leads to the reduction of soil fertility and ecosystem stability. Young plants and seedlings are particularly vulnerable to drought due to their limited resources for adapting to water shortages.

Authors C. Ingrao *et al*. (2023) noted that there are several causes of water deficit, such as low rainfall, extreme temperatures, high light intensity, and salinity. On the other hand, the water in the soil may be sufficient, but the plants cannot absorb it. Water stress of this type is referred to as physiological drought (or pseudo-drought). Drought stress leads to changes at the physiological, morphological, ecological, biochemical, and molecular levels in plants. Researchers G.G. Haile *et al*. (2019) demonstrated that drought negatively impacts the quantity and quality of plant growth and yield. The response of plants to water deficit depends on the duration and intensity of the deficit, as well as on the species, age, and stage of plant development. Scientists A. Gupta *et al*. (2020) proved that most plants have developed drought stress resistance mechanisms, which are diverse and species-dependent. Author T.R. Ault (2020) demonstrated that drought also has significant global consequences, affecting various aspects of ecosystems and human life. One of the primary issues is the decline in agricultural productivity, which can lead to food shortages, a food crisis, higher food prices, and famine, especially in crop-growing regions. Simultaneously, drought causes soil degradation, impairing its long-term fertility. Climate change, including rising temperatures and decreases in the amount of precipitation, can contribute to the spread of deserts and the expansion of arid zones.

Authors X. Yang *et al*. (2021) observed that drought stress results in a loss of biodiversity, as many plants and animals cannot adapt to such conditions and are forced to migrate or become extinct. The study of the impact of drought on the morphometric parameters of plants has become an urgent issue, as water deficits cause significant changes in plant biomass, size, and organ shape. In this context, it is important to consider various aspects of the impact of drought on the morphometric parameters of plants to understand the adaptation strategies of plant organisms to extreme climatic conditions and to develop effective measures to preserve plant cover in the face of climate change. A pressing task in agrotechnology is to study the methods and mechanisms for modulating stress resistance in agricultural plants under water deficit conditions. Researchers S.V. Prylutska *et al*. (2022) proved that structured water-soluble carbon nanoparticles, specifically fullerene C_{60}, are promising in this regard due to their unique biological and physicochemical properties, including hydrophobicity.

Scientist N. Gopalakrishnan (2018) noted that carbon nanomaterials are widely used across various industries, particularly as lubricants, in fuel cells, electronics, cosmetics, theranostics, food additives, and antioxidants, among others. The C_{60} molecule, due to its antioxidant activity, can absorb oxygen and nitrogen free radicals and influence plant growth and development, particularly by reducing abiotic-induced oxidative stress, such as water deficit. In addition, authors A. He *et al*. (2021) showed that the hydrophobic C_{60} molecule can interact with cell membranes and affect their permeability; whether this influence is positive or negative depends on the concentration and mode of administration of the nanoparticles. Researchers P.R. Riley & R.J. Narayan (2021) and M. Gaur *et al*. (2021) noted that fullerene C_{60} have promising applications in medicine and pharmaceuticals due to their ability to deliver drugs in a targeted manner and control their release. Thus, fullerene C_{60} is a unique molecule with numerous potential applications in materials science, as well as in both biological and agricultural research. Consequently, the aim of the study was to evaluate the impact of fullerene C_{60} on the morphometric parameters of microgreen peas (*Pisum sativum*) of the ECO variety under drought conditions. The objectives of the study were to assess the dose-dependent effect of fullerene C_{60} on the growth of microgreen peas under regular watering or water deficit when germinated on different substrates, as well as to evaluate the potential phytotoxic and protective effects of the nanoparticles.

MATERIALS AND METHODS

Synthesis and characterisation of structured C_{60} molecules. An aqueous colloidal solution of fullerene C_{60} (150 μg/mL initial concentration) was synthesised and characterised at the Institute of Chemistry and Biotechnology of the Technical University of Ilmenau (Germany) following the method of transferring the C_{60} molecule from an organic solution into the aqueous phase, followed by ultrasonic treatment (Schuetze *et al*., 2011). The structure and size of C_{60} nanoparticles, as well as their stability in aqueous solution, were estimated using microscopic and spectroscopic measurements. An aqueous colloidal solution of fullerene C_{60} was prepared in concentrations of 0.1, 0.2, 0.5, and 1 μg/mL. Pea (*Pisum sativum*) of the ECO variety was chosen as *the object of the study*. Category CH1 seeds (certified seeds of the first generation) from the 2022 harvest, supplied by the private enterprise "ABINA", were used. The drought resistance of this variety is rated at 8.3-8.5 points out of 9, indicating high drought resistance (Sowing peas, 2018).

Conditions of the experiment. Pea seeds were washed to remove contaminants under running water. Damaged seeds with visible signs of pest damage or shell imperfections were discarded. The seeds were then soaked in a 20 g/L NaCl solution for 10 minutes. The seeds that settled at the bottom of the NaCl solution were used for cultivation. To promote faster germination, the seeds were soaked in a 0.02% H_3BO_3 solution for 25 minutes and then washed three times under running water. Subsequently, the seeds were sterilised in 3% H_2O_2 for 15 minutes and washed three times under running water (Fig. 1).

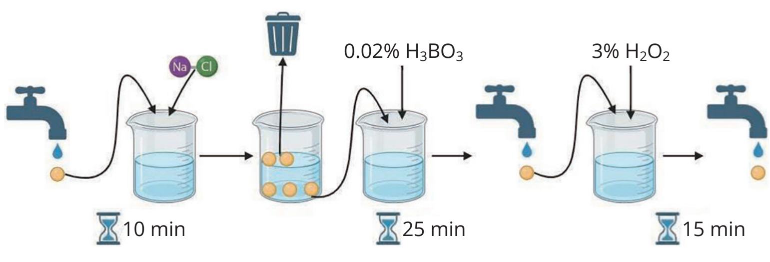

Figure 1. Scheme of pea seed preparation (*Pisum sativum*)
Source: created by the authors

Each sample contained 30 g of seeds, which were pre-soaked for 3 hours in the following solutions: control – distilled H_2O, experimental samples – fullerene C_{60} colloidal aqueous solution at concentrations – 0.1, 0.2 µg/mL, 0.5, and 1 µg/mL. The pea seeds were then transferred to the appropriate containers on the substrate and evenly distributed over the surface (Fig. 2). Containers with seeds pre-treated with carbon nanoparticles (CNPs) were stored for 4 days at room temperature (24°C) in a dark place to allow for germination, then exposed to light/dark conditions of 12/12 hours.

Figure 2. Scheme of treatment and planting of pea seeds (*Pisum sativum*)
Source: created by the authors

To evaluate the growth advantage of microgreen peas, seeds were planted on different substrates: a linen mat (Experiment No. 1) and a universal soil mixture from the manufacturer "Florida" (Experiment No. 2) consisting of turf, raised peat, lowland peat, river sand, mineral fertilisers (Potassium, Sodium, Phosphorus), and microelements (Iron, Zinc, Boron, Copper, Molybdenum, Manganese), with a pH value of 5.5-7.5 (Universal soil mixture, n.d.).

Simulation of drought under laboratory conditions. For the germination of microgreen peas with limited moisture on linen mats (Experiment No. 1), spraying with water was carried out after 3 days. When growing pea microgreens on the soil mixture (Experiment No. 2), drought was simulated using Tumanov's wilting method (Kirichenko *et al.*, 2016), with spraying stopped on the 2nd day after seed germination. Watering of the samples was resumed 1 day before the experiment. Spraying of experimental samples without drought simulation was carried out regularly – every other day. *The assessment of the morphometric indicators of peas* was conducted on the 14th day after seed planting (Fig. 3).

Experiment #1 (linen mats) Experiment #2 (soil mixture)

Figure 3. Photos of microgreen pea plants (*Pisum sativum*) on the 14th day of the experiment in the control groups (under regular watering)
Source: created by the authors

The following parameters were evaluated: plant height, diameter of the main shoot, number of leaves, root length, total plant weight, and leaf weight. Additionally, the ratio of leaf weight to plant weight (as an indicator of photosynthetic effort), the ratio of shoot length to plant weight (as an indicator of relative shoot growth), and the ratio of shoot length to its diameter were determined (Penkovska, 2019). The sample size for the experiments was 10-15 seedlings. The research was conducted in the Educational Scientific Laboratory of Biochemistry and Phytobiotechnology, Department of Physiology, Plant Biochemistry and Bioenergetics, Faculty of Plant Protection, Biotechnology and Ecology at the National University of Life and Environmental Sciences of Ukraine. Experimental studies with plants were conducted following ethical standards (United Nations Convention No. 995_030 "On the Conservation of Biological Diversity", 1992).

Statistical analysis of the obtained research results was conducted using methods of variational statistics, particularly with the t-test and one-/two-way analysis of variance (ANOVA). Differences in indicators with values of $p \leqslant 0.05$ were considered statistically significant (Prylutskyi *et al.*, 2017). Experiments were conducted in at least three replicates for each variant. Graphing and data analysis were performed using Microsoft Excel 2010 and GraphPad Prism 7 software. The results are presented as M (Arithmetic Mean) ± SD (Standard Deviation).

RESULTS AND DISCUSSION

Microgreens are a resilient crop, requiring minimal attention to weather conditions, environmental composition, and growing circumstances, making them easy and quick to cultivate under home or laboratory conditions (Michell *et al.*, 2020). The physiological state of plants was studied during the germination stage, which does not require large reserves of nutrients, water, or light. Accordingly, the objective of this study was to evaluate the effect of biotic factors such as carbon nanoparticles (CNPs) and drought on the early stages of plant growth, particularly the vegetative organs of peas (root, stem, leaves) and the mass of plants and leaves. The research showed that by the 14th day after pea seed germination, differences in morphometric indicators were observed between the control group (under regular watering) and different substrates (Table 1, Table 2).

Table 1. Morphometric indicators of microgreen peas on the 14th day after germination on a linen mat (Experiment No. 1) under regular watering and water deficit (M ± m, n = 15)

Morphometric indicators	Regular watering (control)	Water deficit
The height of the main shoot, cm	10.2 ± 1.7	6.5 ± 0.58
The diameter of the main shoot, mm	1.8 ± 0.255	1.7 ± 0.03
The number of leaves, pcs	4.0 ± 0.35	2.0 ± 0.17
The total plant mass, g	0.812 ± 0.065	0.715 ± 0.680
The mass of all leaves, mg	44.0 ± 0.039	20.0 ± 0.18
The root length, cm	15.4 ± 3.4	12.6 ± 1.7

Source: created by the authors

The morphometric indicators for plants grown on the soil mixture (Experiment No. 2) were significantly higher compared to those grown on the linen mat (Experiment No. 1). Specifically, the height of the main shoot was 1.5 times greater, the diameter of the main shoot and the number of leaves were 1.7 times greater, the total plant mass was 1.9 times higher, and the leaf mass was 4.3 times greater in Experiment No. 2 compared to Experiment No. 1 (Table 1, Table 2). Therefore, microgreen pea germination is more effective in the soil mixture, which allows for the production of higher-quality plant material under laboratory conditions.

Table 2. Morphometric indicators of microgreen peas on the 14th day after germination in the soil mixture (Experiment No. 2) under regular watering and water deficit (M ± m, n = 15)

Morphometric indicators	Regular watering (control)	Water deficit
The height of the main shoot, cm	15.5 ± 1.46	5.7 ± 0.54

Table 2. Continued

Morphometric indicators	Regular watering (control)	Water deficit
The diameter of the main shoot, mm	3.0 ± 0.29	2.2 ± 0.17
The number of leaves, pcs	7.0 ± 0.66	5.0 ± 0.376
The total plant mass, g	1.543 ± 0.11	0.795 ± 0.055
The mass of all leaves, mg	191.0 ± 18.6	46.0 ± 3.82
The root length, cm	13.2 ± 1.29	8.3 ± 0.75

Source: created by the authors

Under water-deficit conditions, on the 14^{th} day after pea seed germination, a significant decrease in the studied morphometric indicators of pea plants was observed both on linen mats (Experiment No. 1) and in the soil mixture (Experiment No. 2). In particular, with insufficient substrate hydration, a decrease was noted in the height of the main shoot by 1.6 times (Experiment No. 1) and by 2.7 times (Experiment No. 2), the diameter of the main shoot by 1.4 times (Experiment No. 2), the number of leaves by 2 times (Experiment No. 1) and by 1.4 times (Experiment No. 2), the total plant mass by 1.9 times (Experiment No. 2), the mass of all leaves by 2.2 times (Experiment No. 1) and by 4 times (Experiment No. 2), and the root length by 1.2 times (Experiment No. 1) and by 1.6 times (Experiment No. 2) compared to plants grown under regular watering conditions. Thus, under simulated water-deficit conditions, a significant decline in the morphometric indicators of microgreen peas was observed.

It is known that drought slows down plant development by reducing the evaporative surface, which inhibits the growth of leaves and shoots (Priadkina *et al.*, 2022). This, in turn, leads to a decrease in plant mass, particularly the leaves. During droughts, the plant allocates its resources towards the development of the root system to search for water at greater depths. It was shown, that under drought conditions, the ratio of root length to shoot height in peas was 1.9 times and 1.5 times greater in Experiment No. 1 and Experiment No. 2, respectively, compared to the control (regular watering) (Table 1, Table 2). Drought poses a significant threat to agriculture, leading to a reduction in crop yields, loss of soil fertility, and ecosystem imbalances (Hoffmann *et al.*, 2018). Water deficit in the soil results in stunted plant growth, reduced crops, leaf loss, and the eventual death of plants (Mukarram *et al.*, 2021). A current priority is the study and modulation of stress resistance mechanisms in plants induced by water deficit. To increase the resistance of agricultural crops to drought, fullerene C_{60} carbon nanoparticles were used.

The effect of fullerene C_{60} on the morphometric parameters of peas germinated on different substrates under regular watering conditions was investigated. It was shown that fullerene C_{60} had a substrate- and dose-dependent effect on pea microgreens (Fig. 4). Thus, under germination conditions on linen mats (Experiment No. 1), a decrease in leaf mass was observed after treatment with 0.2, 0.5, and 1 μg/mL fullerene C_{60} by 29%, 32%, and 31%, respectively, compared to the control without the addition of CNPs (Fig. 4). Under germination conditions on the soil mixture (Experiment No. 2), after treatment with 0.5 and 1 μg/mL fullerene C_{60}, a decrease in total plant weight by 24% and 32% and in leaf weight by 23% and 24%, respectively, was observed compared to the control without the addition of CNPs (Fig. 4). In addition, fullerene C_{60} at 0.1 and 0.2 μg/mL concentrations did not affect the studied morphometric indicators of microgreen peas.

Therefore, fullerene C_{60} in the studied concentration range (0.1-1 μg/mL) did not cause a negative phytotoxic effect on pea microgreens. Next, the effect of fullerene C_{60} on the morphometric parameters of peas under water-deficit conditions was investigated (Fig. 5). For this, a model of drought tolerance of microgreen peas on a soil mixture was used, as plant growth indicators were more markedly reduced on this substrate. The physiological state of microgreen peas of the ECO variety, induced by drought, was restored after the seeds were pre-treated with fullerene C_{60} (Fig. 5). The protective effects of CNPs are evidenced by the increase in the studied morphometric indicators. Thus, the height of the main shoot and the weight of the leaves after treatment with 0.1, 0.2, and 0.5 μg/mL fullerene C_{60} were twice as high compared to the respective indicators under water deficit without the addition of CNPs. It was also noted that the root length in plants treated with 0.1 μg/mL, 0.2 μg/mL, and 0.5 μg/mL fullerene C_{60} was 51%, 22%, and 20% longer, respectively, compared to untreated plants. The diameter of the main shoot increased slightly (Fig. 5). Fullerene C_{60} helps maintain water balance and assists plants in better retaining water, improving the condition of cell membranes and increasing the efficiency of water use. Together, these mechanisms provide plants with better drought tolerance by preserving or even improving morphometric indicators (Kovač *et al.*, 2021).

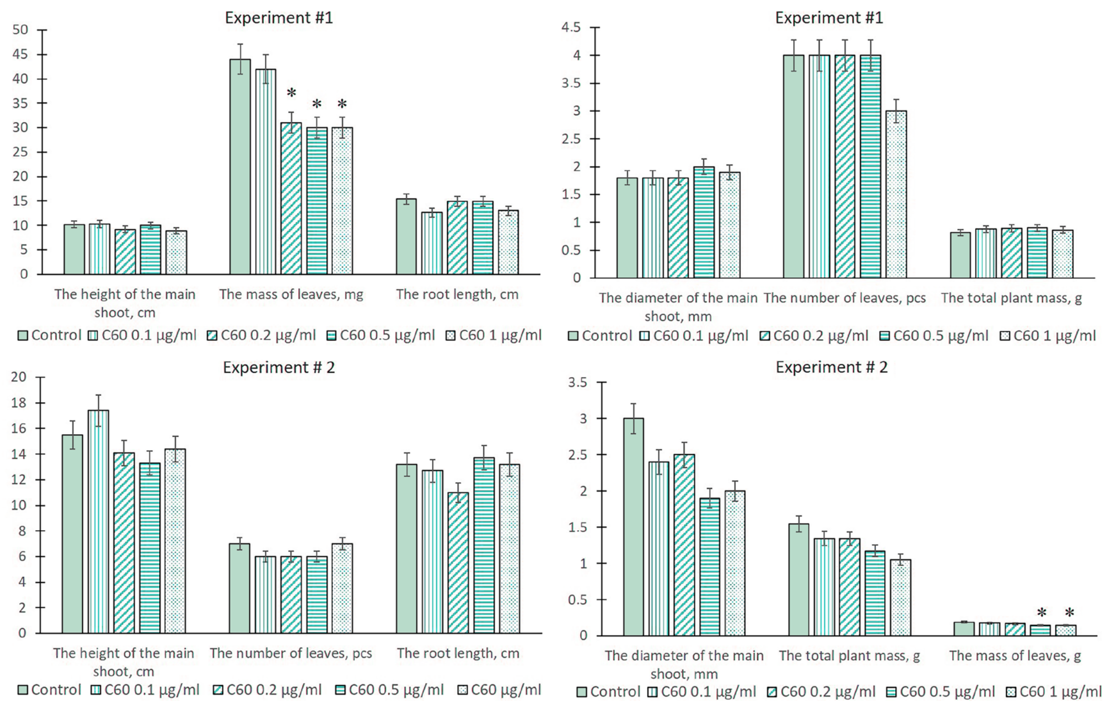

Figure 4. Morphometric indicators of microgreen peas on the 14th day after treatment with fullerene C_{60} and germination on a linen mat (Experiment No. 1) and soil mixture (Experiment No. 2) under regular watering

Note: *P < 0.05 in comparison with control

Source: created by the authors

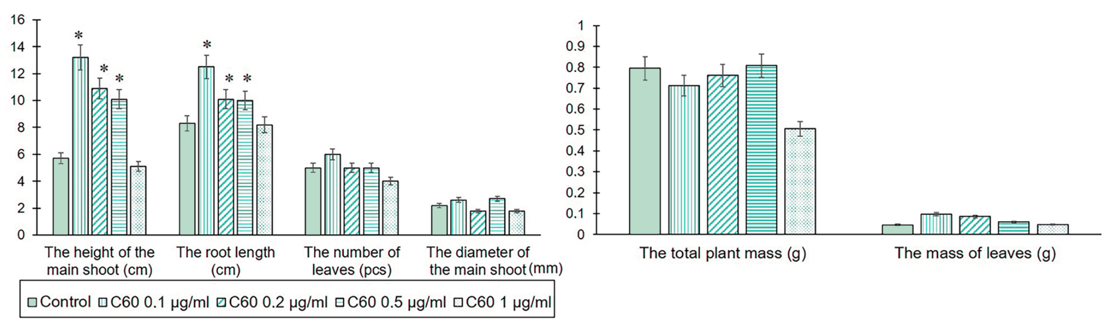

Figure 5. Morphometric indicators of microgreen peas on the 14th day after treatment with fullerene C_{60} and germination on the soil mixture under water deficit

Note: *P < 0.05 in comparison with control

Source: created by the authors

An important task of agricultural technologies is the development of comprehensive approaches that allow for the study of the impact of drought on the physiological state of agricultural plants and their mechanisms of adaptation to extreme climatic conditions, as well as the development of effective biotechnological strategies for plant preservation in the context of climate change. Water deficit causes significant changes in the biomass, size, and shape of plant organs; thus, the study of the impact of drought on the morphometric parameters of plants is particularly relevant (Seleiman *et al*., 2021). Long roots are one of the key morphological features that facilitate adaptation to water deficit. This morphological feature plays an important role in providing drought resistance (Wasaya *et al*., 2018). Long roots ensure stable access to water for plants, even in challenging climatic conditions, thereby contributing to their survival and productivity (Lynch, 2018). In particular, authors M. Rezayian *et al*. (2018), and O. Basal *et al*. (2020) showed that plants under the influence of drought develop a long root system and exhibit better stress tolerance and drought resistance, while plants with short roots are more vulnerable to drought. The authors S. Jafarnia *et al*. (2018) demonstrated that the height of seedlings of the Persian oak (*Quercus brantii*) significantly decreased under water deficit.

The study of the effect of nanosized and structured carbon nanoparticles (CNPs) on plants is a significant and promising area of modern agrobiotechnologies. CNPs, due to their nanoscale size and unique physicochemical properties, are capable of altering the morphological, physiological, and biochemical parameters of plants. The results of the research by S.K. Khorrami *et al*. (2020) indicated that MWCNTs at low and medium concentrations (50 and 100 mg/L) had a positive effect on the morphological and anatomical characteristics of both varieties of *Hibiscus esculentus* L. (Bamia and Emerald). However, at high concentrations of MWCNTs (200 mg/L), a negative effect was observed on both varieties of bamia. The authors suggest that the positive effects of MWCNTs can be attributed to their ability to promote water absorption, cell division, and elongation in both okra varieties, while the negative effects may be linked to the destructive impact of MWCNTs on different parts of the cells, particularly on membranes and biological organs. When comparing the effects of natural and synthetic MWCNTs, the authors G. Juárez-Cisneros *et al*. (2020) demonstrated that CNPs formed after forest fires lead to better plant growth and development than chemically synthesised MWCNTs.

Moreover, literature data indicates the potential of carbon nanoparticles to reduce the negative effects of drought and increase the resistance of plants to adverse climatic conditions. The authors J.-L. Xiong *et al*. (2018) studied the germination of *Brassica napus* L. seeds under water deficit conditions after treatment with a water-soluble fullerol derivative at different concentrations (0.01; 0.1; 1; 10; 100; 500; 1,000 mg/L). To simulate drought, rapeseed was germinated in a 15% PEG 6000 medium. It was shown that fullerol in the concentration range of 0.1-100 mg/L had a positive effect on seed germination, while no such effect was observed at high concentrations (500 and 1,000 mg/L). Additionally, fullerol in *B. napus* reduced water stress during seed germination, growth, and photosynthesis.The authors S.Z. Ahmadi *et al*. (2024) demonstrated that, following the application of carbon nanomaterials such as graphene nanotubes and nanoplates at concentrations of 100, 200, and 1,000 mg/L, the number of flowers of *Capsicum annuum* L. increased at both 50% and 100% soil moisture content compared to the control group, which did not receive nanomaterials. Moreover, after the treatment of leaves with carbon nanomaterials, the negative impact of water deficit stress on the mass of roots in both fresh and dry conditions was reduced. After using 200 mg/L GNMs and 1,,000 mg/L MWCNTs at 100% soil moisture content, the number of fruits increased compared to the control group under the same watering conditions. MWCNTs at a concentration of 1,000 mg/L significantly increased the photosynthetic rate and stomatal conductance in both stressed and non-stressed conditions compared to the control. The application of carbon nanomaterials, namely 1,000 mg/L fullerene C_{60}, 200 mg/L graphene nanoplates, and 100 mg/L MWCNTs, increased the amounts of chlorophyll *b*, total phenols, and flavonoids.

MWCNTs have been shown to induce seed germination in oats (Joshi *et al*., 2018b), and wheat (Joshi *et al*., 2018a). It is assumed that MWCNTs, by penetrating through the seed coat and cell walls, form new pores, which increases water absorption and promotes seed germination. Thus, the results obtained and the literature confirm the potential of carbon nanoparticles for regulating the growth, development, and yield of agricultural plants. The effect of carbon nanomaterials on plants depends on the type and concentration of nanoparticles. However, the physiological and biochemical mechanisms underlying the action of these carbon nanostructures are insufficiently understood, which necessitates further research.

CONCLUSIONS

The influence of abiotic factors, particularly fullerene C_{60} and drought, on the physiological state of microgreen peas (*Pisum sativum*) germinated on two substrates, namely linen mats and a soil mixture, was investigated under laboratory conditions. It was shown that the germination of microgreen peas is more effective on the soil mixture than on linen mats. Morphometric indicators of the vegetative organs of peas (roots, stems, and leaves), as well as the mass of the plants and leaves at the initial stages of growth, were evaluated 14 days after seed germination. Under conditions of simulated water deficit, a significant decrease in the morphometric parameters of microgreen peas was noted, namely, the height of the main shoot decreased by 1.6 times (Experiment No. 1) and by 2.7 times (Experiment No. 2); the diameter of the main shoot decreased by 1.4 times (Experiment No. 2); the number of leaves decreased by 2 times (Experiment No. 1) and by 1.4 times (Experiment No. 2); the total plant mass decreased by 1.9 times (Experiment No. 2); the mass of all leaves decreased by 2.2 times (Experiment No. 1) and by 4 times (Experiment No. 2); and root length decreased by 1.2 times (Experiment No. 1) and by 1.6 times (Experiment No. 2) compared to plants germinated under conditions of regular watering.

Fullerene C_{60} exhibited a substrate- and dose-dependent effect on pea microgreens. Fullerene C_{60} at concentrations of 0.1 and 0.2 μg/mL did not exhibit any negative effects. The study showed that the physiological state of microgreen peas induced by drought was restored after pre-treatment with fullerene C_{60}. The protective effect of CNPs was evidenced by a twofold increase in the height of the main shoot, the mass of leaves, and the length of the pea root compared to the indicators under water deficit without the addition of CNPs. Thus, the absence of phytotoxic effects and the protective effects of fullerene C_{60} against induced drought resistance in microgreen peas indicate the promising use of structured carbon nanoparticles in agrobiotechnologies for regulating stress resistance mechanisms in agricultural plants, which requires further physiological and biochemical research.

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CONFLICT OF INTEREST

None.

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Вплив фулерену C_{60} на морфометричні показники мікрозелені гороху (*Pisum sativum*) за умов водного дефіциту

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Анотація. Актуальність роботи полягає у вивченні впливу абіотичних чинників, зокрема фулерену C_{60} та посухи на морфометричні показники мікрозелені гороху. Посуха негативно впливає на ріст і розвиток сільськогосподарських рослин, що знижує їх врожайність. Вуглецеві наночастинки, зокрема фулерен C_{60}, можна використовувати як модулятор стійкості стресових умов для покращення перебігу фізіологічних та біохімічних процесів як на рівні клітини, так і організму рослин. Однак, внутрішньоклітинні механізми взаємодії вуглецевих наночастинок з рослинами є недостатньо вивчені. Метою роботи було дослідити вплив фулерену C_{60} на морфометричні показники мікрозелені гороху (*Pisum sativum*) сорту ECO за водного дефіциту. У роботі використано структуровані водорозчинні вуглецеві наночастинки фулерену C_{60}. Було оцінено такі морфометричні показники мікрозелені гороху як висота пагона, діаметр пагона, кількість листя, маса листя, маса рослини та довжина кореня. Дослідження проводилися на 14 день після обробки насіння гороху водним розчином фулерену C_{60} за концентрацій 0.1, 0.2, 0.5 та 1.0 мкг/мл та висаджених на різних субстратах – лляних килимках і ґрунтосуміші. У роботі було використано хімічні, фізичні та фізіологічні методи. Було показано, що на 14 день після пророщення насіння гороху у контролі (за умов регулярного поливу) на різних субстратах спостерігаються відмінності у морфометричних показниках. Фулерен C_{60} за концентрацій (0.1-1.0) мкг/мл не спричиняв негативного фітотоксичного впливу на мікрозелень гороху. Тоді як фізіологічний стан мікрозелені гороху сорту ECO індукований посухою відновлювався за попередньої обробки насіння розчином фулерену C_{60}. Відсутність фітотоксичних ефектів та захисні ефекти фулерену C_{60} від індукованої посухи у мікрозелені гороху свідчить про перспективність використання структурованих вуглецевих наночастинок у агробіотехнологіях для регуляції механізмів стресостійкості у сільськогосподарських культур

Ключові слова: сільськогосподарські рослини; вуглецеві; наночастинки; посуха; молоді паростки

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Protection of pine plantations in Ukrainian Polissia

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Abstract. The protection of pine plantations in Ukrainian Polissia is one of the most significant environmental problems that have a significant impact on biodiversity, ecological balance and climatic conditions. The aim of the study was to investigate the effect of the bioorganic composition "Meganit Nirbator" on the growth and development of Scots pine (*Pinus sylvestris* L.) planting material. The methods used in the study were: field method and laboratory method. According to the results of the study, it was found that for the period 2022-2024, a positive impact on the growth of seedlings and young pine trees was recorded on the territory of the Volodymyr-Volyn Forestry branch of the State Enterprise "Forests of Ukraine". It was found that the bioorganic composition based on biochemical fractions of basidiomycetes stimulated the growth and development of seedlings and young trees of Scots pine. According to the results of laboratory and field studies, it was noted that an increase in the solution concentration contributed to a significant improvement in plant growth parameters: height, boot diameter, number of new shoots and chlorophyll content. The most effective were solution concentrations of 2% and 5%, which indicated increased resistance to diseases and a decrease in the number of pests. A decrease in the level of disease infections and the number of pests was noted, which indicated the high efficiency of the composition as a biofungicide and insecticide. It was demonstrated that a 5% solution of the biopreparation contributed to a 90% increase in disease resistance 12 months after the field studies, while after 24 months the resistance rate was 80%. The results of the study can be used to develop and implement environmental measures and programmes aimed at restoring Scots pine trees in the Volyn Region

Keywords: *pinus sylvestris*; entomopathogenic fungi; biological preparation; biological method; apical bark beetle; luboid

INTRODUCTION

One of the key environmental challenges facing Ukraine is the destruction of pine forests in Volyn Region due to diseases and pests. The loss of pine plantations can lead to ecosystem degradation and the loss of numerous species of flora and fauna. The problem of the study is that the impact of human activity, climate change and water supply disruptions contribute to an increase in the area of forests affected by disease and pest epidemics. In conformity with the Polissia branch of the Ukrainian Order of the Badge of Honour of the H.M. Vysotskyi Research Institute of Forestry and Agroforestry in Volyn Oblast, more than 7,300 hectares of pine forests have been affected by pests, including the pine beetle (Volyn foresters sound..., 2017).

L. Romanchuk & P. Didenko (2019) found that Scots pine (*Pinus sylvestris* L.) was highly susceptible to the disease under conditions of high

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humidity and warm temperatures, which created favourable conditions for the development and spread of fungi that cause the disease. According to the results of this study, it was noted that the most affected by the Shute disease were forest areas in the Zhytomyr Region. V.F. Drozda & M.S. Karpovich (2020) noted the need for systematic monitoring of temperature conditions in the natural places of diapause of phytophages for the effective use of Boverin in the control of pest populations. In conformity with the findings, it was found that at elevated temperatures (above 17°C), the time of caterpillar death was significantly reduced.

P.V. Didenko *et al*. (2019) noted the need for further research to better understand the mechanisms of action of "Bioecofungus-1" and cerium dioxide nanoparticles on loblolly pine plants. According to the results of their work, it was found that the use of biopreparation and a complex with cerium dioxide nanoparticles contributed to a significant acceleration of the growth and development of Scots pine seedlings. The authors O. Furdychko *et al.* (2020) identified the problem of viral diseases in agricultural and forest crops. The authors recommend the use of basidiomycete fungal compositions for the prevention of viral infections in plants, as well as the use of simulated microgravity processes to heal plants from viral diseases.

Scientists V. Levchenko *et al*. (2023) pointed out the problem of the spread of necrotic and cancerous diseases of boots and branches of forest plantations and putrefactive diseases. They noted that the proposed phytopathological methodology proved to be effective in carrying out forestry preventive measures to localise fungal, bacterial, vascular, necrotic and cancerous pathology. Researchers A.D. Vorobei *et al*. (2023) studied the problem of determining the effectiveness of different pheromone trap designs and their ability to capture pine beetles in the natural conditions of Ukraine. As a result of the scientific work performed by the researchers, it was noted that type A (Funnel) traps with funnels demonstrated the highest efficiency in catching two species of bark beetles, while type B (Theyson) and C (Crosstrap® mini) traps caught a smaller number of pests.

O.A. Boyko *et al*. (2020) noted that the development of methodological approaches is important for the production of growth stimulants and plantation development using components from basidiomycetes. In biotopes with high levels of radiocesium in the soil, populations of fungi such as *Agaricus bisporus* and *Armillariella mellea* have been found to have reduced resistance to pathogens, which has led to a decrease in their density. O.E. Sakici *et al.* (2023) addressed the need to study the unexplored long-term effects of mistletoe infestation on pine tree growth. It is noted that it is relevant to develop strategies aimed at reducing the level of infection, such as timely removal of infected trees and implementation of preventive measures to reduce the spread of mistletoe in the future.

The problem raised in the study by O. Maaß & H. Kehlenbeck (2024) was that the economic feasibility of insect pest monitoring and aerial insecticide spraying is not yet well understood, especially in the context of massive outbreaks of insect pests such as the pine beetle (*Dendrolimus pini* L.). It is noted that the effectiveness and cost-effectiveness of these measures was confirmed by high net cost and cost values, which made them attractive to forest owners in the context of massive outbreaks of insect pests. J. Lee *et al.* (2023) outlined the problem that indicated the need for research to improve the composition of abamectin formulations for more effective control of the pine stem nematode (*Bursaphelenchus xylophilus*), a species of nematode from the family *Aphelenchidae* or *Parasitaphelenchidae*. It was proved that pest paralysis occurred at a dose of 0.06 μg/mL or higher.

The technological parameters of the use of preparations based on biochemical fractions of the basidiomycetes "Meganit Narrator" for forest plantations are insufficiently studied. The need to conduct research on the use of biological products can be justified by several key aspects: reducing the use of chemical pesticides; maintaining biodiversity; and reducing the cost of forestry treatment. The aim of the study was to verify the feasibility of using an organic composition based on biochemical fractions of basidiomycetes to protect pine seedlings and young trees from pests and diseases. Objectives were analysing long-term observation of the growth and physiological state of trees; studying the effect of the organic composition on the growth and resistance of Scots pine plants.

MATERIALS AND METHODS

When studying the feasibility of using the entomopathogenic fungal biological product "Meganit

Nirbator" to protect pine plantations from diseases and pests, the methodology was applied, which is regulated by the Law of Ukraine No. 180-XIV "On Plant Protection" (1998), FSC national forest management system standard for Ukraine (2018). The study used a field method to collect samples of plant litter on the soil surface from the near-stem circles of model trees and to collect caterpillars from tree crowns. The laboratory method was used for species identification and physiological monitoring of caterpillars. The study used an entomopathogenic fungal biological product with a titer of 6 billion spores/g and in concentrations of 1, 2 and 5%.

The collected materials were placed in gauze cages with branches of Scots pine. The caterpillars of three pest species were selected: the larvae of the larvae beetle (165 caterpillars, 55 individuals for each age, in three replications), the apical bark beetle, and the pine chermes (the total number of their caterpillars was 120 individuals, 40 individuals for each species), which were diapaused on tree boles and in the soil. In the experiments with the bark beetle, the experiments were carried out using caterpillars of the third, fourth and fifth instars. The field research was conducted in the period 2022-2024 on plots located in the Volodymyr-Volyn Forestry branch of the state enterprise "Forests of Ukraine", which is located in the southern part of Volyn Region.

During the laboratory stage, preliminary testing of the composition's effectiveness was carried out on seedlings grown under controlled conditions. The field stage included the application of the composition on seedlings and young trees in natural conditions to combat diseases such as pine rust, fusarium and pine bark cancer. A total of 100 seedlings were selected for the study. The chlorophyll content was measured using the gasometrical method. The laboratory stage included: the preparation of seedlings for treatment; treatment of seedlings with different concentrations of the organic composition; and assessment of the growth and physiological condition of seedlings for three months. The field stage involved: selecting a site for planting the seedlings; treating the seedlings with the organic composition before planting; monitoring the growth and physiological condition of the trees for three months, one and two years.

The following parameters were used to evaluate the treatment effectiveness: seedling height (cm); boot diameter (mm); number of new shoots; photosynthetic activity (determined by chlorophyll content); and resistance to diseases and pests. For effective protection of Scots pine plantations from third instar caterpillars, the consumption of *B. bassiana* spores should be at least 8.05 billion/m^2 with the titre of "Meganit Nirbator" 6 billion/g. To determine the amount of "Meganit Nirbator" per hectare, it is necessary to calculate the required number of grams per square metre, and then convert this value to hectares. When calculating the number of grams of the biopreparation per square metre, the following data were obtained (1):

$$\frac{8.05 \text{ billion spores}}{6 \text{ billion spores/g}} = 1.34167 \text{ g/m}^2. \quad (1)$$

Converting this value to a hectare yields the corresponding values (2):

$$W1.34167 \frac{\text{g}}{\text{m}^2} \times \frac{10000 \text{ m}^2}{\text{ha}} = 13.4167 \text{ g/ha}. \quad (2)$$

The laboratory study was carried out in the laboratory of "Monitoring of carbon-absorbing capacity of phytocoenoses" of the Western Ukrainian National University, located at the following address: 3 Peremohy Square, Ternopil, Ukraine, building No. 3. Saplings and young trees of Scots pine (*Pinus sylvestris*), aged from one to three years, were used for the study. The organic composition was created on the basis of biochemical fractions of the basidiomycetes "Meganit Nirbator". The composition included 4 bacterial strains: *Azotobacter chroococcum* (nitrogen-fixing aerobic bacteria, pH optimum – 7-7.5; *Azospirillum Lipoferum* (rhizosphere nitrogen-fixing bacteria with a pH optimum of 7-7.5, and temperature 20-30°C); *Bacillus subtillis* or "blue bacillus" (spore-forming bacterium with amylolytic properties, capable of hydrolysing polysaccharides to simple carbohydrates, effective against Fusarium, Aspergillosis, Rhizoctonia, phytopathogenic pseudomonas; *Bacillus megatherium* – rod-shaped bacterium, optimum pH – 6.5-7.5 and a temperature of 28-35°C, has amylolytic activity, immobilises phosphorus from complex compounds in the form of P_2O_5, and has antifungal properties. The manufacturer is Raktarbazisz-Transit Limited Liability Company (Hungary, Nirbator).

To control the number of pests in the samples, the following equipment and products were used:

Gruntek HS-1.5 hand-held sprayers (manufactured in China), pheromone traps for *Scolytidae* pests (manufactured in China) and biological pathogenic agents *Beauveria bassiana* (manufactured in the USA). The chlorophyll content was measured using a portable fluorometer "Floratest" developed by the Glushkov Institute of Cybernetics of the National Academy of Sciences of Ukraine.

RESULTS

Forest protection is a major part of the environmental policy of many countries. One of the areas of this policy was to ensure the healthy growth of seedlings and young trees, in particular pine. In this context, organic compositions based on biochemical fractions of basidiomycetes can play a pivotal role. *Basidiomycota* is a class of fungi with significant potential in the natural environment, as they are able to produce various biologically active compounds such as antibiotics, enzymes, antioxidants and other metabolites. They can be used to control pathogens and pests in various agronomic and ecological systems due to their natural properties, such as: antagonistic properties – certain species of basidiomycetes can inhibit the growth of pathogens by releasing antimicrobial compounds or by competing for resources. This can reduce plant diseases and improve soil health; pathogenic infestation: several species of basidiomycetes can parasitise other fungi or pathogens, which helps control their populations; bioinsecticide production – basidiomycetes can produce compounds that act as bioinsecticides, killing pests or reducing their numbers.

In forests, Scots pine is often affected by pine rust disease and pine fusarium. Damage to pine plantations is caused by the pine beetle, as well as by the pine top borer and pine chermes. Pine rust (*Peridermium pini*) is a fungal disease that affects pine trees. The pathogen is a fungus from the genus *Cronartium*. It causes the formation of rusty spots and growths on the trunks and branches of trees, which leads to a decrease in their viability and death. *Fusarium* pine blight is a fungal disease that caused browning of needles, wilting and drying of shoots, discolouration of the bark in the areas of infection and damage to the root system of pine trees. The bark beetle can damage trees by making passages under the bark, leading to nutritional disorders and tree death.

According to previous studies, it became known that the treatment of seedlings and young pine trees with the bioorganic composition "Meganit Nirbator", created on the basis of biochemical fractions of basidiomycetes and recommended for use by the Department of Physiology, Plant Biochemistry and Bioenergy of the National University of Life and Environmental Sciences of Ukraine, can be a preventive measure against the pathogen of pine rust, fusarium and pine bark cancer, as well as pests (Sus *et al.*, 2020). The results of the laboratory stage showed a positive effect of the organic composition on the growth and physiological state of pine seedlings (Table 1).

Table 1. Effect of bioorganic composition on the growth of pine seedlings (after 3 months)

Group	Height (cm)	Barrel diameter (mm)	Number of new shoots	Chlorophyll content (mg/g)
Control	25.3 ± 1.2	4.1 ± 0.3	3.2 ± 0.4	1.4 ± 0.1
Composition of 1% solution of the biological product	27.8 ± 1.5	4.5 ±0.3	3.7 ± 0.5	1.7 ± 0.2
Composition of a 2% solution of the biological product	30.2 ± 1.4	4.8 ± 0.2	4.1 ± 0.4	1.9 ± 0.2
Composition of a 5% solution of the biological product	33.1 ± 1.3	5.2 ± 0.3	4.6 ± 0.5	2.1 ± 0.2

Source: compiled by the author

The bioorganic composition stimulated the growth and development of Scots pine plants. With increasing solution concentration (from 1% to 5%), a gradual increase in height, boot diameter, number of new shoots and chlorophyll content was observed. The greatest positive effect was achieved when using a 5% solution of the biological product. After the field research, the results of laboratory studies were confirmed and a long-term positive effect of the organic composition on the growth and stability of young pine trees was demonstrated (Table 2).

Table 2. Effect of bioorganic composition on the growth of young pine trees (after 12 months)

Group	Height (cm)	Barrel diameter (mm)	Number of new shoots	Chlorophyll content (mg/g)	Disease resistance (%)
Control	38.5 ± 2.1	5.4 ± 0.4	4.2 ± 0.5	1.5 ± 0.2	60
Composition of 1% solution of the biological product	42.3 ± 2.2	5.9 ± 0.4	4.8 ± 0.4	1.8 ± 0.2	70
Composition of a 2% solution of the biological product	46.7 ± 2.4	6.3 ± 0.3	5.4 ± 0.5	2 ± 0.2	80
Composition of a 5% solution of the biological product	52.1 ± 2.5	6.8 ± 0.4	6.1 ± 0.5	2.3 ± 0.2	90

Source: compiled by the author

According to the results of the study, a positive effect of the bioorganic composition of the solution on seedlings and young pine trees was noted. Higher concentrations of the solution (2% and 5%) were especially effective, as they stimulated an increase in height, boot diameter and number of new shoots compared to the control group. The high chlorophyll content of the treated plants indicated increased photosynthetic activity, which contributed to their better growth. Disease resistance also increased significantly in the treated plants. This could be due to the biologically active substances contained in the biochemical fractions of basidiomycetes, which were capable of acting as natural fungicides and insecticides.

The study was continued to confirm the long-term effect of the biopreparation on pine trees and the possibility of its widespread use in forestry. An additional observation was conducted for two years to assess the stability and effectiveness of the formulation on mature pine trees. The following parameters were measured: height, boot diameter, number of new shoots, chlorophyll content, disease resistance, and number of pests (Table 3).

Table 3. Effect of bioorganic composition on the growth of adult pine trees (after 24 months)

Group	Height (cm)	Barrel diameter (mm)	Number of new shoots	Chlorophyll content (mg/g)	Disease resistance (%)	Number of pests (individuals/ tree)
Control	65.4 ± 3.2	8.3 ± 0.5	5.2 ± 0.6	1.6 ± 0.3	50	70
Composition of 1% solution of the biological product	70.3 ± 3.4	8.7 ± 0.5	5.8 ± 0.6	1.9 ± 0.2	60	50
Composition of a 2% solution of the biological product	75.8 ± 3.5	9.2 ± 0.4	6.4 ± 0.6	2.1 ± 0.2	70	35
Composition of a 5% solution of the biological product	81.5 ± 3.6	9.7 ± 0.5	7.1 ± 0.6	2.4 ± 0.2	80	20

Source: compiled by the author

According to the data obtained, the positive effect of the bioorganic composition continued for two years. The best results were observed in the groups with a concentration of 2% and 5%, which confirmed the effectiveness of these concentrations in stimulating the growth and resistance of pines. Increases in boot height and diameter were greatest in the 5% solution group. The difference between control and treated trees remained significant. The number of new shoots and chlorophyll content were significantly higher in the treated plants, indicating increased photosynthetic activity. A high level of disease resistance was found in the treated plants, especially in the group with 5% solution, which reduced the risk of infections. The number of pests was significantly reduced, which confirmed the effectiveness of the bio-organic solution as a natural insecticide. The pine trees were treated by applying the composition created by mixing the extracted fractions with organic solvents to the surface of the tree bark (Table 4).

Table 4. Results of pine treatment against pests

Pest	Control group (no treatment)	Processed group	Reduction in the number of pests
The mushroom (*Tomicus piniperda* L.)	50 individuals per tree	10 individuals per tree	80%
The treetop bark beetle (*Scolytus mali*)	30 individuals per tree	5 individuals per tree	83.3%
Pine Hermes (*Pineus pini Hermes*)	20 individuals per tree	3 individuals per tree	85%

Source: compiled by the author

It was found that the treatment with the composition against all three types of pests: the larvae of the pine tree, the pine apex beetle and the pine tree chermes, provided a significant reduction in their number (from 80% to 85%). According to the results of the study, it was found that the organic composition based on biochemical fractions of basidiomycetes reduced the level of disease infections among pine trees (Table 5).

Table 5. Results of pine treatment against diseases

Disease	Control group (no treatment)	Processed group	Reduction of infection
Rusty pine (*Peridermium pini*)	40% of infected trees	10% of infected trees	75%
Fusarium	35% of infected trees	8% of infected trees	77.1%
Pine bark cancer (*Cronartium ribicola*)	25% of infected trees	5% of infected trees	80%

Source: compiled by the author

Pine rust infection decreased from 40% in the control group to 10% in the treated group, representing a 75% reduction in infection. Fusarium infection rates decreased from 35% in the control group to 8% in the treated group, representing a 77.1% reduction in infection. The infection rate of pine bark cancer decreased from 25% in the control group to 5% in the treated group, resulting in an 80% reduction in infection. Due to its environmental safety, this formulation can be used as an alternative method of plant protection compared to chemical pesticides and fungicides. Table 6 shows the mortality rates of caterpillars of herbivorous phytophages of the *Luboid phytophagous* beetle infected with the biopreparation solution, depending on age and temperature.

Table 6. Indicators of mortality dynamics of caterpillars infected with the biological product depending on age and temperature

Types of herbivorous phytophages	Time required to kill 50% of the LT_{50} caterpillar population (days) at temperature					
	Phase of development of the phytophage	13°C	18°C	23°C	28°C	33°C
The mushroom (*Tomicus piniperda* L.)	L_3	10.5	8.4	5.2	2.5	1.2
	L_4	17.2	11.3	7.5	5.5	2.5
	L_5	20.5	19.3	14.5	9.6	7.4
The treetop bark beetle (*Scolytus mali*)	L_3	31.7	30.2	25.3	19.2	5.2
NIR_{05}		N/A	N/A	N/A	N/A	N/A
Pine Hermes (*Pineus pini Hermes*)	L_5	19.1	15.6	11.3	4.5	4.9
NIR_{05}	N/A	N/A	N/A	N/A	N/A	N/A

Notes: L_3, L_4, L_5 – phytophagous caterpillars of different ages
Source: compiled by the author

According to the results of the study, it was found that the population of third instar caterpillars collected from leaf litter and soil surface is particularly vulnerable to the action of *B. bassiana*, the biological basis of the biological product. With increasing temperature, the value of LT_{50} (days) (the time required for the death of 50% of the population) decreased. This indicated that the effectiveness of the bioorganic composition increased at higher temperatures. The air temperature in the range of 13-18°C for a period of 8.4-10.5 days contributed to the death of 50% of the population of caterpillars of the broomrape. With increasing temperature, the period of caterpillar death was reduced. *Luboid caterpillars* in later stages of phytophagous development (L_4, L_5) require more time to die under the influence of the biological product. In conformity with the data on the dynamics of mortality of third instar caterpillars, it is advisable to take measures to apply the solution to the places of diagnosis of the phytophagous phage. The diagnosing caterpillars of the fifth instar pine chermes, as well as the caterpillars of the third instar of the apex bark beetle, were much more resistant to the action of the biological product, and, accordingly, the period of their death was longer. Thus, it can be concluded that the effectiveness of the bioorganic composition depended on the temperature of the environment, the developmental stage of the caterpillars and the type of herbivorous phytophages. Higher temperatures resulted in faster caterpillar death, and caterpillars at later stages of development were more resistant to infection. In comparison with the top bark beetle and pine chermes, it was found that the caterpillars of the larvae of the larvae were more favourable to the effect of the preparation. Table 7 shows the toxicological parameters of the biological product activity against caterpillars of different age groups of phytophages.

Table 7. Toxicological parameters of the biological product activity against caterpillars of different ages of phytophages

Types of herbivorous phytophages	Phase of development of the phytophage	Physiological state of the caterpillars	Concentration of a bio-organic agent that causes the death of 50% of the LK_{50} caterpillar population (days)	
			% by drug	The term of the decisive days
The mushroom (*Tomicus piniperda* L.)	L_3	Active, healthy	0.025%	14
	L_4	Development of organs	0.04%	18
	L_5	Big, ready to fly	0.05%	22
The treetop bark beetle (*Scolytus mali*)	L_3	Active, newly hatched	0.03%	12
Pine Hermes (*Pineus pini Hermes*)	L_5	Mature, with a large supply of nutrition	0.045%	20

Notes: L_3, L_4 L_5 – phytophagous caterpillars of different ages
Source: compiled by the author

The biopreparation demonstrated different toxicological parameters (LK_{50}) for different stages of caterpillar development of different phytophagous species. For example, for caterpillars of the bark beetle in the phases L_3, L_4 and L_5, the concentration of the bioorganic agent that causes the death of 50% of the caterpillar population LK_{50} (days) percentage by the preparation increased from 0.025% to 0.05% due to the increase in the physiological state of caterpillars from active to ready to fly. As for the pine top bark beetle and pine chermes, the LK_{50} value also varied depending on the stage of caterpillar development, indicating a variation in the effectiveness of the preparation depending on the stage of the phytophage life cycle. Table 8 shows the consumption rates of the biological product.

Table 8. Indicators of consumption rates of a biological product for the protection of pine plantations depending on the age of the bark beetle caterpillars

Age of the caterpillars of the bowhead beetle	Concentration of a bio-organic agent that causes the death of 90% of the population of LK_{50} caterpillars$_{90}$ (billion spores/mL) as a function of phytophage mortality		Parameters of *B. bassiana* spore consumption, billion/m^2	
	Crop residues – soil surface	Total mortality rate	For plant protection needs	To destabilise the effective part of the phytophage
The second	0.025	0.005	1.5	0.41
The third	0.04	0.003	8.05	0.35
The fourth	0.55	0.07	12.55	1.2

Source: compiled by the author

The number of grams of the biological product per m^2 was 13.4167 g/ha. Thus, for effective protection of Scots pine plantations from third instar caterpillars, 13.42 g/ha of the biological product with a titre of 6 billion/g should be used. The bioorganic composition stimulated the growth and development of Scots pine plants. With increasing concentration of the solution, a gradual increase in height, boot diameter, number of new shoots and chlorophyll content was observed. The use of the bio-organic composition has reduced the use of chemical pesticides and fungicides, which has had a positive impact on the region's environment. The high effectiveness of the biological product in controlling pests and diseases, as well as stimulating plant growth, makes it an attractive solution for forestry enterprises seeking to ensure sustainable use of forest resources. Protecting pine plantations from pests and diseases is critical to maintaining the ecological balance in the region. Pine trees affected by diseases and pests can cause significant environmental problems, including reduced biodiversity, poor air and water quality, and increased risk of forest fires.

The use of a bio-organic composition based on biochemical fractions of basidiomycetes helped to solve a number of local environmental problems: the replacement of chemical plant protection products with biological ones reduced the negative impact on the environment, in particular soil and water pollution; the composition contributed to increasing the resistance of pine trees to diseases and pests, which helped to maintain healthy forests and reduce tree mortality; treatment with the composition increased the chlorophyll content and photosynthetic activity of trees, which had a positive impact on the environment. Thus, the application of the biopreparation contributed to solving local environmental problems, ensuring healthy growth and sustainability of pine plantations, which, in turn, improved the overall environmental condition of the region and the state of forest ecosystems.

DISCUSSION

Analysing the results of the study, it can be concluded that it is possible to apply the technology of coniferous trees treatment with a bioorganic composition based on biochemical fractions of basidiomycetes, since the treatment of seedlings and young trees has shown high efficiency.

It was found that for the effective protection of Scots pine trees from third instar caterpillars, the required dose was 13.42 g/ha of a biological product with a titer of 6 billion g. A similar issue was raised by C.J. Fettig *et al*. (2020). They found that lower doses of SPLAT® Verb verbenone (7 grams per tree) can be effectively used to protect pine trees from the mountain pine beetle (*Dendroctonus ponderosae Hopkins*). This statement can be agreed with, as really low doses of verbenone can be effective for a long time, providing long-term protection of trees from pests.

It was noted that the infection with pine rust decreased from 40% in the control group to 10% in the treated group. A similar problem was studied by J. Kuang *et al.* (2024). They found that the use of multi-temporal satellite data significantly improved the accuracy of monitoring forest stands affected by pine wilt disease compared to the use of single satellite images. The results of the current study did not coincide with the conclusions of the researchers as they conducted a detailed study at the local level using additional ground monitoring methods and laboratory analysis, which allowed them to identify different stages of the disease and apply effective control measures in a timely manner.

It was found that *Fusarium* infection decreased from 35% in the control group to 8% in the treated group. A similar issue was studied by K. Youssef (2023). In the course of the study, the researcher found that latent root infections of loblolly pine trees in areas with a low risk of infection with the root sponge disease *Heterobasidion annosum* L. were quite common. In addition, these infections were more common in plots with a higher site index, indicating a higher probability of infection of trees on more fertile soils. This statement should be accepted, as a higher site index may indicate a higher tree density and greater competition for resources, which can weaken trees and make them more vulnerable to infections.

According to the results of the study, it was shown that the treatment of pines with an organic composition based on biochemical fractions of basidiomycetes effectively reduced the number of pests of the apex bark beetle. A similar issue was studied by O. Golan *et al*. (2022). According to the results of their work, it was found that the use of trap trees and their treatment with bifenthrin reduced the number of dead trees in the plantations, which indicated the effectiveness of such measures to prevent the spread of bark beetles. The study came to similar conclusions, which revealed that after the application of the bio-organic composition, the number of apex bark beetle pests decreased by 83.3%.

The caterpillars of the third age of the apex bark beetle were much more resistant to the biological product, so the time of their death was longer. The results of these indicators were reviewed by N. Erbilgin *et al.* (2021), who noted that pine trees exposed to bark beetle attacks and drought showed a significant decrease in carbohydrate reserves, which limited their ability to synthesise protective diterpenes and increased the likelihood of tree death due to physiological exhaustion and mechanical damage to vascular tissue by beetles. Thus, both studies present different aspects of the study of pine forest mortality: determination of resistance to the drug of the caterpillar of the apex bark beetle in this study and analysis of the impact of environmental factors on the phytosanitary state of forests in the scientific work of the researchers.

It was demonstrated that air temperature in the range of 28-33°C for a period of 5.2-19.2 days contributed to a reduction in the death of caterpillars of the top bark beetle. A similar study was conducted by M. Haseeb (2023). According to the results of this scientist's work, it became known that the invasions of the southern pine beetle and black terpentine beetle were limited to trees that were damaged by fire, lightning or other pests, but pines that were stressed due to drought or shading were rarely attacked. This statement can be agreed with, as beetle infestations are often observed in conditions where trees are vulnerable due to physical or biological damage.

It was recorded that the treatment with the bio-organic composition demonstrated effectiveness in the fight against the apex bark beetle, reducing their number to 5 individuals per tree, compared to the control group, where their number was 30 individuals per tree. A similar study was conducted by E.M. Hansen *et al*. (2023). It was noted that climatic water scarcity significantly affected the probability of infestation, showing that periods of high-water stress could contribute to the spread of beetles and further damage to the pine forest. The results obtained in the present study differed from the conclusions drawn by the researchers, which could be due to differences in their research methods, such as the method of assessing abiotic conditions (temperature, precipitation) using *Daymet* interpolation in the analysed study and the field method in the present study. The fifth instar caterpillars of the pine hermes (*Pineus pini Hermes*) were much more resistant to the biological product, and, accordingly, the time of their death was longer. The issue under discussion was studied by L. Heiðarsson *et al*. (2020). According to the results of their work, it was noted that lodgepole pines of Icelandic origin were less susceptible to woolly aphid (*Pineus pini*) invasion compared to pine plantations of non-Icelandic origin, which can be explained by natural selection, which contributed to the adaptation of lodgepole pine in Icelandic conditions. The results of my study did not coincide with the findings of the researchers as they analysed the resistance to the effects of a bio-organic composition.

The effectiveness of treating pine trees with a bioorganic composition against pine rust disease showed a 75% reduction in infection. A similar issue was studied by K. Zhang & J. Stenlid (2023). Their study found that the protocols for monitoring and managing the lodgepole pine rust epidemic, developed to detect the rust fungus (*Cronartium pini*), were effective and specific for identifying this fungus in lodgepole pine samples. These protocols were used not only to detect the presence of this pathogen in asymptomatic and symptomatic trees, but also to track the occurrence of Scots pine rust in Northern Europe. The conclusions presented in this study differed from those of the researchers, as this study focuses on the use of a bio-organic composition based on biochemical fractions of basidiomycetes to control pine rust disease, while the researchers' investigated protocols for monitoring and managing the epidemic of this disease. An organic composition based on biochemical fractions of basidiomycetes effectively reduced the level of disease infections among pine trees by 77.1%. A similar issue was studied by L. de Vos *et al*. (2024). The results of this work revealed that one of the pine *fusarium* strains (*Fusarium circinatum*) showed significant differences in the length of this chromosome, with large parts of its distal and proximal regions missing. These regions were characterised by a lower gene density and an increased prevalence of repetitive elements, which can be contrasted with syntenic segments and core chromosomes. It is worth agreeing with the results of this study, as the detection of significant differences in chromosome length and the absence of large parts of the distal and proximal regions indicates genetic instability or specific adaptive mechanisms of this strain.

The infection of pine bark cancer decreased from 25% in the control group to 5%. A similar issue was studied by D. Martin *et al*. (2022). According to the results of their work, it can be stated that Raman spectroscopy has become an effective tool for the early detection of biochemical changes associated with pine bark cancer caused by the fungus *Fusarium circinatum*. The study showed that this method can be used to select trees resistant to pine resin canker, in particular due to lignin as a key component of resistance. The results of this study should be accepted, as Raman spectroscopy can detect changes in the molecular composition of tissues at very early stages, making it extremely valuable for detecting pathogenic changes before visible symptoms appear. Lignin, as one of the main components of wood, plays an important role in fungal resistance, as its structure and chemical properties affect the ability of the fungus to penetrate and grow in wood. The use of a bioorganic composition based on biochemical fractions of basidiomycetes helped to effectively reduce the level of Fusarium infections in pine trees. A similar problem was investigated by T. Wendhausen *et al.* (2019). The results of this scientific study showed that the use of an antagonistic biological product *Trichoderma* sp. for the treatment of pine seeds effectively reduced the incidence of Fusarium root rot and also contributed to an increase in the number of seedlings and plant growth. Agreeing with the statement, it should be noted that the opinion of the researchers and colleagues is correct, as the use of the biological product *Trichoderma* sp. had several confirmed advantages. Firstly, this fungus is able to inhibit the growth of pathogenic fungi, such as *Fusarium*, due to its antagonistic properties. The biological product *Trichoderma* sp., together with other microorganisms, releases enzymes and antibiotics that inhibit pathogenic microflora. Secondly, *Trichoderma* sp. helps to improve plant growth, as it stimulates the development of the root system, improves the availability of nutrients and increases plant resistance to stressful conditions.

As a result of using the bio-organic composition, the infection of pine bark cancer decreased from 25% in the control group to 5% in the treated group, thus reducing the infection rate by 80%. A similar issue was investigated by F. Ratsoma *et al*. (2024). They presented results that showed that the pine resin cancer pathogen Fusarium circinatum formed biofilms with increased extracellular matrix (ECM) production in response to abiotic factors such as temperature and pH, which highlighted the adaptability of this phytopathogen to environmental changes. The findings of the researchers should be accepted, as the extracellular matrix of biofilms can indeed provide protection for pathogen cells from adverse environmental conditions such as high or low temperatures and extreme pH levels. In the presented study, it was noted that the created organic composition demonstrated effectiveness due to the content of phenolic compounds (bioactive substances capable of exhibiting antioxidant and antibacterial properties). A similar issue was studied by I. Amri *et al*. (2022). In the course of the study, they found that pine essential oils were characterised by significant phytotoxic and antifungal activity due to the high content of monoterpene hydrocarbons, among which limonene, α-pinene and β-pinene were the dominant components. The highest herbicidal activity was demonstrated by the essential oil of *Pinus pinea*, while the highest antifungal activity was demonstrated by the essential oils of *Pinus pinea* and *Pinus nigra*. It is worth agreeing with this formulation of the researchers' conclusions, as the researchers' findings are based on specific experimental data indicating a significant content of monoterpene hydrocarbons in pine essential oils, which may indeed have pronounced phytotoxic and antifungal properties.

After the treatment with the bioorganic composition, an increase in disease resistance of Scots pine plants was observed. The present topic was studied by A.H. Wani *et al*. (2010). According to the results of the study, it was noted that the application of rhizobacteria of the genus *Bacillus* to the seeds of blue pine (*Pinus wallichiana*) led to an improvement in the growth and health of seedlings. It is possible to agree with the opinion of the researchers, as *Bacillus* rhizobacteria have an antagonistic effect on plant pathogens, producing antibiotics or other antimicrobial compounds, which reduces the risk of disease. A high level of disease resistance was found in the treated Scots pine plants in the group with a 5% solution of the composition. This topic was studied by E. Karlsen-Ayala *et al*. (2022). The results of their work showed that pine seedlings grown in soil with natural mycorrhizal communities showed better growth and health indicators compared to those grown using commercial inoculants. The conclusion of the researchers, as natural mycorrhizal communities consist of many species of fungi that interact with plants on a deeper level, providing better water and nutrient absorption and increasing resistance to stress and disease.

In 12 months after the field research, a high level of disease resistance was found in the treated loblolly pine plants, which amounted to 90%. A similar topic was studied by R.A. Sniezko *et al*. (2023). According to the results of their study, it was noted that after inoculation with the fungal pathogen *Cronartium ribicola*, a high level of genetic resistance to blister rust was found in certain populations of whitebark pine. The results of my research coincided with the findings of the researchers, as they also conducted a detailed analysis of the genetic resistance of loblolly pine to various pathogens. The composition of the composition for the treatment of pine seedlings included fungal extracts enriched with proteins from basidiomycetes. A similar topic was studied by H. Patel *et al*. (2023). The results of this study showed that fungi of the *Basidiomycetes* group were collected and stored for further use, in particular in important areas such as medicine, education and agriculture, which highlighted their importance for local development and the ecosystem. It is worth agreeing with the conclusion of the researchers as these fungi do have significant potential as a source of medicinal substances and for biodegradation of organic matter in soils. In 24 months after the field trials, high disease resistance of the treated Scots pine plants was observed, which amounted to 80%. The same topic was studied by T. Persson *et al*. (2024). According to the results of the study presented by the authors, it was shown that the selection of loblolly pine to increase resistance to blister rust can be successful without the risk of adversely affecting other critical breeding traits, such as vitality, which was 0.17, and height, which reached a value of 0.25. The conclusions of the researchers should be accepted, as they confirm the feasibility of such selection in terms of preserving valuable genetic resources of Scots pine, which is critical for the further development of pine variety improvement programmes.

CONCLUSIONS

According to the results of studies of the effect of bioorganic composition on the growth and development of Scots pine planting material, it was shown that the treatment of seedlings and young pine trees with an organic composition based on biochemical fractions of basidiomycetes had a positive effect on their growth and development. The composition concentration of 2% and 5% showed the best results. The use of such an organic composition can be recommended to increase the efficiency of pine cultivation in forest nurseries and forest plots. The effectiveness of the product depended significantly on the temperature. At higher temperatures, the death of caterpillars occurred in a shorter period of time. For the caterpillars of the broomrape (*Tomicus piniperda* L.) in the L_3 phase, the LT_{50} (time to death of 50% of the population) decreased from 10.5 days at 13°C to 1.2 days at 33°C. It was found that caterpillars at later stages of development (L_4, L_5) require more time to die under the influence of the biological product. For example, for caterpillars in the L_5 phase, the LT_{50} value at 13°C was 20.5 days, which is significantly higher compared to the L_3 phase (10.5 days). It was determined that the caterpillars of the larvae of the larvae were more favourable to the effect of the drug compared to the apical bark beetle and pine chermes. For pine chermes, even at the highest temperature (33°C), LT_{50} is higher than for the larvae of the bark beetle.

In the review of studies of forest areas of the Volodymyr-Volyn Forestry Enterprise destroyed by pests and diseases, it should be noted that some data on the condition of pine plantations are not available for analysis. The study did not take into account the impact of environmental factors, such as climatic conditions, soil characteristics, pollution levels and other external factors that can also affect the health of forest stands. Further research may include the introduction of additional field observations to develop methods for the mass production of biochemical fractions of basidiomycetes. The research may include improving the composition of the composition and studying its effect on other woody plant species.

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CONFLICT OF INTEREST

None.

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Захист соснових насаджень Українського Полісся

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Анотація. Захист соснових насаджень Українського Полісся є однією з найбільш значущих екологічних проблем, які мають суттєвий вплив на біорізноманіття екологічну рівновагу та кліматичні умови. Метою дослідження було вивчення впливу біоорганічної композиції «Меганіт Нірбатор» на ріст і розвиток посадкового матеріалу сосни звичайної (*Pinus sylvestris* L.). В ході дослідження застосовувались методи: польовий метод, лабораторний метод. По результатам дослідження виявлено, що за період 2022-2024 років на території філії «Володимир-Волинське лісове господарство» державного підприємства «Ліси України» зафіксовано позитивний вплив на ріст саджанців та молодих дерев сосни. Виявлено, що біоорганічна композиція на основі біохімічних фракцій базидіоміцетів стимулювала ріст і розвиток саджанців та молодих дерев сосни звичайної. За результатами лабораторних і польових досліджень відзначено, що збільшення концентрації розчину сприяло значному покращенню параметрів росту рослин: висоти, діаметру стовбура, кількості нових пагонів та вмісту хлорофілу. Найбільш ефективними були концентрації розчину 2 % та 5 %, що свідчило про підвищену стійкість до хвороб та зниження кількості шкідників. Відмічено зниження рівня інфекцій хворобами та кількості шкідників, що свідчило про високу ефективність композиції як біофунгіциду та інсектициду. Продемонстровано, що 5 % розчин біопрепарату сприяв збільшенню стійкості до хвороб через 12 місяців після проведення польових досліджень на 90 %, тоді як через 24 місяці показник стійкості становив 80 %. Результати дослідження можуть бути використані з метою розробки та впровадження екологічних заходів та програм, спрямованих на відновлення дерев сосни звичайної в Волинській області

Ключові слова: *pinus sylvestris*; ентомопатогенні гриби; біопрепарат; біологічний метод; верхівковий короїд; лубоїд

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Diagnostics and bioremediation of soils affected by military operations in Ukraine

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Abstract. As a result of the full-scale invasion, extensive areas of agroecosystems suffered from toxic substances, which made the issue of restoring damaged land through the destruction of pollutants relevant. The purpose of this study was to substantiate and systematise diagnostic indicators for characterising soil bioactivity and to develop scientific approaches to bioremediation of damaged land. The study employed the method of analysing existing approaches to solving problems, the method of comparing data on the number of microorganisms of the main ecological and functional groups, which was determined according to the method of sowing soil suspensions on agarified nutrient media. The indices of microbial processes were calculated. Bioremediation measures were performed by introducing microbial biomass and culture fluids into the soil: *Dietzia maris* IMV B-7350, *Rhodococcus erythropolis* IMV B-7351, *Bacilus subtilis* IMV B-7349, *Pseudomonas aureofaciens* IMV B-7558, and *Streptomyces violaceus* IMV Ac-5027. The findings of the study showed that the level of microbiota

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repression in the soil affected by the explosions was exceedingly high compared to the undamaged soil. The most sensitive were phosphate-mobilising, nitrogen-fixing, amylolytic (including streptomycetes) microorganisms. In the soil from the bomb crater, the number of nitrogen-fixing and streptomycetes was 7% and 8% of the control, respectively, oligotrophic and amylolytic – 19.2% and 23.6%; phosphate-mobilising – 26%. In the soil damaged by an anti-tank shell explosion, the number of amylolytic, phosphate-mobilising, nitrogen-fixing, oligotrophic, and streptomycetes bacteria was 1.5%-10% of the control soil. The microbiota was repeatedly analysed 6 months after soil bioremediation. In the soil from the bomb crater, the number of phosphate-mobilising and nitrogen-fixing microorganisms increased and was 1.5-3.8 times greater than in the undamaged control, while the number of streptomycetes increased 12 times compared to the damaged soil. In the soil from the unexploded ordnance crater, the amount of phosphate mobilisers was 2.5 times higher than in the control, while the number of streptomycetes and nitrogen fixers increased 5.9 and 20.5 times, respectively, compared to the damaged soil. After bioremediation measures, the soil samples under study can be classified as either slightly toxic or non-toxic. The practical value of this study lies in the effectiveness of the proposed bioremediation approach, which is crucial for the further development of soil remediation measures in the context of the destruction of the Ukrainian agroecosystem

Keywords: military impact; damage; restoration; biological products; biological activity; soil microbiota

INTRODUCTION

Due to Russia's full-scale aggression, active hostilities are ongoing on the territory of Ukraine, which are a severe stress factor for ecosystems, including soil biological activity. The military impact has led to the destruction of arable land, which is Ukraine's most valuable natural resource. Toxic substances present in the soil, overcoming the barrier of the rhizosphere microbiota, get into plants and agricultural products, and then through trophic chains – into the human body. Determining the level of soil damage and developing biotechnologies for its remediation is an urgent problem.

According to statistics from the World Bank, the Government of Ukraine, and the European Commission, the territories of Luhansk (100%), Kherson (95%), Chernihiv (80%), Zaporizhzhia (74%), Sumy (70%), and Donetsk (64%) regions were the most affected by the Russian full-scale invasion in 2022, while the share of Ukraine's territory affected by occupation, mining, and hostilities in the total area of the country was 31.74% (Nykolyuk *et al.*, 2024). Total losses from soil disturbance are currently estimated at over UAH 980 billion. During a full-scale invasion, many toxic substances, including heavy metals (plumbum, cadmium, aluminium, copper, cobalt), are released into the soil as a result of military equipment, explosions, and combustion. Explosive substances also enter the ground: 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, nitroglycerin, nitrocellulose, 2,4-dinitrotoluene, and various perchlorate compounds used in rocket and gunpowder (Zaitsev *et al.*, 2022). The explosion of a single ballistic missile produces more than 60 kg of toxic poisonous substances that enter the soil. I. Greaves & P. Hunt (2022) reported that the harmful emissions usually include carbon monoxide, nitrogen dioxide, heavy metals, and solid rocket fuel residues.

The use of phosphorus bombs produces phosphoric acids, phosphine, and white phosphorus particles wrapped in oxides that can persist in the soil for years. These toxic substances interact with soil organic matter and its absorption complex, on which fertility depends most of all. The transportation and storage of large quantities of fuels and lubricants can be added to the list of negative military impacts, which become an environmental problem due to large fuel spills that often occur in the middle of agricultural land and cover large areas (Tauqueer *et al.*, 2021). Against the backdrop of anthropogenic factors, global climate change has been added, which acts as a stressor in plant growth and development and prevents high and quality yields. Pollution by toxic substances released into the soil, disruption of genetic horizons after explosions, and changes in organic matter content have a repressive effect on microbiocenosis, which raises reasonable concerns about possible disruptions to ecological balance in agroecosystems (Wang *et al.*, 2024).

Soil functioning is ensured by the interaction of three components: physical, chemical, and biological. As biological components, microorganisms play a vital role in maintaining soil fertility and stabilising biological activity. Soil microbial communities perform crucial ecosystem functions such as regulatory, maintenance, and biosynthetic (Ortiz & Sansinenea, 2022). Managing these functions is a significant biotechnological challenge in modern agrocenoses. Especially significant are the functions of microbial cenoses in soils affected by military operations in Ukraine. X. Wang *et al*. (2024) noted that the bacterial biomass of soil in temperate meadows reaches 1-2 t/ha. The study of the microbiome showed that 1 g of soil contains about 1,000 thsd bp of microbial genomic sequences. The soil of agrocenoses is one of the most diverse anthropogenic environments.

Research was conducted at the D.K. Zabolotnyi Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine to investigate the functioning of microbiocenoses of various soil types, microbial biodiversity, and to identify microorganisms that are indicators of soil ecological status. The study showed the role of agronomically useful rhizosphere and phytosphere bacteria in biological nitrogen fixation, transformation of hardly soluble soil phosphates into a form accessible to plants, provision of plants with biologically active compounds and phytoprotective effect. According to M. Dimova *et al*. (2020), this helps to improve the conditions for plant growth and development, increase their immunity, and improve the quality of agricultural products. According to the findings of F.W. Leal *et al*. (2024), the current problem is to overcome the agrarian crisis by restoring soils damaged by military actions, destroying toxic substances, restoring the biological potential of the native microbiota, increasing plant resistance to adverse biotic and abiotic factors through the biosynthetic potential of microorganisms.

To assess the damage caused to soils and carry out a set of remediation measures, it is necessary to determine the environmental indicators and bioactivity of soils. As of 2024, no comprehensive scientific approaches have been developed to monitor the impact of adverse military factors on the biological properties of soils, including microbiocenosis, nor has the list of indicators to be studied been determined or the relevant methods described. The lack of assessment of the environmental damage and its toxic consequences determined the purpose of the study – to substantiate and systematise biodiagnostic indicators for characterising the condition and biological activity of soils and to develop scientific approaches to bioremediation of land damaged by hostilities. To this end, it was necessary to determine the number of the key agronomically useful groups of microorganisms in damaged soils, calculate the ratio between the main ecological and functional groups of microorganisms, develop bioremediation measures based on the latest microbial biotechnologies and determine their effectiveness.

MATERIALS AND METHODS

Soil sampling for the study was performed according to DSTU GOST 17.4.4.02:2019 (2019). The location of the source of contamination/damage/explosion, test sites, and sampling sites were marked on a map or plan of the damage. For microbiological analysis, 10-20 spot samples were taken from one test site at a depth of 0-20 cm, mixed and formed into a pooled sample, packed in cooler bags and submitted for analysis, and soil samples were stored at 4°C for no more than 24 hours. Samples of military-affected soil from Chernihiv region were examined: soil from a crater after a 250 kg bomb explosion; and a control of undamaged soil from the same location outside the affected area.

Soil from the crater of an exploded anti-tank shell near a burnt-out tank and a control of undamaged soil from this location outside the affected area. The count of microorganisms of the main ecological and functional groups in the studied samples was determined by conventional methods of sowing soil suspension on agarified nutrient media: ammonification – on meat-peptone agar, pedotrophic – on agar with soil extract, amylolytic – on starch-ammonia agar, phosphate-mobilising – on Naum's medium with phenolphthalein sodium phosphate, nitrogen-fixing – on Ashby's selective medium, and oligotrophic – on starvation agar. The counts of microorganisms in the soil were expressed in colony forming units per 1 g of absolutely dry soil (CFU/1 g), considering its moisture content in the initial samples. The replication of the analyses was triplicate. To determine the focus of microbial processes in the soil, the ratio of the number of individual groups of microorganisms (m.o.) – indices of pedotrophicity (PI), oligotrophicity (OI), and mineralisation/immobilisation (MII) – was calculated as follows: PI = number of pedotrophic m.o./number of ammonifying m.o.; PI > 1 indicated soil recovery in the presence of organic matter. The PI > 6 indicated that the soil quality was approaching virgin lands, OI = number of oligotrophic m.o./number of amylolytic m.o. OI > 1 indicated unfavourable degradation processes in the soil, MII = number of amylolytic m.o./number of ammonifying m.o. An MII > 1 indicates the predominance of mineralisation processes over the immobilisation of soil organic matter.

To develop the key approaches to bioremediation of the affected soils, laboratory experiments were conducted with the introduction of microbial biomass and culture fluid of microorganisms into the soil, i.e., bioaugmentation of soil samples with biological products: a complex biological preparation (BP 1) based on microorganisms – destructors of toxic substances: *Dietzia maris* IMV B-7350, *Rhodococcus erythropolis* IMV B-7351, *Bacilus subtilis* IMV B-7349, *Pseudomonas aureofaciens* IMV B-7558 and a biological preparation (BP 2) based on the biomass and culture fluid of *Streptomyces violaceus* strain IMV Ac-5027, which is characterised by high phosphate-mobilising and cellulosolytic activity and antagonistic effect against phytopathogens (Ukraine Patent No. 107240, 2006; Iutynska, *et al.*, 2017). Both biological preparations were mixed in the following volumes: 1-2 l of BP 1 diluted in water and 0.5-1 l of BP 2 per 1 ha. The microbial preparations were used to stimulate soil microbiota and activate the destruction of chemical pollutants, after which the microbiological characteristics and the level of soil damage were reassessed.

As the most sensitive microorganisms to stress factors, it was proposed to use the number of nitrogen-fixing, phosphate-mobilising bacteria, and streptomycetes as bioindicators. It was proposed to determine the level of damage (repression, R) of the soil microbiota by military actions (toxic substances, thermal explosion, etc.) by the percentage of the above groups of microorganisms in relation to the control undamaged soil according to Equation 1:

$$R = B : O \times 100\%, \qquad (1)$$

where *P* is the level of repression of the bioindicator group of microorganisms; *B* is the number of microorganisms of the bioindicator group from the affected area; *O* is the number of microorganisms of the bioindicator group outside the affected area, control. That is, the lower the percentage of diagnostic groups of microorganisms in the soil, the higher the level of damage. Accordingly, the levels of damage gradation were used and proposed (Table 1).

Table 1. Gradation of soil damage levels

Damage level	Repression coefficient value	Repression level characteristics
I	under 20%	Critical damage
II	21-30%	High damage
III	31-40%	Average damage
IV	41-70%	Weak damage
V	over 71%	Fluctuations in the number of microorganisms within statistically acceptable limits

Source: created by the authors

The distribution of soil microbiota damage levels by gradations allows determining both the condition of the original samples of the affected soil and the effectiveness of remediation measures. The comparative method was applied to determine changes in the microbial communities of the affected soils after remediation.

RESULTS AND DISCUSSION

Explosives are known to adversely affect soil microbiota. The widespread use of 2,4,6-trinitrotoluene, the primary component of explosives, pollutes the environment and causes severe harm to human health. For example, TNT in soils inhibits nitrogen fixation, dehydrogenase, and other microbial activity even at low levels of contamination of 10 mg/g (Lamba *et al.*, 2024). It was found that the impact of shell explosions had a considerable negative effect on the counts of microorganisms of the main ecological and functional groups (Table 2).

Table 2. Counts of microorganisms in the soil damaged after explosions and after bioremediation, mn CFU/1 g of absolutely dry soil

Experimental variant	Ammonifying	Pedotrophic	Nitrogen-fixing	Phosphate-mobilising	Oligotrophic	Amylolytic	
						Total	Incl. streptomycetes
Soil from a bomb crater							
Source sample	12.4 ± 1.2	74.6 ± 2.9	3.8 ± 0.7	2.6 ± 0.5	6.4 ± 0.8	6.5 ± 0.8	1.3 ± 0.4
After bioremediation	100 ± 3.3	154 ± 4.3	68.8 ± 2.8	37.8 ± 2.1	67 ± 2.7	118.4 ± 3.6	15.6 ± 1.3
Control intact	14.6 ± 1.2	42.7 ± 2.2	47.4 ± 2.2	2.9 ± 0.6	33.3 ± 1.9	27.5 ± 1.7	19.8 ± 1.5
Soil from an exploded ordnance crater							
Source sample	3.5 ± 0.6	17.0 ± 1.4	2.0 ± 0.3	0.8 ± 0.2	1.2 ± 0.4	0.4 ± 0.2	0.19 ± 0.1
After bioremediation	188.5 ± 4.6	28.5 ± 1.8	41.0 ± 2.1	25.0 ± 1.7	38.4 ± 2.1	32.4 ± 1.8	11.2 ± 1.1
Control intact	14.6 ± 1.3	42.7 ± 2.2	47.4 ± 2.2	9.9 ± 1.0	33.3 ± 1.9	27.5 ± 1.7	19.8 ± 1.4

Source: compiled by the authors

In the soil of the bomb crater, the count of bacteria decreased and was as follows relative to the control: nitrogen-fixing bacteria and streptomycetes, 7% and 8%, respectively, oligotrophic and amylolytic bacteria, 19.2% and 23.6%; phosphate-mobilising bacteria, 26%; the number of pedotrophic and ammonifying microorganisms changed insignificantly. In the soil affected by the explosion of an anti-tank shell, a catastrophic inhibition of the development of amylolytic, phosphate-mobilising, nitrogen-fixing, oligotrophic bacteria, and streptomycetes was recorded; their number was 1.5-10% of the control variant. The number of ammonifying and pedotrophic microorganisms, which was 23.9% and 39.8% of the control values, decreased. According to the findings of the study, the data obtained revealed that the sensitivity of microorganisms to these factors and their ecological functions in the soil were considered to determine bioindicator groups that characterise the impact of explosives. Nitrogen-fixing, phosphate-mobilising bacteria, and streptomycetes were the most sensitive to these stress factors, which shows the sensitivity of these microorganisms to chemical polythenes and high-temperature combustion.

It was argued that nitrogen-fixing bacteria play a major role in optimising the nitrogen regime of soils due to their ability to fix biological nitrogen by binding molecular atmospheric nitrogen into ammonium compounds available to plants (Sharma *et al.*, 2023). Nitrogen is an essential chemical element responsible for photosynthesis and chlorophyll content, synthesis of plastic substances, and overall crop productivity. It was noted that phosphate-mobilising bacteria are a significant ecological and functional group, capable of decomposing hard-to-reach organophosphates using phosphatases. Inorganic phosphates, through the production of organic acids and their conversion into plant-absorbable forms of phosphorus, mobilised it by releasing phosphorus from insoluble and fixed forms in the soil. This resulted in increased availability for plants, changes in the pH of the environment, and the production of chelating substances (Ammar *et al.*, 2023).

It was found that amylolytic microorganisms perform hydrolytic decomposition of carbohydrate components (oligo-, polysaccharides, and starch) contained in plant residues and soil organic matter. These processes result in the transformation of residual carbohydrates, which are subsequently involved in the formation of the aliphatic part of the molecules of humus and humic compounds, thereby increasing soil fertility (Putri & Nakagawa, 2020). Among amylolytic agents, it is important to determine the count of streptomycetes, as this is a common group of mycelial bacteria that perform active functions in the mineralisation and transformation of organic matter due to their ability to synthesise a wide range of enzymes and other biologically active compounds, and play a vital role in the formation of productive microbial-plant systems and increase soil suppression of phytopathogens (Loboda *et al.*, 2024).

According to Table 1, the level of damage to microorganisms of bioindicator groups in the soil of the bomb crater averaged 13.7%; in the soil from the exploded munition crater – 7.3%. These values belonged to the 1st critical level of damage, which indicated an urgent need for reclamation measures. Earlier, the effectiveness of the destruction of toxic organochlorine compounds and the cleanup of contaminated soils using selected microorganisms-destructors was shown (Levchuk *et al.,* 2022). Microorganisms can decompose explosives such as trinitrotoluene. For the bioremediation of the affected soil, microbial preparations based on *D. maris* IMV B-7350, *R. erythropolis* IMV B-7351, *B. subtilis* IMV B-7349, *P. aureofaciens* IMV B-7558, and a metabolic biopreparation based on *S. violaceus* IMV Ac-5027 were used in the samples.

6 months after the application of the biological products, the soil microbiota was repeatedly analysed, focusing on the counts of biodiagnostic groups of microorganisms, according to Table 2. In the soil from the bomb crater, the number of phosphate-mobilising and nitrogen-fixing microorganisms increased and was 1.5-3.8 times greater than in the undamaged control. The number of streptomycetes also increased by 12 times compared to the damaged soil and amounted to 79% of the number in the undamaged control sample. In the soil from the unexploded ordnance crater after bioaugmentation, the number of phosphate-mobilising microorganisms exceeded the value in the undamaged control sample by 2.5 times, the number of streptomycetes and nitrogen fixers increased by 5.9 and 20.5 times, respectively, compared to the damaged soil, and amounted to 57% and 86% of the undamaged control. The study showed that as a result of the application of biological products, the samples can be classified as slightly toxic or non-toxic. The calculation of ecological and physiological indices helped to determine the focus of biochemical processes in the soil and the activity of the microbiota involved in the overall process of decomposition-synthesis of organic matter (Table 3).

Table 3. Ecological indices of microbial communities

Experiment variant	Pedotrophicity index	Oligotrophicity index	Mineralisation-immobilisation index
Soil from a bomb crater			
Source sample	6.0	1.0	0.52
After bioremediation	1.5	0.6	1.18
Control intact	2.9	1.2	2.0
Soil from an exploded ordnance crater			
Source sample	4.9	3.0	0.1
After bioremediation	0.2	1.2	0.2
Control intact	2.9	1.2	2.0

Source: calculated by the authors

The pedotrophicity index shows the activity of transformation of water-soluble humus fractions. The oligotrophicity index shows the activity of the oligotrophic part of the soil microbiocenosis; an increase in this index may generally indicate a slowdown in the destruction of organic matter and a decrease in the content of nutrients in the soil. The mineralisation-immobilisation index shows how actively the processes of decomposition of soil organic matter are taking place (increase in the coefficient) or, conversely, the incorporation of organic matter into biological assimilation and a decrease in its mobility (decrease in the coefficient) (Demyanyuk *et* *al*., 2020). Under the conditions of this experiment, the initial samples of the damaged soil were characterised by the highest pedotrophicity indices, which, combined with high oligotrophicity indices, showed the danger of humus compounds destruction. After bioremediation, both indices decreased simultaneously with an increase in the immobilisation index, which indirectly indicated positive changes in the focus of biochemical processes involved in the preservation of soil organic matter. The positive effect of bioaugmentation can also be explained by the fact that the use of microbial preparations, apart from introducing beneficial microorganisms into the soil, provides it with biologically active substances synthesised by introduced producers, which activates the native microbiota.

The experience of the First and Second World Wars, the hostilities in Vietnam and Myanmar, and the civil war in Yugoslavia showed that military operations cause severe damage to soils, disturbing the topsoil and subsoil layers, and polluting with toxic heavy metals and explosives (Appau *et al*., 2021; Shukla *et al*., 2023). The self-restoration of damaged soils is extremely slow, and even today, European soils where hostilities were fought in the First World War contain toxic heavy metals in levels hundreds of times greater than the permissible levels. Thus, there is a necessity for bioremediation measures. This is especially true for Ukraine's fertile soils. Analysts emphasise that the Russian-Ukrainian war has far-reaching adverse effects on the interconnected systems of food production, energy supply, and the overall ecosystem (Chowdhury *et al*., 2023).

The use of microorganisms to clean soils from heavy metals, pesticides, and industrial pollution is an effective and environmentally safe measure, since the relevant microorganisms present in contaminated soils are resistant to and capable of degrading pollutants (Tufail *et al*., 2022, Sarker *et al*., 2023). This approach is quite reasonable and effective, which has been proven by the findings of studies on the isolation of active organochlorine degraders from a toxic waste landfill near the city of Kalush. Microbial bioremediation using selected active microbial degraders has been successfully used to remediate soil from pesticides, organic contaminants, explosives, and radioactive residues (Gupta *et al*., 2020).

The successful use of microorganisms for explosives remediation is known, specifically the use of micromycetes for mycoremediation. The biotransformation of explosives in soil by the fungus *Phanerochaete chrysosporium* near an army ammunition depot (USA, Oregon) was effective, and strains of *Mucor* sp. T1-1 and *Aspergillus niger* N2-2 degraded trinitrotoluene by 70-100% in dark and red soils (USA, Georgia, USA) (Geris *et al*., 2024). However, micromycetes require the development of special biotechnologies for cultivation under industrial conditions to produce the necessary volumes of biomass sufficient for practical use. Furthermore, some species of aspergillus fungi recommended for remediation may not meet toxicological and hygienic requirements, as bacterial strains are more promising.

Among prokaryotic microorganisms, particularly non-spore-forming bacteria, explosive destructors are also being searched for to bioremediate militarily disturbed soils. For example, 41 strains of bacteria, 14 strains of microscopic fungi, and 3 strains of yeast were isolated from soil from explosive testing sites, and their ability to degrade TNT was established in experiments where degrading microorganisms used these toxicants as the sole source of carbon (C) and nitrogen (N) (Kvesitadze & Khatisashvili, 2023). These microorganisms were characterised by the presence of a highly active enzyme, nitroreductase, which catalyses the primary conversion reactions of TNT and hexogen, reducing their toxicity and increasing the water solubility of explosives (Tauqueer *et al*., 2021). The experiments of these researchers showed that the native soil microflora is capable of decomposing TNT by 30% under optimum humidity and temperature conditions, while the introduction of microbial degraders further increases the level of destruction by another 25-30%. The greatest effect was achieved with the introduction of *Pseudomonas* sp. TNT-44 and *Rhodococcus* sp. TNT-74 strains into the soil, which contributed to the 70-75% remediation. However, the researchers do not provide data on how the state of the soil microbiota changed under the influence of these measures. The complete mineralisation of explosives, primarily TNT, through bacterial degradation as an effective method of remediation of contaminated areas is of great research interest. Biological treatment of contaminated soil is an effective and economical method of complete mineralisation of TNT and reduction of the total amount of intermediate products formed at the following stages (Serrano-González *et al*., 2018; Xu *et al*., 2021). Several bioremediation technologies are used to remove or stabilise contaminants from a contaminated environment by applying bioremediation at the site of contamination – so-called *in situ* bioremediation – or by transferring contaminated material (e.g., soil) to a remote treatment facility – *ex situ* methodology. *In situ* processes include biosparging, biostimulation, bioaugmentation, bioventilation, natural attenuation, bioleaching, bioremediation, phytoremediation, while *ex situ* methods include bioburial, composting, agriculture, biofilters, and bioreactors. Among the above methods, according to the findings of the present study, the most affordable and low-cost is bioaugmentation, which is characterised by the use of microorganisms that are environmentally friendly, have pronounced biodegrading activity, are resistant to high concentrations of pollutant, and do not have undesirable side effects (Bala *et al*., 2022).

The bioaugmentation of soils contaminated with polychlorinated dibenzo-p-dioxins and dibenzofurans using the micromycetes *Stropharia rugosoannulata* or *Phanerochaete velutina* can reach 62-64% of the initial concentration of pollutants (Kvesitadze & Khatisashvili, 2023). Thus, microorganisms are considered as one of the biological agents that perform remediation (Bala *et al*., 2022). However, despite the general approaches, the use of bioaugmentation requires correction considering the specifics and degree of contamination, characteristics of the native microbiota, and soil and climatic conditions. That is why this study was conducted, which showed the positive effect of the bioaugmentation used in the study – the introduction of a consortium of microorganisms that decompose contaminants and reduce their adverse effects on biota: it activated native soil microorganisms and facilitated the purification process. Based on the data obtained, it was argued that the introduced microorganisms initiate degradation processes, which are subsequently performed not only by the introduced strains, but also by the native microbiota. The involvement of soil microbiocenoses in the remediation of contaminated soils has not been sufficiently covered in the literature and requires more extensive research. The effectiveness of the applied biological products in the study was confirmed by the improvement of biodiagnostic indicators of soil biota.

CONCLUSIONS

Considering the extensive destruction of arable land in Ukraine as a result of military impact and the necessity of determining the state of their damage, diagnostic indicators for characterising soil microbiota were substantiated and systematised. Summarising the diagnostics of the state of microbial communities of the soil affected by military actions, the counts of the main ecological and functional groups were determined. In the epicentre of the explosion, compared to relative controls outside the affected area, a sharp decrease in the count of prokaryotes involved in the transformation of nitrogen, phosphorus, and soil organic matter was found. In monitoring the military impact on microbiocenoses, it was proposed to use the number of the most sensitive groups – nitrogen-fixing and phosphate-mobilising bacteria and streptomycetes – as biodiagnostic indicators, and to calculate the indices of mineralisation/immobilisation, oligotrophicity, and pedotrophicity, which characterise the focus of microbial processes. Gradations of five levels of damage (from critical to weak) to the soil microbiota were developed, based on the determination of the repression of each of the bioindicator groups of microorganisms. The prospects of using the bioaugmentation method of biological products based on bacteria capable of degrading toxic substances and increasing the adaptive potential of plants to stress factors were proved. These measures showed a substantial activation of soil microbial communities, which was confirmed by an increase in the count of agronomically useful groups of microorganisms. Based on the findings, it was concluded that the bioremediation measure is effective for restoring microbial communities in the soil damaged by military operations.

The prospect of this study and further case studies is to include microbiological monitoring in the state system of environmental monitoring of militarily disturbed soils. Further development of measures to restore the affected soils involves identifying those microbial agents that form the most active microbial-plant systems in complex biological products, considering the specifics and degree of soil damage, as well as identifying resistant crops for use in integrated phyto-microbial bioremediation. A necessary stage of integrated research is the correction of the soil nutritional regime and its humus state. The body of such research will serve as a basis for developing measures to restore soils damaged by military operations in production conditions.

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CONFLICT OF INTEREST

None.

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Діагностика і біоремедіація ґрунтів, постраждалих внаслідок воєнних дій в Україні

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Анотація. Внаслідок повномасштабного вторгнення значні площі агроекосистем постраждали через пошкодження токсичними речовинами, що зробило актуальним питання відновлення ушкоджених земель шляхом деструкції полютантів. Метою дослідження було обґрунтувати і систематизувати діагностичні показники для характеристики біоактивності ґрунтів та розробити наукові підходи до біоремедіації пошкоджених земель. Використано метод аналізу існуючих підходів для вирішення задач, метод порівняння даних чисельності мікроорганізмів основних еколого-функціональних груп, яку було визначено за методикою посіву ґрунтових суспензій на агаризовані поживні середовища. Розраховано індекси спрямованості мікробних процесів. Біоремедіаційні заходи вносили в ґрунт мікробну біомасу і культуральні рідини: *Dietzia maris* IMB B-7350, *Rhodococcus erythropolis* IMB B-7351, *Bacilus subtilis* IMB B-7349, *Pseudomonas aureofaciens* IMB B-7558 і *Streptomyces violaceus* IMB Ac-5027. Отримані результати дослідження показали, що рівень репресії мікробіоти у ґрунті, постраждалому після вибухів, був надзвичайно високим, порівняно з неушкодженим. Найбільш чутливими були фосфатмобілізувальні, азотфіксувальні, амілолітичні (у т.ч. стрептоміцети) мікроорганізми. У ґрунті з вирви від бомби чисельність азотфіксаторів і стрептоміцетів становила 7 % і 8 % відповідно від контролю, оліготрофних і амілолітичних – 19,2 % і 23,6 %; фосфатмобілізувальних – 26 %. У ґрунті, пошкодженому від вибуху протитанкового снаряду чисельність амілолітиків, фосфатмобілізаторів, азотфіксаторів, оліготрофів і стрептоміцетів становила 1,5 %-10 % від контрольного ґрунту. Через 6 місяців після біоремедіації ґрунту проведено повторний аналіз мікробіоти. У ґрунті із вирви від бомби кількість фосфатмобілізувальних і азотфіксувальних мікроорганізмів зросла і була вищою, ніж у неушкодженому контролі у 1,5-3,8 рази, чисельність стрептоміцетів підвищилась у 12 разів порівняно з ушкодженим ґрунтом. У ґрунті із воронки розірваного боєприпасу кількість фосфатмобілізаторів була у 2,5 рази більшою порівняно з контролем, чисельність стрептоміцетів і азотфіксаторів зросла у 5,9 і 20,5 разів відповідно порівняно з ушкодженим ґрунтом. Після біоремедіаційних заходів, досліджувані зразки ґрунту можна віднести до слаботоксичних, або нетоксичних. Практична цінність роботи полягає в ефективності запропонованого біоремедіаційного підходу, який є важливим для подальшої розробки комплексу заходів з відновлення грунтів в умовах руйнування агроекосистеми України

Ключові слова: мілітарний вплив; пошкодження; відновлення; біопрепарати; біологічна активність; ґрунтова мікробіота

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Edaphic aspect in the ecological structure of the dendroflora in the "Nova Sofiyivka" park

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Abstract. The conflict between the needs of plants and the ecological characteristics of edaphotope directly affects the aesthetics of the landscape. In this connection, the compliance of the needs of woody plants with edaphic conditions was investigated. The purpose of the study was to determine the edaphic component of the ecological structure of the dendroflora of "Nova Sofiyivka" park to predict the potential viability of plant communities. The paper used descriptive, statistical, and comparative research methods. The description of the park's dendroflora was made according to the instructions for the inventory of green spaces in localities of Ukraine. The species composition was determined using plant identification guides, and the relevance of species names was verified according to the current international classification. Ecological analysis of dendroflora was performed based on ecological scales, in which plants were divided into 7 ecological groups depending on the soil moisture regime, moisture variability, aeration, acidity, total salt regime, carbonates and nitrogen content. In the

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dendrology of the park, mesophytes predominate in terms of sensitivity to the soil moisture regime, hemihydrocontrastophobes predominate in terms of variability, hemiaerophobes predominate in terms of air regime, neutrophils predominate in terms of acidity, mesotrophs predominate in terms of salt regime, acarbonatophiles predominate in terms of carbonates content, and nitrophiles predominate in terms of nitrogen content. The survey revealed a rational solution to the problem of providing appropriate soil moisture conditions for plants belonging to the eco-groups of hydrocontrastophobes, hygromesophytes, and hygrophytes. To meet the needs of eco-groups of subacidophiles and acidophiles in low soil acidity, the root-containing soil layer was replaced with a peat mixture with the appropriate pH before planting. The practical significance of the results obtained lies in the possibility of their use by gardening specialists to optimise plantings, considering edaphic conditions that affect the increase in plant resistance and reduce the intensity of plant care. Plants for which planting sites are selected based on their environmental needs are more vital and durable

Keywords: species composition; edaphotope; eco-group; coenosis; urbanised environment

INTRODUCTION

The expansion of urbanised territories leads to disruption of the natural ecosystem by changing the structure and processes of its functioning as a result of anthropogenic activities. In order to at least partially compensate for destructive natural phenomena to reduce negative environmental, socio-economic, and other consequences, integrated green areas are created in the urban environment.

The context of sustainable development of urbanised territories is based on the establishment of residential garden and park landscapes using plant communities formed from highly decorative and sustainable plants (Orlóci & Fekete, 2023). Urban greening is not only aesthetically pleasing, but its role in *ex situ* plant biodiversity conservation strategies is equally important (Ismail *et al*., 2021; Wang *et al*., 2024). The creation of decorative compositions contributes to the introduction and maintenance of valuable collections of woody and herbaceous plants, which leads to the establishment of a unique artificial phytocenosis. As indicated by S. Didenko & O. Shynder (2023), mature artificial phytocenoses are the most resistant to colonisation by undesirable native species and many rare plants are found in such plant communities, which successfully form stable populations. Results of the study by T. Krivomaz *et al*. (2022) indicate that green spaces improve microclimatic conditions in an urbanised environment, enrich the air with oxygen, promote the absorption of excess carbon dioxide, purify dust, and reduce the number of pathogenic microorganisms.

According to J. Konarska *et al*. (2023), woody plants provide numerous ecosystem services, including mitigation of urban heat. However, the cooling effect of plantings is often reduced due to reduced growth of vegetative organs and poor sanitary condition, which is associated with difficult growing conditions in an urbanised environment, which can be compensated by an increase in the area of green areas. Recreational, cognitive, educational, and cultural functions of parks are of great social importance. The study by J. Cubino & J. Retana (2023) concluded that biodiversity and the quantitative composition of plantings have a high level of correlation with human life expectancy. A high level of biodiversity and the saturation of space with plant communities increases human viability. In this regard, increasing the area occupied by green spaces is a promising area for optimising urban space. The species composition of urban flora depends on the bio-ecological characteristics of plants and the microclimatic conditions of green areas (Muzychenko e*t al*., 2020). Environmental monitoring of flora is especially important in conditions of anthropogenically altered landscapes to assess the current state and predict trends in changes in its components in the future (Stakhiv *et al*., 2021; Optasyuk & Hrygorchuk, 2021; Oliferchuk *et al*., 2023).

The stability of the created coenosis can be predicted based on the analysis of the ecological structure of the dendroflora. Ecological analysis determines the ecological and biological features of species and their relationships with the habitat. T. Marucha *et al*. (2023) point out that in the conditions of significant anthropogenic impact on the natural environment, issues related to determining the current state of the vegetation cover of protected areas are of particular relevance, because the development of scientific foundations for rational use of natural resources and protection of the plant world is impossible without a deep study of the flora. When creating new garden and park facilities, it is important to consider the compliance of the range of plants used with the ecotope. Problems of conflict between plant needs and environmental conditions strongly affect the aesthetic condition of plantings. According to N. Melnychuk & Ya. Henyk (2019), climate-forming properties of the main sinusia unifiers should be used for the development of park and forest park phytocoenoses with high aesthetic value and favourable sanitary and hygienic conditions. S. Rogovskyi *et al*. (2023) indicate that the viability of trees, in addition to abiotic factors, is also affected by excessive thickening of the stand and competition between plants for light, moisture, and mineral nutrition elements. Edaphic conditions affect the natural restoration of plant communities. The importance of this statement is highlighted in the paper by T. Murphy *et al*. (2024), where the authors scientifically confirmed the critical importance of soil moisture for plant survival at an early stage of ontogenesis.

Edaphotope directly affects the structure and productivity of plant communities, their stability, durability, and aesthetic appeal. The purpose of the study was to investigate the edaphic components of the ecological structure of the dendroflora in the park under study to assess the potential viability of the site's ecosystems. To achieve this goal, the following tasks were defined: to perform an inventory of dendroflora; to analyse the fastidiousness of woody plants to edaphic environmental conditions; to identify the compliance of the growing conditions of woody plants with their edaphic needs.

MATERIALS AND METHODS

As of 2020, the territory of the city of Uman is 4,787 hectares, of which 1,239.6 hectares are green spaces, including city parks, squares, street greenery, educational institutions, memorial areas, household plots, etc. "Nova Sofiyivka" park is a new recreational facility that was opened to the public on 14 July 2019. The Park is part of the National Dendrological Park "Sofiyivka" of the National Academy of Sciences of Ukraine (Fig. 1).

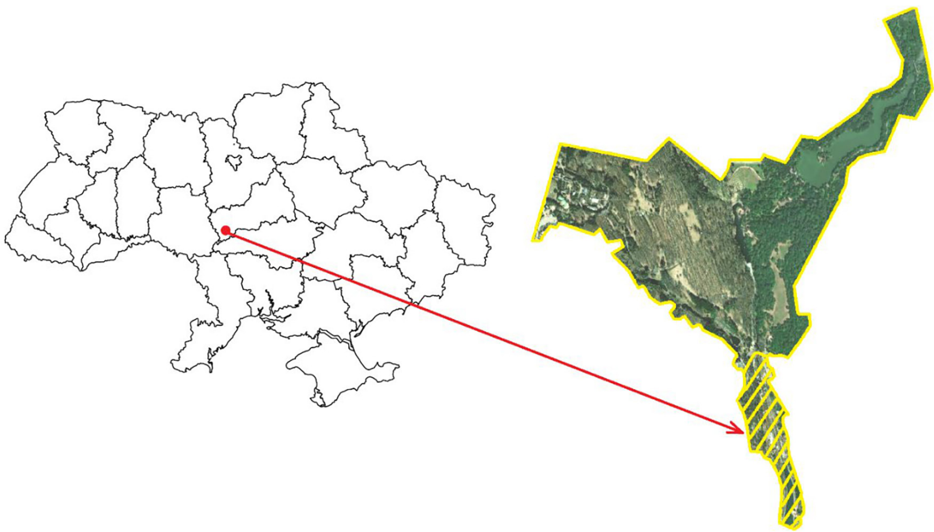

Figure 1. Location of the "Nova Sofiyivka" park
Source: compiled by the authors

All compositions on its territory were created during 2018-2019 on an area cleared of previous vegetation using a modern range of ornamental plants. The park, with an area of 10.7 hectares, was created in the lower reaches of the Kamianka River, outside the historical part of Sofiyivka. The new part is built in an eclectic style, consists of nine separate ideological and planning compositions placed along the riverbed and combined into one complex using an alley and trail network. The study was conducted during 2020-2023. The inventory control of the park's dendroflora was carried out in accordance with the Instructions for the Inventory of Green Spaces in Settlements of Ukraine (Order of the State Committee for Construction, Architecture and Housing Policy of Ukraine No. 226 "On the Approval of the Instructions for the Inventory of Green Spaces for the Inventory of Green Spaces in Settlements of Ukraine", 2001). The preparatory stage included determining the territory to be inventoried, preparing a map scheme for accounting for plantings, developing an inventory plan, and selecting accounting methods. The continuous inventory method was chosen for the study. The actual field survey consisted of establishing the boundaries of the inventoried plots. A visual inspection of each tree plant was carried out, and data on species, age, sanitary condition, height, trunk diameter at a height of 1.3 m, crown diameter, and plant location on a map were recorded. The stage of processing the results for our study included the creation of a summary list of the number of plants, grouping of plants by species composition, summing up the amount and percentage of participation in the plantation.

Species identification was performed using Plants of the World Online (n.d.), and the relevance of species names was checked according to the international classification The Word Flora Online (n.d.). The ecological analysis of dendroflora was performed based on ecological scales developed by Ya.P. Didukh (Didukh, 2011). The park's dendroflora in the edaphotope category was divided into eco-groups according to the ratio of plants to the soil moisture regime, moisture variability, soil aeration, acidity, total salt regime, carbonates and nitrogen content. The paper used a descriptive method that consisted in determining the edaphic conditions optimal for successful growth and development of plants, a statistical method that helped to identify the frequency of occurrence of a species in the structure of dendroflora, and a comparative method that evaluated whether the requirements of plants meet the conditions of edaphotope. The research was carried out in accordance with the current legislation of Ukraine, international standards for editors and authors, recommendations of the Committee on Publication Ethics (COPE) and according to the Ethical Code of a Scientist of Ukraine (Resolution of the National Academy of Sciences of Ukraine No. 2 "On the Ethical Code of a Scientist", 2009). The principles of the UN Convention on Biological Diversity (Convention on Biological Diversity, 1992) were not violated. The paper contains original data provided directly by the authors, who are listed in the authorship of the publication.

RESULTS AND DISCUSSION

The territory of "Nova Sofiyivka" park is dominated by regraded chernozem, which is concentrated on the slopes of ravines, while meadow-bog soil dominates in the lower reaches along the riverbed. Regraded chernozem contains 3.7-4% humus, has a high level of absorbed cations, which contain the most calcium – 21.0-28.0 mg-Eq per 100 g of soil. The content of metabolic magnesium is 4.0-4.7 mg-Eq per 100 g of soil. Content of SiO_2 is approximately 80.0%, Fe_2O_3 – 3.1-3.2%, AL_2O_3 – 10.0-11.0%, CaO – 1.9-2.5%, MgO – 0.9-1.5%, P_2O_5 – 11.0-14.1 mg/100 g, K_2O – 8.6-11.8 mg/100 g. The share of total phosphorus is 0.1-0.15%, potassium – 1.9-2.7%, nitrogen – 0.1-0.2%. The degree of base saturation is high – over 90%. According to the pH of the salt extract, the regraded chernozem is neutral – 5.7-6.4%. Meadow-marsh soil contains 3.0-4.1% humus. The sum of exchange cations varies deep into the profile in the range of 14.2-31.9 mg-Eq per 100 g of soil.

The degree of saturation of the soil absorption complex is about 90%. Content of SiO_2 – 62.7-86.2%, Fe_2O_3 – 1.0-2.6%, AL_2O_3 – 3.2-6.5%, CaO – 3.2-10.5%, MgO – 0.4-0.8%, nitrogen – 0.08-0.30%, P_2O_5 – 1.73-2.52 mg/100 g, K_2O – 0.68-0.85 mg/100 g. The acidity of salt extract is 7.0-7.2%. An inventory of the dendroflora of "Nova Sofiyivka" park revealed that 112 taxa of woody plants were used to form plant compositions. The range of woody plants was selected to create a cultural phytocenosis focused on the dominance of mesophytes – plants adapted to fresh forest and meadow habitats (Fig. 2).

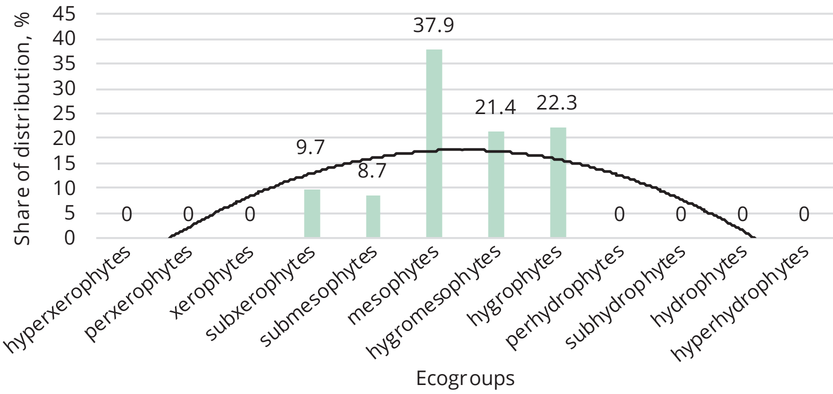

Figure 2. Distribution of dendroflora species in "Nova Sofiyivka" park into eco-groups in relation to the soil moisture regime
Source: compiled by the authors

It was found that the groups of hygromesophytes – plants of moist forest-meadow habitats and hygrophytes adapted to grey-earth forest-meadow ecotopes – occupy almost the same share, differing by less than 1%. Types of subxerophytes characterised by the ability to grow in meadow-steppe conditions with low moisture content and submesophytes – adapted to fairly dry forest-meadow habitats with moderate moisture content of the soil layer, have the smallest share of participation in the coenosis. No other groups were found in the park's dendroflora. The use of dendroflora with such a composition is a rational solution, since the dominance of mesophytes is characteristic of most of the flora of the temperate zone, to which the "Nova Sofiyivka" park geographically belongs. Significant share of hygromesophytes and hygrophytes require waterlogged areas, the requirements of these plant groups are met by taking into consideration the terrain in the selection of planting sites and regular artificial irrigation.

Relation of species to soil moisture conditions has one of the priority values when creating plantings, because an improperly selected range of plants can lead to the collapse of the plantings and disrupt the entire compositional design. The dendroflora of "Nova Sofiyivka" park is dominated by a group of hemihydrocontrastophobes, which are in the central part of the ecological scale (Fig. 3).

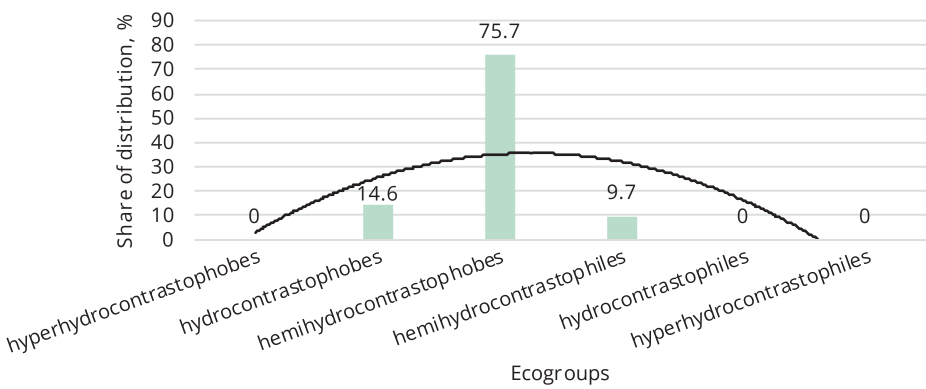

Figure 3. Distribution of dendroflora species in "Nova Sofiyivka" park into eco-groups in relation to changes in humidification conditions
Source: compiled by the authors

Plants of this group need moderate uneven moistening of the root layer of the soil with its complete soaking. The eco-group of hydrocontrastophobes (plants of ecotopes with uniform stable moistening by soil and partially surface waters of the root layer) is significantly inferior in quantity to the previous group. Hemihydrocontrastophiles have the smallest proportion in the composition of dendroflora, they successfully withstand dry forest-meadow and meadow-steppe conditions with uneven moisture of the root-bearing soil layer. Other eco-groups are not represented in the dendroflora.

The fastidiousness of selected woody plants to the stability and intensity of soil moisture is fully satisfied on the territory of the park, especially for hydrocontrastophobes for which low-lying planting sites with surface ground water are selected and an irrigation system is provided. Analysis of the ratio of park dendroflora to soil aeration (Fig. 4) showed the absolute dominance of the group of hemiaerophobes – plants that grow on moderately aerated soils. The location of the polynomial trend indicates the emergence of a range of plants from species that do not require specific wetlands or highly aerated soils. In terms of soil acidity, the park's dendroflora is dominated by neutrophils – species that successfully grow on soils with an acidity of pH = 6.5-7.1 (Fig. 5).

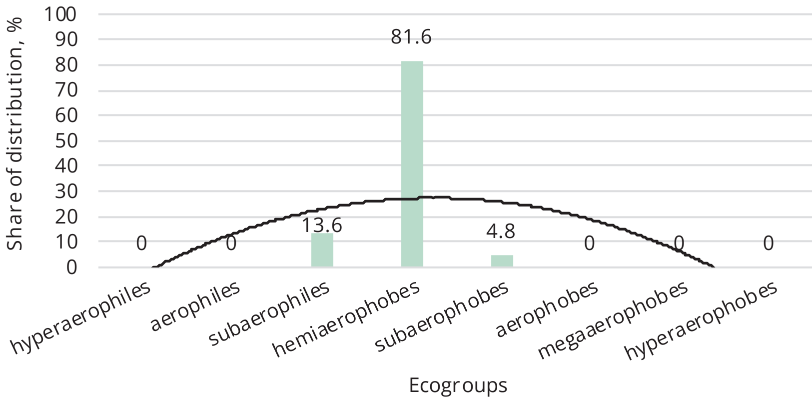

Figure 4. Distribution of dendroflora species in "Nova Sofiyivka" park into eco-groups in relation to soil aeration

Source: compiled by the authors

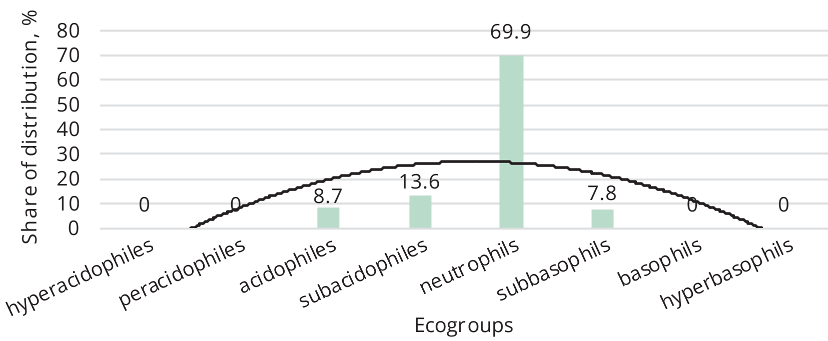

Figure 5. Distribution of dendroflora species in "Nova Sofiyivka" park into eco-groups in relation to soil acidity

Source: compiled by the authors

The predominance of such plants in the selection of the assortment for landscaping facilitates their adaptation to new conditions, as the soils of the region of implementation are neutral. Acidophiles and subacidophiles together occupy a fairly significant share – 22.3%, due to their bioecological characteristics, they require acidic (pH = 4.5-5.5) and slightly acidic (pH = 5.5-6.5) soils. To meet the needs of these plants, the soil was replaced with a peat mixture with the appropriate acidity, which is especially important for growing rhododendrons, hydrangeas, and magnolias. Subbasiphils make up only 7.8%, these species grow best on alkaline soils with pH = 7.2-7.7, but they also withstand neutral conditions quite well and generally do not require replacing the soil mixture and creating special soil conditions. The distribution of species by salt regime revealed absolute dominance of the mesotrophic group in the composition of dendroflora (Fig. 6) that require non-saline sod-podzolic, grey forest and meadow-swamp soils with a washing regime.

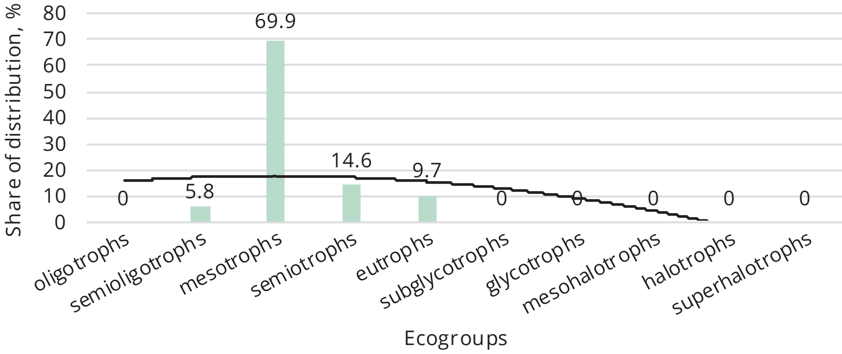

Figure 6. Distribution of dendroflora species in "Nova Sofiyivka" park into ecological groups in relation to the general salt regime of the soil

Source: compiled by the authors

The second place with a share of 14.6% is occupied by a group of semieutrophs growing on dark grey forest soils, podzolic chernozems enriched with salt (150-200 mg/L) with the content of HCO_3^- – 4-16 mg/100 g of soil, and traces of SO_4^- and Cl^- in some types. Such soils are characterised by leaching and podzolisation processes. Groups of eutrophs and semioligotrophs occupy a small share, plants of the first group need soils enriched with salts of upper chernozems, developed sod-podzolic soils with no signs of salinity, the second group can withstand washing poor in salt, strongly leached sod-podzolic and brown forest soils. The deviation of the polynomial trend indicates that there are no species in the range of used plants that require soils with chloride salting, because the soil conditions of the object of greening implementation are not able to meet the requirements of eco-groups of plants placed on the right side of the graph. According to the sensitivity to the content of carbonates in the soil, the park's dendroflora is divided into 7 eco-groups (Fig. 7).

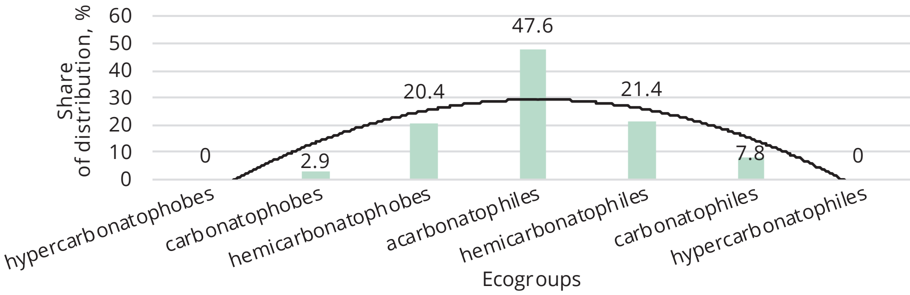

Figure 7. Distribution of dendroflora species in "Nova Sofiyivka" park into ecological groups in relation to the content of carbonates in the soil

Source: compiled by the authors

According to these data, the dominant group of acarbonatophiles requires neutral habitats and is tolerant to a small content of carbonates in the soil (CaO, MgO = 0.5-1.5%), which do not rise to the upper layers due to the washing regime. These are the characteristics of podzolised grey forest soils formed on loessial soils.

The groups hemicarbonatophobes and hemicarbonatophiles differ by 1% and occupy an intermediate position. The first group includes plants that avoid carbonate substrates and grow on podzolic, meadow, and gley soils, while the second group grows on washed – out, sufficiently carbonate-rich chernozems (CaO, MgO = 1.5-5%). The proportion of carbonatophiles (plants that prefer to grow on carbonate soils where CaO, MgO = 5-10%) and carbonatophobes (plants that do not grow on carbonate soils (CaO, MgO = 0.05%), but on soils formed on acidic silicate soils (sands, granites, gneiss, etc.) is not significant and is less than 10%. A slight deviation of the polynomial trend to the right indicates the predominance of carbonatophilic species in the dendroflora, which corresponds to the natural edaphic conditions of the "Nova Sofiyivka" park, which is located on the granite batolite, the main parent rock of most of the territory is a forest rich in calcium carbonates and modern alluvial-deluvial deposits. The content of nitrogen compounds is one of the most important components of soil trophicity, and 3 groups of plants are distinguished in the park's dendroflora by their tolerance to this edaphic factor (Fig. 8).

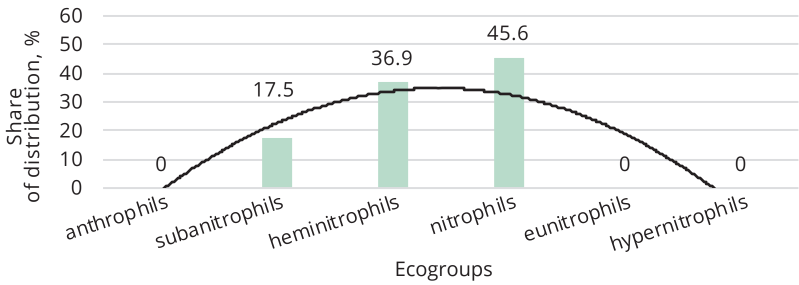

Figure 8. Distribution of dendroflora species in "Nova Sofiyivka" park into ecological groups in relation to the nitrogen content in the soil

Source: compiled by the authors

The leading place belongs to nitrophiles, which need soils sufficiently provided with mineral nitrogen. The second place is occupied by geminitrophils – plants that grow on soils that are moderately provided with mineral nitrogen. The smallest proportion is allocated to subanitrophiles that can withstand oligotrophic soils poor in mineral nitrogen. The detected trend deviation to the left indicates that the range of woody plants can grow successfully in soils with a low content of nitrogen compounds. The detailed analysis of the compliance of the edaphic needs of woody plants with the conditions of the ecotope applied in the study allowed assessing the adequacy of the selection of an assortment of plants for the created landscaping object.

The detailed analysis applied in this paper helped to find out whether the edaphic needs of woody plants correspond to the conditions of the park's ecotope. As indicated by V. Melnyk & N. Denysyuk (2020), to implement the tasks of the modern environmental policy of Ukraine and other legal documents regulating the issues of greening urbanised territories, it is important to conduct research on the taxonomic and ecological structure of dendroflora of parks and squares. The researchers prove that the compliance of growing conditions with the ecological requirements of woody and shrubby plants is the basis for the creation of durable green spaces, so in Rivne, the predominant group in terms of moisture demand is mesophytes, which accounted for 70.0% of all described plants, the percentage of xerophytes, xeromesophytes, and mesohygrophytes varied from 13.0-17.0%, the smallest share was hygrophytes – about 2.0%.

This ratio of ecological groups, according to the researchers, is conditioned by the lack of optimal humidification conditions. Mesophytes are a fairly common ecological group, which is confirmed in the studies by M. Tsybulia (2021) dedicated to the flora of the "Male Polissia" National Nature Park and A. Dzyba (2023), who investigated the ecological classification of dendroflora of parks-monuments of landscape art. The dominance of this group of plants is proved in the descriptions of the flora of the tracts of the Vizhnytskyi National Nature Park by T. Morozova (2024) and the study by A. Lisnichuk & N. Hutsalo (2020) dedicated to the analysis of plant expositions of the Kremenets Botanical Garden. To create the "Nova Sofiyivka" park, preference is given to woody mesophytes adapted to fresh forest habitats with complete moistening of the root layer of the soil by rain and meltwater. The use of mesophytes is a rational solution, because they dominate the flora of the Right-Bank Forest-Steppe of Ukraine (Dayneko, 2020).

During the period of climate change, as noted by T. Hwang *et al*. (2020), it is expected that temperatures will rise and hydrological extremes will increase, despite the fact that warming has some positive effects on plants, contributes to an increase in the duration of the growing season and an increase of CO_2 concentrations in the atmosphere, the increased variability of the hydrological component of the climate will lead to an increase in the duration of dry periods, which can cause mass death of woody plants. For these reasons, the use of drought-resistant plants will allow creating long-lasting decorative park spaces.

The study by O. Ivanchenko & V. Bessonova (2020) emphasised that in the conditions of the central moderately arid agroclimatic region, xerophytes predominate in the humidification regime – 39.0%, mesophytes – 28.5% – are in second place, and hygrophytes – 0.7% – have the smallest share. The expediency of using xeromesophytes is clearly substantiated by T. Boiko *et al.* (2020), who revealed the problems of resistance of woody plants in the conditions of southern Ukraine, and proves the prospects of using this group of plants. The "Nova Sofiyivka" contains two groups of plants resistant to arid conditions – subxerophytes (9.7%) and submesophytes (8.7%), which can later form the basis of park compositions.

Eco-groups of hygrophytes (22.3%) and hygromesophytes (21.4%) have a significant share in the park's dendroflora, but all these plants are located in areas with surface underground water and in conditions of constant artificial irrigation. In general, the park's dendroflora is correctly selected in relation to the soil moisture regime, dominated by the eco-group of mesophytes, which is successfully combined in compositions with subxerophytes and submesophytes. The created plantings are stable in arid conditions, which is quite promising due to the aridisation of the Forest-Steppe (Kalashnikova & Doroshenko, 2020). Soil moisture affects the distribution of woody plants (Davis *et al.*, 2022), is important for drought monitoring and climate modelling (Gallego-Elvira *et al.*, 2019; Li & Sawada, 2022). In terms of tolerance to the variability of soil moisture, the park is dominated by hemihydrocontrastophobes, plants of this ecological group can withstand uneven moisture, which fully corresponds to the weather and climatic conditions of the area of introduction. Most of the park's plants (81.6%) belong to hemiaerophobes, which do not require specific soil air conditions for successful growth and development.

Soil acidity is essential for the absorption of phosphorus, which is an essential element for plant nutrition and affects the growth of vegetative organs. However, M. Wavrek *et al.* (2023) indicate that the availability of this element is limited by pH, it decreases above 7.5 and below 6.5, with the most optimal pH for accumulation considered to be 6.5. The pH values of the park's soils indicate a sufficient proportion of phosphorus for the successful growth and development of cultigenic dendroflora. The level of soil acidity has a significant impact on growth processes, for example O. Didur *et al*. (2023) they prove the existence of a close relationship between pH levels and soil buffering values, that is, the potential of the soil that controls the accumulation and consumption of a certain element of fertility. In terms of soil pH, neutrophils dominate the park – 69.9%, which corresponds to the existing soil acidity. However, acidophiles and subacidophiles have a fairly significant proportion of dendroflora and require completely different soil pH values for successful nutrition and functioning. Non-compliance of acidity indicators with the needs of plants for these groups affects the functioning of the roots, which leads to chlorosis, slowing growth and low viability (Turner *et al.*, 2020). To meet the needs of subacidophilic and acidophilic plants, the soil was replaced with a peat mixture with a pH of 4.5-6.5 before planting.

As noted by Ü. Ateş & D. Demiroğlu (2024), the creation of sustainable green areas is crucial in urban design and is not possible without considering environmental factors and environmental characteristics of the implementation area. The researchers suggest that plants with high environmental tolerance should be used for this purpose. In the study devoted to woody plants in modern and historical parks in Kilis (Turkey), the researchers stressed that almost half (46.8%) of plants in resistant plant communities had a high tolerance to soil salinity. The salt regime directly affects the processes of soil formation and plant adaptation. The basis of the dendroflora of the "Nova Sofiyivka" park was created with the participation of mesotrophs. The selected group of plants prevails in stable dendrocenoses with average soil trophism, which was proved by I. Komarova & E. Yevtushenko (2021), and S. Pototska (2024), so high viability of these woody plants in the park is to be expected. Mineral nitrogen is an integral element of plant nutrition, nitrophils and geminitrophils are the basis of plantings due to the demand for soil nitrogen content in the park, and the edaphic conditions of the park for these groups of plants are adequate and meet their needs. It is important to control the level of nitrogen in the soil, because plant groups in areas with a low nitrogen content are more sensitive to fluctuations in soil moisture than plant groups in areas rich in nitrogen (Shovon *et al.*, 2020).

Due to the fact that "Nova Sofiyivka" park was created on a completely modified site, almost 90% of the territory was subject to complete reconstruction and optimisation, so in the next 10-20 years a number of floral changes should be expected, which are outlined and substantiated in the study by K. Molozhon *et al*. (2023), which indicates that under reconstruction conditions, soil aeration increases, the level of variability in the moisture regime and acidity of edaphotope increases, and the content of carbonates and nitrogen increases. The conditions of the reconstructed object become more suitable for hemihydrocontrastophobes, acarbonatophiles, eunitrophiles, and hemiaerophobes. Based on the analysis of the ecological structure of the dendroflora, it was determined that the edaphic conditions meet the needs of the park's woody plants. Further observations on the adaptation and success of plant growth and development and studies of edaphic indicators that affect the phytoindication assessment will reveal phytocenotic processes in park communities in more detail.

CONCLUSIONS

The ecological structure of the Dendroflora of "Nova Sofiyivka" park was investigated in the context of the edaphic aspect. The flora included 112 taxa of woody plants. In relation to the water regime of the soil, the largest number of taxa belonged to mesophytes, which need complete moistening of the root layer. Plants of this group met the conditions of edaphotope, which simplifies agricultural care techniques. Hygrophytes (22.3%) and hygromesophytes (21.4%) are most vulnerable to moisture deficiency, which led to the selection of low-lying planting sites and additional use of artificial irrigation. Subxerophytes (9.7%) and submesophytes (8.7%) are the most drought-resistant of the existing eco-groups and the most promising in the conditions of climate aridisation. In terms of tolerance to soil moisture variability, hemihydrocontrastophobes dominate (75.7%), which can withstand moderately uneven soil moisture in the park. It was found that hemiaerophobic plants (81.6%), which are fully adapted to the existing moderately aerated soil, are most demanding for soil aeration. The distribution of dendroflora in relation to soil acidity showed a preference for neutrophils (69.9%), which successfully grow on neutral soils of the park. For acid-dependent plants – acidophiles (8.7%) and subacidophiles (13.6%), specific soil conditions have been created (artificial substrate with peat dominance), plants of these groups need constant monitoring of the soil pH level.

The dominance of the mesotrophic group in the dendroflora composition (69.9%) is consistent with the existing soils with average trophic content. The carbonate content in the park's soils fully meets the needs of acarbonatophilic plants (47.6%). Nitrophiles predominate in soil nitrogen (45.6%) – plants are typical for soils sufficiently provided with mineral nitrogen, but a significant proportion of geminitrophiles (36.9%) and subanitrophiles (17.5%) indicates tolerance to soils with a low content of nitrogen compounds, which will positively affect the stability of the plant group. Further study of the viability and mechanisms of plant adaptation to changing conditions of the "Nova Sofiyivka" park's environmental habitat as an object of intensive recreation during the period of climate change will reveal the mechanisms of regulating the balance in the human-plant-soil system. Detailed research is required to investigate changes in the mechanical and biochemical composition of soils depending on the age structure of the dendroflora and the stage of reconstruction of the park.

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CONFLICT OF INTEREST

None.

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Анотація. Конфлікт між потребами рослин та екологічними характеристиками едафотопу безпосередньо впливає на естетику ландшафту. У зв'язку з чим досліджено відповідність потреб деревних рослин едафічним умовам. Мета дослідження передбачала визначення едафічної складової екологічної структури дендрофлори парку «Нова Софіївка» для прогнозування потенційної життєздатності рослинного угрупування. У роботі застосовано описовий, статистичний та порівняльний методи дослідження. Опис дендрофлори парку виконано згідно інструкції з інвентаризації зелених насаджень у населених пунктах України. Видовий склад визначено за допомогою визначників рослин, актуальність видових назв узгоджено відповідно до сучасної міжнародної класифікації. Екологічний аналіз дендрофлори виконано на основі екологічних шкал, у яких рослини було розділено на 7 екогруп залежно від режиму зволоження ґрунту, мінливості зволоження, аерації, кислотності, загального сольового режиму, вмісту карбонатів та азоту. У дендрофлорі парку за вибагливістю до режиму зволоження ґрунту переважають мезофіти, за мінливістю – гемігідроконтрастофоби, за повітряним режимом – геміаерофоби, за кислотністю – нейтрофіли, за сольовим режимом – мезотрофи, за вмістом карбонатів – акарбонатофіли, за вмістом азоту – нітрофіли. Обстеження виявило раціональне вирішення проблеми забезпечення відповідними умовами ґрунтового зволоження рослин, що належать до екогруп гідроконтрастофобів, гігромезофітів та гігрофітів. Для задоволення потреб екогруп субацидофілів та ацидофілів у низькій кислотності ґрунту перед посадкою рослин було застосовано заміну кореневмісного шару ґрунту на торфосуміш з відповідним pH. Практична цінність отриманих результатів полягає у можливості їх використання фахівцями садово-паркового господарства для оптимізації посадок з врахуванням едафічних умов, що впливають на підвищення стійкості рослин та зменшують інтенсивність догляду за насадженнями, адже рослини для яких підібрані місця посадки з врахуванням їх екологічних потреб більш життєві та довговічні

Ключові слова: видовий склад; едафотоп; екогрупа; ценоз; урбанізоване середовище

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