Assessment of heavy metal and pesticide contamination in organic crops using *Allium cepa*

Oleksandr Babych[*]

Master, Assistant
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0002-4271-3629

Lubov Khonenko

PhD in Agricultural Sciences, Associate Professor
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0002-5365-8768

Valentina Gamajunova

Doctor of Agricultural Sciences, Professor
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0002-4151-0299

Ihor Bulba

PhD in Agriculture Sciences, Senior Lecturer
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0009-0004-9545-8475

Anna Salnikova

PhD in Agriculture Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0001-6706-2140

● **Abstract.** Increasing requirements for the environmental safety of agricultural systems necessitate reliable bioindicative methods for assessing the toxicity of soil contaminants to plants. This study aimed to compare the phyto-, cyto-, and genotoxic effects of binary combinations of heavy metals (Cd+Pb, Cu+Zn) and glyphosate using the *Allium cepa* test system. A controlled laboratory experiment with 20 onion bulbs per group, five repetitions, and one control and three experimental treatments was conducted. Morphometric analysis of root growth (root length and fresh weight), visual evaluation of morphological damage, cytogenetic analysis of apical meristem cells (mitotic index and chromosomal aberration frequency and spectrum), and Student's t test were used to assess. EC50 was calculated using nonlinear regression, and compared to maximum permissible concentration standards. The highest phytotoxicity was observed for the Cd+Pb combination, which reduced root growth by 55.1%, followed by Cu+Zn (47.2%), while glyphosate showed the lowest effect (39.0%). Cytogenetic analysis revealed significant inhibition of mitotic activity (54.2%, 38.5%, and 26.3%, respectively) and increased chromosomal aberrations, with heavy metals showing predominantly clastogenic effects and

*Corresponding author

glyphosate characterised by a higher proportion of chromosomal bridges. Experimentally determined EC50 values for Cu, Zn, Pb, and Cd were 3-25 times lower than current maximum permissible concentrations, indicating biologically significant effects at concentrations considered permissible. The results demonstrated the high sensitivity of the *Allium cepa* test system and indicated that existing environmental standards for heavy metals may underestimate risks to plant organisms, particularly in agricultural systems where copper-containing products are permitted

Keywords: phytotoxicity; genotoxicity; cytotoxicity; cytogenetic analysis; apical meristem

Introduction

Against the backdrop of the increasing demands for soil quality and environmental safety in agriculture, the problem of toxic contamination is becoming acute. Human activity leads to the accumulation of persistent heavy metals and pesticides in the soil, which inhibit cellular processes and cause disease. Bioindication, a method of assessing the state of the environment using living organisms, is used to identify these threats. Various biological objects have been used in studies of soil toxicity bioindication. D.S. Gangwar (2024) analysed current trends in the development of biosensor technologies for monitoring pollutants in agricultural systems. The potential of biosensors for the accurate determination of toxic substances, including pesticides, organic pollutants and heavy metals, has been established. The results justified the need to integrate biosensor technologies into environmental monitoring systems for agricultural soils. P. Osyczka *et al.* (2023) investigated the suitability of measuring peroxidation of membrane lipids in *Cladonia rei* lichen as a biomarker for predicting elevated levels of toxic trace elements in soil. It was found that the level of peroxidation did not increase linearly with the pollution index value, indicating the activation of protective mechanisms in lichens. The data obtained confirmed the effectiveness of using physiological indicators of lichens to assess the ecological state of soil ecosystems.

Microbial communities as indicators of soil quality were studied by N. Malik *et al.* (2023), who conducted a comparative analysis of soil toxicity in organic and conventional farm fields. The results showed that alternative soil management practices reduced heavy metal toxicity and supported the microbial population. The differences in microbial composition between different farming systems indicated the sensitivity of microorganisms to soil cultivation methods. The long-term impact of sustainable soil management practices on heavy metal concentrations was assessed by Y. Chen *et al.* (2025). Organically managed soils contained 10.8-73.7% less heavy metals than conventional systems and were characterised by lower geoaccumulation indices. Microbial sequencing revealed increased richness and diversity of bacteria and fungi in organic soils, demonstrating the positive impact of organic practices on soil biota. Vertebrates as bioindicators of the ecological status of agroecosystems were studied by M. Verderame & R. Scudiero (2019), who analysed the health status of the *Podarcis siculus* lizard from agriculturally managed areas. A comparison with populations from non-anthropogenic areas showed that lizards from organic farms had lower levels of

toxic heavy metal accumulation in their tissues. The differences observed confirmed that less intensive agricultural management systems created a less toxic environment for wildlife compared to conventional farming methods.

The variability and succession of microbial communities under conditions of persistent heavy metal pollution were analysed by M. Shuaib *et al.* (2021). It was found that heavy metals (copper, lead, mercury, nickel, cadmium, zinc, and arsenic) caused significant changes in the composition of microbial communities and activated specific mechanisms of microorganism survival. The adaptive processes identified revealed the role of microbes in the biogeochemical cycles of polluted ecosystems and the mechanisms of their tolerance to toxic stress.

The biogeochemical characteristics of heavy metals in the agroecosystems of the Forest-Steppe zone of Ukraine were studied by I. Shumyhai *et al.* (2022). It was found that a significant amount of chemical elements entered the soil with mineral and organic fertilisers, negatively affecting its physical and chemical properties. The results justified the need to control the quality of fertilisers and develop measures to reduce the anthropogenic load on regional agroecosystems. The effectiveness of exogenously applied melatonin in increasing pepper tolerance to chromium stress was investigated by M. Rizwan *et al.* (2024). It was found that melatonin improved photosynthetic parameters and antioxidant enzyme activity in plants under chromium stress conditions. The recorded effects demonstrated the promise of using natural bioregulators to increase plant resistance to the toxic effects of heavy metals in agricultural production. The interaction of heavy metals and pesticides in the soil environment was studied by W. Jiang *et al.* (2021), who analysed the effect of cadmium and lead ions on the enantioselective degradation of α-cypermethrin. The results showed that heavy metals significantly inhibited the degradation of the pesticide, increasing its half-life and suppressing the activity of key soil enzymes.

The combined negative effect of pollutants on biochemical processes in the soil is important for understanding the fate of pesticides in contaminated agroecosystems (Khassanova *et al.*, 2024). The mechanisms of combined soil contamination with heavy metals, microplastics and pesticides were analysed by S. Fang *et al.* (2025). Complex synergistic interactions between pollutants through electrostatic adsorption, surface complexation and physical absorption were identified. The established changes in the bioavailability and toxicity of individual components when

they interact emphasised the need to take into account the multicomponent nature of pollution when assessing environmental risks. A systematic analysis of the toxic effects of heavy metals and pesticides on agricultural soils and plants was carried out by A. Alengebawy *et al.* (2021). The mechanisms of pollutant accumulation in plant tissues and their impact on plant physiological parameters, including photosynthesis, water exchange and growth, were analysed. The summarised data substantiated the need to develop comprehensive strategies for monitoring and reducing the negative impact of pollutants on the productivity and safety of agricultural products.

Despite the achievements, there is a lack of long-term and comprehensive assessments of the impact of pollutants on ecosystems, as well as standardised methods of biodiagnostics. In addition, there is a need for in-depth study of the mechanisms of interaction between different stressors and the development of innovative monitoring technologies. The identified gaps necessitated the current study. The aim of the study was to conduct a comprehensive bioindicative assessment of the toxic effects of different types of pollutants on agroecosystems – binary combinations of heavy metals and glyphosate – on the morphophysiological and cytogenetic in dicators of the test organism *Allium cepa* to establish their relative danger and mechanisms of toxic action.

Materials and Methods

The present work was an experimental laboratory study conducted between September and November 2024. Data collection was carried out under controlled laboratory conditions. *Allium cepa* L. bulbs of the "Stuttgart Riesen" variety were used as biological test objects. *Allium cepa* was selected due to its high sensitivity to genotoxic and cytotoxic effects, large chromosomes ($2n = 16$) facilitating cytogenetic analysis, extensive validation in the scientific literature for assessing heavy metal and pesticide toxicity, simple cultivation protocol, and demonstrated correlation with other biological test systems. The sample was selected deliberately, using clear inclusion criteria: only healthy bulbs of the same size (diameter 3.5 ± 0.5 cm) without mechanical damage, signs of sprouting or disease were used. Bulbs that did not meet these criteria were excluded from the study. The experimental design included one control and three experimental groups, each consisting of 20 bulbs in five replicates. Analytically pure chemical reagents were used for the model solutions: cadmium chloride ($CdCl_2$), lead nitrate ($Pb(NO_3)_2$), copper sulphate ($CuSO_4 \bullet 5H_2O$), zinc sulphate ($ZnSO_4 \bullet 7H_2O$) and a commercial glyphosate preparation (in the form of isopropylamine salt, 360 g/l). Specifically, the Cd + Pb solution contained cadmium and lead at a 1:1 molar ratio, and the Cu + Zn solution contained copper and zinc at a 1:1 molar ratio.

The bulbs were germinated in individual 200 mL glass containers containing 100 mL of the corresponding test solution at a temperature of 22 ± 2 °C and a 12-hour photoperiod. Bulbs were placed with the basal plate facing downward so that only the basal part was immersed (~3-5 mm) and

were fixed at the container mouth using a cardboard/foam support ring (no substrate was used). Aqueous exposure was necessary to achieve the study objectives: direct comparison of toxic effects between pollutants requires identical exposure conditions and precise concentration control, which is unattainable in soil systems where complex sorption, precipitation, and microbial processes create variable and unpredictable bioavailability.

This method enabled accurate determination of EC_{50} values – quantitative parameters essential for comparing experimentally observed toxicity with established regulatory maximum permissible concentration (MPC) standards for soils, including DSanPiN 2.2.7.029-99 (1999) for heavy metals and Regulation of the European Parliament and of the Council No. 396/2005 (2005) for pesticide residues. Furthermore, direct solution contact provided optimal conditions for cytogenetic analysis by ensuring sufficient pollutant uptake to root meristem cells, allowing detection of chromosomal aberrations and differentiation between clastogenic and aneugenic mechanisms. The experimental design prioritized mechanistic understanding and relative toxicity ranking over field simulation, providing fundamental data on intrinsic toxic potency independent of site-specific soil characteristics. For quantitative assessment of phytotoxicity at the macroscopic level, the length of all roots of each bulb was measured using a millimetre ruler and the wet weight of the root bundle was determined on Sartorius CPA225D analytical scales (Germany) (accuracy ±0.1 mg). Based on these data, the phytotoxic effect (PE) was calculated using formula (1):

$$PE = ((L_c - L_e) / L_c) \times 100\%, \qquad (1)$$

where L_c – the average root length in the control group; and L_e – the average root length in the experimental group. A visual analysis of morphological changes was also carried out, recording changes in colour, turgor, and the presence of necrosis and deformations. This comprehensive approach made it possible to obtain data on general growth inhibition and specific external manifestations of toxicity.

Cytogenetic analysis methods were used to elucidate the cellular mechanisms of toxicity. Root tips were fixed in Clark's fixative (ethanol : acetic acid, 3:1), after which temporary pressure preparations were prepared by acid hydrolysis in 1N HCl and staining with 2% aceto-orsenine. The preparations were analysed using a Leica DM500 light microscope (Leica Microsystems, Germany). Cytotoxicity was assessed by calculating the mitotic index (MI) as the proportion of cells in mitosis per 1,000 cells analysed. Genotoxicity was determined by the frequency and spectrum of chromosomal aberrations (bridges, fragments, lagging chromosomes, C-mitoses) in the anaphase and telophase stages, analysing at least 200 anaphases and telophases for each group. These methods allowed to differentiate the effect of pollutants on cell division processes and the integrity of the chromosome apparatus.

The interpretation of the results was based on statistical data processing in the R software environment (version

4.2.1). To compare the mean values between groups, the two-sample Student's *t* test was used, and differences were considered statistically significant at a level of $p < 0.05$. Quantitative assessment of toxicity was performed by determining the effective concentration (EC_{50}). EC_{50} values were estimated by fitting a nonlinear regression, which accounts for the lower and upper asymptotes, slope, and inflection point of the dose-response relationship. The EC_{50} value was derived as the inflection point of the fitted curve corresponding to 50% growth inhibition. The experimentally determined EC_{50} values were compared with the regulatory MPC (DSanPiN 2.2.7.029-99, 1999; Regulation of the European Parliament and of the Council No. 396/2005, 2005) allowed to assess the adequacy of existing environmental standards.

Analysis of macroscopic indicators allowed the level of phytotoxicity of the studied pollutants to be established

and compared. Cytogenetic analysis revealed the cellular mechanisms of this toxicity, differentiating between cytotoxic and genotoxic effects. The calculation of EC_{50} and its comparison with MPC provided a quantitative basis for assessing the environmental risks associated with soil contamination in agricultural systems. Thus, the consistent application of these methods provided a comprehensive bioindicative assessment of the impact of heavy metals and glyphosate on plant organisms.

Results

The effect of heavy metals and glyphosate on the growth processes of the test object *Allium cepa* L. was assessed by analysing morphometric parameters and visual morphological changes in the root system. Quantitative and qualitative results characterising the phytotoxic effect are presented in Table 1.

Table 1. The effect of heavy metals and glyphosate on macroscopic indicators of the root system of *Allium cepa* L

Test group	Average root length, mm ($M \pm SD$)	Phytotoxic effect (*PE*), %	Average root wet weight, mg ($M \pm SD$)	Ratio to control, %	Visual morphological changes
Group 1 (Cd+Pb)	8.12 ± 0.45	55.1	315 ± 25	56.3	Necrosis of tips (dark brown colour), thickening, brittleness, deformities
Group 2 (Cu+Zn)	9.55 ± 0.61	47.2	380 ± 31	67.9	Browning of tips, loss of turgor, curvature, reduction in root hairs
Group 3 (Glyphosate)	11.03 ± 0.52	39.0	442 ± 28	78.9	Retention of white colour, reduction in diameter, general growth inhibition without local necrosis
Control	18.08 ± 1.15	-	560 ± 42	100	Elastic, white roots with a well-developed root hair zone

Note: *M* – mean value; *SD* – standard deviation; *PE* was calculated using the root growth inhibition formula
Source: compiled by the authors

Analysis of the data obtained demonstrates statistically significant ($p < 0.001$) inhibition of growth processes in all experimental groups compared to the control. This confirms the phytotoxic effect of the studied combinations of heavy metals and glyphosate at the given concentrations. The most pronounced inhibitory effect was recorded in Group 1 (Cd + Pb). The average root length in this group was 8.12 ± 0.45 mm, which corresponds to the maximum *PE* calculated by formula (1) at 55.1%. This reduction in length was accompanied by a proportional decrease in the average raw weight of the roots to 315 ± 25 mg, which is only 56.3% of the control value. Qualitative analysis confirmed high toxicity: intense necrotic processes were observed at the tips of the roots (darkening to dark brown), their thickening and increased brittleness, indicating deep structural damage to the tissues.

In Group 2 (Cu + Zn), the phytotoxic effect was slightly lower but remained at a high level (F = 47.2%). The reduction in root length to 9.55 ± 0.61 mm correlated with a decrease in root mass to 380 ± 31 mg (67.9% of the control). The morphological changes in this group were different in nature compared to Group 1: instead of pronounced necrosis, browning of the tips and a noticeable loss of turgor were observed, indicating a disturbance in the water balance of

the cells and the initial stages of damage. A decrease in the number of root hairs was also noted.

Group 3 (Glyphosate) showed the lowest level of phytotoxicity among the experimental groups (*PE* = 39.0%). Growth inhibition to $1,103 \pm 0.52$ mm was statistically significant, but visual damage was minimal. The roots retained their normal white colour and showed no signs of necrosis or deformation. The main effect was manifested in general growth inhibition and a decrease in root diameter, which may indicate a systemic rather than a local mechanism of toxic action directed at the biochemical processes of growth. In the control group, active growth of the root system was observed to an average length of 18.08 ± 1.15 mm. The roots were elastic, white in colour, with a well-developed zone of root hairs, indicating optimal conditions for growth and the absence of toxic stress.

Analysis of the variability of indicators (*SD*) indicates the highest homogeneity of response in Group 1 (*SD* = 0.45), which may be due to the strong and universal inhibitory effect of the Cd+Pb combination. Conversely, the greatest variability in the control group (*SD* = 1.15) reflects the natural variability of biological processes in the absence of stress factors. Thus, the analysis of macroscopic indicators allowed to establish a clear gradation of phytotoxicity of the

studied pollutants: (Cd+Pb) > (Cu+Zn) > Glyphosate. The morphological changes detected indicate different mechanisms of damage. For a deeper understanding of these mechanisms at the cellular level, cytogenetic analysis was performed. To elucidate the cellular mechanisms underlying macroscopic growth inhibition, cytogenetic analysis of apical meristem cells was performed. Detailed results are presented in Table 2.

Table 2. Cytotoxic and genotoxic effects in cells of the apical meristem of *Allium cepa* L

Indicator	Group 1 (Cd+Pb)	Group 2 (Cu+Zn)	Group 3 (Glyphosate)	Control
MI, %	48.5 ± 3.1	65.2 ± 4.0	78.1 ± 3.5	106.0 ± 5.8
MI inhibition, %	54.2	38.5	26.3	-
Total frequency of aberrations, %	12.4 ± 1.5	8.9 ± 1.1	5.1 ± 0.9	1.2 ± 0.4
Spectrum of aberrations, % of total number:				
Chromosome fragments	62.9	58.4	49.0	66.7
Chromosome bridges	37.1	41.6	51.0	33.3
Lagging chromosomes	3.2	2.1	1.5	0.5
C-mitosis	1.8	0.9	0.2	0

Note: several types of aberrations could be observed simultaneously in one cell, therefore the total percentage exceeds 100%
Source: compiled by the authors

The results of cytogenetic analysis revealed disturbances in cell division processes and structural damage to chromosomes in all experimental groups. The cytotoxic effect, assessed by changes in the MI, directly correlates with the data of macroscopic analysis. Group 1 (Cd+Pb) showed the most profound suppression of mitotic activity: the intensity of cell division decreased by more than half (54.2% inhibition) compared to the control. This indicates the cytotoxic effect of the combination of cadmium and lead, which blocks the transition of cells to mitosis or stops it in the early stages. In Group 2 (Cu+Zn), MI inhibition was less pronounced but still significant, reaching 38.5%. Glyphosate (Group 3) showed the least cytotoxic effect, with a 26.3% reduction in MI. Thus, in terms of cytotoxicity, the studied pollutants are ranked in the same order as in terms of phytotoxicity.

The genotoxic effect, characterised by the frequency and spectrum of chromosomal aberrations, also shows a clear dependence on the type of pollutant. The overall frequency of aberrations in Group 1 exceeded the control level by more than 10 times, indicating the high mutagenic potential of the Cd+Pb combination. Analysis of the spectrum of abnormalities in this group showed that almost two-thirds (62.9%) of all aberrations were chromosomal fragments. This dominance indicates a predominantly clastogenic (chromosome-fragile) mechanism of action. In Group 2, the frequency of aberrations exceeded the control by 7.4 times. The spectrum of abnormalities was similar to Group 1, with a predominance of fragments (58.4%), indicating a similar, albeit less intense, clastogenic mechanism of action of the Cu+Zn pair.

Group 3, which includes glyphosate, was identified within the study. Although the overall frequency of aberrations here was the lowest among the experimental groups (4.2 times higher than the control), the spectrum of disorders was different. This group had the highest relative proportion of chromosomal bridges (51.0%), which even exceeded the proportion of fragments. A mechanism of genotoxicity was established, covering not only chromosome breaks but also disturbances in the functioning of the apparatus of their separation. Additional evidence of the specificity of heavy metal action is the detection of C-mitoses, a marker of spindle dysfunction. This type of aberration was most pronounced in the heavy metal groups (Group 1 – 1.8% and Group 2 – 0.9%), while glyphosate showed a minimal level of C-mitoses (0.2%), indicating a significantly higher aneugenic potential of heavy metals compared to glyphosate at the concentrations studied. Thus, microscopic analysis not only confirmed the toxicity gradation established at the macro level, but also revealed differences in the mechanisms of toxic action of the studied pollutants. Heavy metals demonstrate a powerful clastogenic and aneugenic effect, while the genotoxicity of glyphosate is associated with other cellular targets. The results of calculating p values for key macroscopic and microscopic indicators are summarised in Table 3.

Table 3. Summary table of p values for comparison of experimental groups

Comparable groups	Root length	Root weight	MI	Frequency of aberrations
Control vs Group 1	< 0.001	< 0.001	< 0.001	< 0.001
Control vs Group 2	< 0.001	< 0.001	< 0.001	< 0.001
Control vs Group 3	< 0.001	< 0.01	< 0.01	< 0.01
Group 1 vs Group 2	< 0.05	< 0.05	< 0.01	< 0.05
Group 1 vs Group 3	< 0.001	< 0.001	< 0.001	< 0.001
Group 2 vs Group 3	< 0.01	< 0.05	< 0.05	< 0.01

Source: compiled by the authors

The data presented in Table 3 provide quantitative statistical confirmation of the observed effects and allow for an objective assessment of the reliability of the differences identified between the groups. Analysis of *p* values is key to moving from qualitative observations to scientifically sound conclusions. The results of the analysis clearly demonstrate that the impact of all pollutants studied led to statistically significant changes compared to the control group. For Group 1 (Cd+Pb) and Group 2 (Cu+Zn), the highest level of statistical significance ($p < 0.001$) was recorded for all four indicators: root length and mass, MI, and frequency of chromosomal aberrations. Such a low *p* value indicates that the probability of such large differences occurring by chance is extremely low (less than 0.1%). This is evidence of the strong and indisputable phyto- and genotoxic effects of both combinations of heavy metals.

For Group 3 (Glyphosate), the differences from the control are also statistically significant, but with a slightly higher p-value for some parameters. While root length inhibition remains highly significant ($p < 0.001$), the *p* value for root mass, MI and aberration frequency is < 0.01. This is consistent with the data in the previous tables, which showed a less pronounced toxic effect of glyphosate. Nevertheless, a significance level of $p < 0.01$ is still considered highly reliable in biological studies and leaves no doubt about the presence of a toxic effect. A key result that highlights the informative nature of the study is the presence of statistically significant differences not only relative to the control, but also between the experimental groups themselves. This allows not only to state toxicity, but also to compare its level.

A comparison of Group 1 (Cd+Pb) and Group 2 (Cu+Zn) revealed significant differences in all analysed parameters. The most significant difference was observed in the MI ($p < 0.01$), indicating a significantly stronger cytotoxic potential of the Cd+Pb pair. The differences in macroscopic indicators (length, weight) and the frequency of aberrations were also significant ($p < 0.05$). This allows to make a reasonable conclusion that, under the conditions studied, the combination of cadmium and lead is more toxic than the combination of copper and zinc. Similarly, a comparison of both groups with heavy metals (Group 1 and Group 2) with Group 3 (Glyphosate) revealed highly significant differences ($p < 0.01$ or $p < 0.001$) in all indicators. This statistically confirms that the toxicity of both combinations of heavy metals is significantly higher than that of glyphosate. Thus, the results of statistical analysis allow to establish a clear, scientifically based hierarchy of toxicity of the pollutants under study. The toxicity gradation is as follows: (Cd+Pb) > (Cu+Zn) > Glyphosate. The presence of reliable differences between all groups indicates not only different levels of toxicity, but also the high sensitivity and differential ability of the *Allium cepa* test system used, which makes it an effective tool for the comparative assessment of the toxicity of various chemical compounds and their combinations.

To provide a quantitative reference for toxicity assessment and regulatory comparison, EC_{50} were calculated for individual components of the studied pollutants. It should be noted that, except for glyphosate, the experimental exposure was conducted using binary mixtures (Cd+Pb and Cu+Zn). Therefore, the obtained EC_{50} values do not represent the intrinsic toxicity of the experimental mixtures as such, but rather serve as comparative indicators reflecting the relative sensitivity of the *Allium cepa* test system and allowing alignment with existing regulatory MPC. The values shown in Table 4 were compiled by integrating the experimentally obtained EC_{50} values from the present dose-response analysis with the corresponding MPC values extracted from applicable regulatory documents, followed by calculation of the MPC/EC_{50} ratios for comparative assessment.

Table 4. Comparison of experimentally determined EC50 values with regulatory MPC values

Pollutant	MPC of mobile forms in soil, mg/kg	Experimental EC_{50}, mg/l	MPC/EC_{50} ratio
Cadmium (Cd)	0.7	0.45	1.6
Lead (Pb)	6.0	2.10	2.9
Copper (Cu)	3.0	0.12	25.0
Zinc (Zn)	23.0	2.55	9.0
Glyphosate	0.1	0.22	0.45

Source: compiled by the authors based on DSanPiN 2.2.7.029-99 (1999), Regulation of the European Parliament and of the Council No. 396/2005 (2005)

A comparative analysis of experimentally determined toxic concentrations and regulatory indicators revealed a systemic discrepancy for a group of heavy metals. This indicates that biologically significant negative effects at the level of primary producers in the agroecosystem may occur at concentrations that are considered acceptable according to current regulations. The most critical discrepancy, demonstrating the potential inconsistency of existing standards with biological effects, was recorded for copper (Cu). The experimentally determined EC_{50} of 0.12 mg/L was 25 times lower than the MPC standard (3.0 mg/kg). Such a significant discrepancy indicates a potentially high environmental risk associated with copper contamination of soils, which may be underestimated when using a regulatory approach alone. In certain agricultural management systems, copper-containing fungicides are widely used plant protection products, which leads to the gradual accumulation of copper in the soil to concentrations that, although not exceeding the MPC, may have a phytotoxic effect (Shahini *et al.*, 2023).

A similar, albeit less pronounced, trend can be observed for other metals studied. For zinc (Zn), the MPC exceeds the EC_{50} by nine times, and for lead (Pb) by almost three times. This confirms that the problem of non-compliance of standards with biological effects is not unique to copper, but is systemic for the group of heavy metals. For cadmium (Cd), a different relationship was observed. Despite its known high toxicity and one of the lowest MPC standards, the experimentally determined EC_{50} was still 36% lower than the standard. This indicates that even for the most dangerous pollutants, existing standards may not have a sufficient safety factor to protect plant organisms from chronic exposure.

A fundamentally different picture is observed for glyphosate. Its experimental EC_{50} (0.22 mg/l) was 2.2 times higher than the MPC standard adopted in the European Union (EU) countries (0.1 mg/kg). This may indicate that: a) the standard for glyphosate has been set with a significant safety margin specifically for acute phytotoxicity to higher plants; b) the *Allium cepa* test system may be less sensitive to this herbicide than other biological objects (e.g., soil microorganisms, aquatic invertebrates), the risks to which were also taken into account when developing the MPC.

Summarising the data obtained, it can be stated that the results of the study cast reasonable doubt on the adequacy of existing MPC standards for heavy metals, especially for copper and zinc, in the context of protecting plant components of agroecosystems. The identified discrepancies, where biologically significant effects occur at concentrations significantly lower than the permissible limits, justify the need to review and possibly tighten environmental standards, which is particularly relevant for ensuring the environmental sustainability and productivity of agricultural systems.

● Discussion

The current study evaluated the toxicity of heavy metals and glyphosate using morphometric growth indicators and cytogenetic changes in *Allium cepa*. The most toxic effect was observed with Cd+Pb exposure, accompanied by a significant reduction in root length and mass, inhibition of mitotic activity by more than 50%, and a high frequency of chromosomal aberrations, especially fragmentation. A systemic discrepancy between biologically active concentrations of toxicants and current standards was recorded, which makes it necessary to review the MPC in organic production soils. In a study by T. Da Silva Martins *et al.* (2024), soil toxicity was bioindicated using enzyme activity (arylsulfatase, urease) under conditions of prolonged pesticide exposure. It was found that increased concentrations of Cd, Cu, and Zn in soils lead to a decrease in enzyme activity, indicating a decline in soil quality. Although the study confirms the negative impact of heavy metals, the methodology is based not on phytotests but on microbiological indicators. Unlike the results with *Allium cepa*, the authors do not analyse genotoxicity and do not question the current standards. The discrepancy in the conclusions may be explained by the lower sensitivity of microbiological indicators to sublethal concentrations or by a different biological object of analysis.

In the experiment, the cadmium-lead combination caused the most severe morphological changes, including intense necrotic processes at the root tips (darkening to dark brown), their thickening and increased brittleness, indicating profound structural tissue damage and a maximum phytotoxic effect of 55.1%. M. Bożym & J. Rybak (2024) established a completely different toxicity hierarchy using *Lepidium sativum*: Se > As > Hg > Sb > Mo > Cd > Co > Zn > Ni. Lead showed stimulating effects at low concentrations instead of high toxicity, and zinc took the place of cadmium as less toxic, fundamentally contrasting with the results found in the current study on serious phytotoxicity. The discrepancies can be explained by the species-specific sensitivity of different plant test objects and concentration effects (Mustafayeva *et al.*, 2011; Lyubchyk *et al.*, 2019).

In the experiments conducted, glyphosate showed the lowest level of phytotoxicity among all groups, with growth inhibition of up to 11.03 ± 0.52 mm with minimal visual damage. The main effect was manifested in general growth inhibition and a decrease in root diameter without signs of necrosis or deformation, which was interpreted as a systemic mechanism of toxic action directed at the biochemical processes of growth. E. Yalçin & K. Çavuşoğlu (2022) documented severe chromosomal aberrations and genotoxicity in *Allium cepa* at a concentration of 500 mg/L, showing direct interactions between deoxyribonucleic acid (DNA) and glyphosate through spectral analysis. The study found an increase in the formation of micronuclei, chromosome fragments, sticky chromosomes, and bridges, along with severe cell damage, including damage to epidermal and cortical cells and irregular vascular tissue. The discrepancies are explained by the use of different concentrations of glyphosate and different focuses on morphological versus cytogenetic effects.

Cytogenetic analysis showed that in the glyphosate group, the highest relative proportion of chromosomal bridges (51.0%) even exceeded the proportion of fragments, indicating a distinct mechanism of genotoxicity associated not only with chromosome breaks but also with disruption of the apparatus of their separation, unlike the potent clastogenic and aneugenic effects of heavy metals. C. Benbrook *et al.* (2023) conducted a meta-analysis of 94 genotoxicity tests, showing that 73% of studies of technical glyphosate and 95% of studies of glyphosate formulations were positive for genotoxicity. Seven positive *in vivo* human studies reported DNA damage, oxidative stress, and chromosomal aberrations, providing strong evidence of clastogenic and aneugenic effects (Ilderbayeva *et al.*, 2024). This contradicts the conclusion of the current study that glyphosate has no clastogenic potential. In cytogenetic analysis, C-mitoses – a marker of spindle dysfunction – were only observed in the heavy metal groups (Group 1 and Group 2), indicating their aneugenic potential, which is absent in glyphosate at the concentrations studied. R. Mesnage *et al.* (2022) demonstrated

that glyphosate-based herbicides activated DNA damage response pathways using ToxTracker tests, revealing mechanisms of oxidative stress and protein unfolding.

Results of the statistical analysis allowed for the establishment of a clear, scientifically grounded hierarchy of toxicity for the studied pollutants, with the gradation (Cd+Pb) > (Cu+Zn) > Glyphosate. The presence of significant differences between all groups indicated not only different levels of toxicity but also the high sensitivity and differential capability of the *Allium cepa* test system used. V.I. Domínguez-Rodríguez *et al.* (2020) presented a modified Organisation for Economic Co-operation and Development (OECD) protocol using biotests with earthworms, which showed different sensitivity patterns compared to plant tests. For oil drilling waste, soil extracts showed 0% mortality, while direct soil contact showed 100% mortality. However, after treatment, mortality dropped to acceptable OECD ranges (3-13%), indicating that methodological approaches significantly influence conclusions about toxicity. The discrepancies are explained by the different sensitivities of the test organisms and methodological peculiarities.

The study's results demonstrate clear gradations of phytotoxicity for the studied pollutants with a progressive increase in toxic effects, confirmed by statistically significant inhibition of growth processes in all experimental groups compared to the control, with the most pronounced inhibitory effect at the highest concentrations. C. Wei *et al.* (2022) demonstrated clear hormesis dose-effect relationships, where low concentrations of zinc (15-30 mg/L) improved wheat growth and the activity of antioxidant enzymes. The maximum stimulatory response occurred at 15 mg/L for root growth, with improved photosynthetic ability and antioxidant enzyme activity, completely contradicting linear dose-effect models of toxicity. The discrepancies are explained by non-linear biological reactions and the essential nature of some metals at low concentrations.

Glyphosate demonstrated a statistically significant growth inhibition to 11.03 ± 0.52 mm, which was interpreted as evidence of a toxic effect and a potential danger to agroecosystems, especially in contexts where its use is restricted but contamination from neighbouring agricultural plots can occur. M. Hagner *et al.* (2019) found that applying Roundup at maximally allowed doses (3 kg/ha of glyphosate) had only minor and temporary effects on soil fauna and functioning, with no glyphosate residues detected at the end of the experiment. The effects on soil functioning were minimal compared to mechanical weed removal, challenging assumptions about glyphosate's persistence and toxicity. The discrepancies may be explained by different doses, environmental conditions, and exposure times.

The study's results treated all investigated heavy metals exclusively as toxicants that cause damage to plant tissues and inhibit growth processes, without considering the possible positive biological roles of some of these elements at low concentrations. Y. Wan *et al.* (2024) provided comprehensive documentation of over 9,000 hormesis models, showing patterns of low-dose stimulation and high-dose inhibition for trace elements and heavy metals in multiple biological systems, demonstrating that environmental pollutants can act as biological regulators rather than exclusively toxic agents. This changes the understanding of the role of metals in biological systems and the need to consider hormetic effects in toxicological assessments.

In the context of alternative agricultural management practices, attention was focused on external contamination by heavy metals and pesticides, without a detailed consideration of potential sources of contamination that may come from the organic materials themselves used in such farming systems. J.O. Olowoyo & L.L. Mugivhisa (2019) demonstrated that organic materials used in farming can contain toxic pollutants that bioaccumulate in plant tissues. Manure contained varying concentrations of arsenic, cadmium, and lead, with higher concentrations in pig manure compared to cow manure, challenging assumptions about the safety of organic fertilisers regarding heavy metal contamination. The discrepancies are explained by the underestimation of internal sources of contamination in organic systems.

The organic farming system was considered an environment with minimal use of synthetic pesticides, where the main sources of contamination are external or associated with the previous conventional use of land, with the expectation of gradual remediation following conversion to organic methods (Zakharchuk *et al.*, 2019; Shuvar *et al.*, 2022). A. Benzing *et al.* (2025) showed that 21 out of 90 chemicals may potentially leave residues in organic food even after two years of conversion, with food residues linked to residual contamination in soil. The research was based exclusively on the results of bio-testing with *Allium cepa* to establish a hierarchy of pollutant toxicity and draw conclusions about the adequacy of regulatory standards. The high sensitivity and differential ability of this test system were seen as an advantage for environmental assessment. Nevertheless, D. Kim *et al.* (2021) demonstrated that the choice of test species at the screening level of ecological risk assessments is critical, as different species can show opposite reactions to the same substances. This indicates the limitations of conclusions based on a single test organism, even one as sensitive as *Allium cepa*.

The problem identified in the current study is the significant phytotoxic and genotoxic effect of heavy metals (Cd+Pb, Cu+Zn) and glyphosate on the growth and structure of the *Allium cepa* L. root system, manifested by growth inhibition, morphological damage, and cellular aberrations. This points to a high ecological risk of soil contamination, especially considering the discrepancy between toxic concentrations and existing standards. One study, namely P. Ziarati *et al.* (2020), examined the bioadsorption of heavy metals from contaminated soils and water using food and agricultural wastes. This research proposes an alternative approach to limiting soil heavy metal toxicity through immobilisation and reduced plant availability, which does not contradict the phytotoxic effects observed in the present study but opens discussion on potential mitigation strategies. Another study,

V.O. Velychko (2020), analysed physiological and ecological monitoring of xenobiotics, including heavy metals, within the food-livestock system, emphasising the systemic effects of heavy metals on living organisms through food chains. The conclusions stress the importance of controlling the migration of toxic elements and accounting for them across different trophic levels, thereby broadening the context of the present research, which is focused mainly on a plant-based test system.

A.O. Splodytel (2019) focused on the distribution and mobility of heavy metals (Cd, Cu, Pb) in national park territories and their seasonal migration with water flow. The detected exceedances of metal threshold levels in water underscore the environmental danger and confirm the need for monitoring not only soil, as in the current study, but also issues of multi-component detoxification and contamination dynamics. O.V. Shabatura *et al.* (2023) determined the change in the long-term content of Cu, Pb, Zn, Cd, and other elements in the air, which correlates with the influence of industrial and agricultural pollution sources. Such data support the broader ecological context of the current research while offering an alternative, atmospheric bio-indication, that complements the assessment of soil toxicity.

According to the present results, the EC_{50} values for copper (0.12 mg/L), zinc (2.55 mg/L) and other metals showed that biologically significant effects occur at concentrations 3-25 times lower than the current MPCs. This suggests that existing regulatory standards for soil contamination, specifically the MPCs for heavy metals established in national sanitary regulations (DSanPiN 2.2.7.029-99) and their alignment with European regulatory approaches governing permitted agricultural inputs, may underestimate ecological risks. This is particularly relevant for for agricultural management systems in which the use of copper-based fungicides is permitted under European regulatory frameworks, leading to the gradual accumulation of copper in soils at concentrations that do not exceed MPC values but may still exert phytotoxic and genotoxic effects on primary producers (Hutorov *et al.*, 2021; Hussain *et al.*, 2022). In the publication by T. Odunjo & E. Thomas (2021), it was found that in soil samples from organic farms, the content of heavy metals (Pb, Cr, Ni) was predominantly in forms with low bioavailability to plants (reserve and reducible fractions). The authors concluded that risks of metal uptake by plants in organic systems are minimal. This contradicts the findings of the present study, where toxicity was observed even at low concentrations. Possible explanations for these discrepancies include differences in assessment methods (fractional analysis versus phytotesting), the use of different test systems, and variation in soil types.

In the course of the present study, glyphosate was found to suppress *Allium cepa* growth, reduce the MI by 26.3%, and increase the frequency of chromosomal bridges. Although these effects were less pronounced than in the Cd+Pb group, they were statistically significant. The experimental EC_{50} exceeded the regulatory MPC by a factor of 2.2,

which may suggest an overestimation of risk or limited sensitivity of the test object. The work of J.L. Gallego & J. Olivero-Verbel (2021) offered an alternative perspective by studying the cytogenetic toxicity of glyphosate and mixtures of heavy metals in soils from organic and conventional crops. It was found that in organic soils, glyphosate did not reach levels that produced marked toxicity, and pesticide contents were below detection limits. By contrast, conventional samples showed an increased frequency of cytogenetic abnormalities. Thus, unlike the present study, this research confirmed the ecological safety of organic systems with respect to glyphosate, which may be explained by lower actual pesticide concentrations under field conditions.

The conducted study therefore established a clear hierarchy of phyto- and genotoxicity for the pollutants under investigation; however, comparative analysis with other scientific works reveals substantial variability and contextual dependence of such results. The established toxicity ranking is not universal and may shift significantly depending on the choice of test organism, specific interactions between metals (e.g. antagonism), and substance concentration. Interpretation of toxicity is further complicated by nonlinear dose response relationships, particularly hormesis, where low concentrations of some metals may have a stimulatory rather than inhibitory effect. Moreover, conclusions about pollutant mechanisms of action, especially glyphosate, remain contentious, as macroscopic manifestations may not fully reflect the spectrum of cytogenetic damage, while broader meta-analyses point to more complex mechanisms of genotoxicity. Assumptions regarding the ecological purity of certain agricultural management practices are also called into question, since the literature indicates prolonged persistence of pesticides in soil and the possibility of contamination via organic fertilisers themselves. Thus, while the findings obtained are valid within the scope of the present experiment, they should be interpreted in light of these factors, underscoring the need for a comprehensive, multisystem approach in ecotoxicological assessments that accounts for the broader ecological context, including pollutant migration pathways and possibilities for bioremediation.

Conclusions

A comprehensive comparative assessment of the phyto- and genotoxic effects of various types of agroecosystem pollutants was carried out, combining macroscopic morphometric analysis and cytogenetic research. A quantitative hierarchy of toxicity of the studied pollutants was established with statistically significant differences ($p < 0.001$): the cadmium-lead combination caused maximum inhibition of root system growth to 8.12 ± 0.45 mm with a phytotoxic effect of 55.1% and a decrease in mass to 56.3% of the control values, while the copper-zinc pair caused less pronounced inhibition to 9.55 ± 0.61 mm (47.2%), and glyphosate showed the weakest effect with a reduction in length to 11.03 ± 0.52 mm (39.0%). Specific morphological damage was identified for each type of contaminant: dark

brown necrosis and thickening for the cadmium-lead combination, browning of the tips with loss of turgor for the copper-zinc pair, and general inhibition without local necrosis for glyphosate.

Cytogenetic analysis revealed the mechanisms of toxic action at the cellular level through the assessment of the MI and the spectrum of chromosomal aberrations. The most profound inhibition of cell division was recorded in the cadmium-lead group with a decrease in the MI to $48.5 \pm 3.1\%$ (54.2% inhibition) and a tenfold increase in the frequency of aberrations to $12.4 \pm 1.5\%$ compared to the control ($1.2 \pm 0.4\%$). Statistical analysis confirmed the reliability of the differences found both between the experimental groups and the control ($p < 0.001$-0.01) and between the experimental groups themselves ($p < 0.001$-0.05). A predominantly clastogenic mechanism of action of heavy metals was established, with a predominance of chromosomal fragments (62.9% for cadmium-lead and 58.4% for copper-zinc combinations), while glyphosate was characterised by a different spectrum with a prevalence of chromosomal bridges (51.0%). A comparison of experimentally determined EC_{50} values with MPC standards revealed critical discrepancies for heavy metals: copper showed a 25-fold, zinc a 9-fold, and lead a 2.9-fold exceedance of MPCs over biologically significant concentrations. The data obtained justify a review of existing environmental standards for heavy metals in the direction of tightening, especially in the context of agricultural systems. Further research should focus on studying the chronic effects of subtoxic concentrations and synergistic interactions of pollutants in field conditions. A limitation of the study was the use of a single test system, which requires verification on additional model organisms.

✔ Conflict of Interest
None.

✔ References

[1] Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., & Wang, M.-Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, 9(3), article number 42. doi: 10.3390/toxics9030042.

[2] Benbrook, C., Mesnage, R., & Sawyer, W. (2023). Genotoxicity assays published since 2016 shed new light on the oncogenic potential of glyphosate-based herbicides. *Agrochemicals*, 2(1), 47-68. doi: 10.3390/agrochemicals2010005.

[3] Benzing, A., Piepho, H.-P., Malik, W.A., Finckh, M.R., & Fantke, P. (2025). Pesticides in soils as a source of residues in food after two years conversion to organic farming. *Environmental Research*, 279(1), article number 121769. doi: 10.1016/j.envres.2025.121769.

[4] Bożym, M., & Rybak, J. (2024). In vitro chronic phytotoxicity of heavy metals and metalloids to *Lepidium sativum* (garden cress). *Ecotoxicology*, 33(1), 94-103. doi: 10.1007/s10646-024-02729-z.

[5] Chen, Y., Guo, J., Zhao, H., Qu, G., Han, S., & Huang, C. (2025). Assessing two decades of organic farming: Effects on soil heavy metal concentrations and biodiversity for sustainable management. *Sustainability*, 17(15), article number 6817. doi: 10.3390/su17156817.

[6] Da Silva Martins, T., *et al.* (2024). Contamination risk by heavy metals and enzymatic stoichiometry in agricultural soils under intense use of pesticides. *Environmental Monitoring and Assessment,* 196(9), article number 805. doi: 10.1007/s10661-024-12965-9.

[7] Domínguez-Rodríguez, V.I., Adams, R.H., Sánchez-Madrigal, F., Pascual-Chablé, J.D.L.S., & Gómez-Cruz, R. (2020). Soil contact bioassay for rapid determination of acute toxicity with *Eisenia foetida*. *Heliyon*, 6(1), article number e03131. doi: 10.1016/j.heliyon.2019.e03131.

[8] DSanPiN 2.2.7.029-99. (1999). *Hygienic requirements for the management of industrial waste and determination of their hazard class for public health*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=47238.

[9] Fang, S., Hua, C., Yang, J., Liu, F., Wang, L., Wu, D., & Ren, L. (2025). Combined pollution of soil by heavy metals, microplastics, and pesticides: Mechanisms and anthropogenic drivers. *Journal of Hazardous Materials*, 485, article number 136812. doi: 10.1016/j.jhazmat.2024.136812.

[10] Gallego, J.L., & Olivero-Verbel, J. (2021). Cytogenetic toxicity from pesticide and trace element mixtures in soils used for conventional and organic crops of *Allium cepa* L. *Environmental Pollution*, 276, article number 116558. doi: 10.1016/j.envpol.2021.116558.

[11] Gangwar, D.S. (2024). Recent trends in biosensor development for climate smart organic agriculture and their role in environmental sustainability. *Environment, Development and Sustainability*. doi: 10.1007/s10668-024-05300-2.

[12] Hagner, M., Mikola, J., Saloniemi, I., Saikkonen, K., & Helander, M. (2019). Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field. *Scientific Reports*, 9, article number 8540. doi: 10.1038/s41598-019-44988-5.

[13] Hussain, T., Ahmed, S.R., Lahori, A.H., Mierzwa-Hersztek, M., Vambol, V., Khan, A.A., Rafique, L., Wasia, S., Shahid, M.F., & Zengqiang, Z. (2022). In-situ stabilization of potentially toxic elements in two industrial polluted soils ameliorated with rock phosphate-modified biochars. *Environmental Pollution*, 309, article number 119733. doi: 10.1016/j.envpol.2022.119733.

[14] Hutorov, A., Lupenko, Y., Sherstiuk, S., Ponomarenko, Y., Hutorova, O., & Yermolenko, O. (2021). Innovative potential of the agrarian sector of Ukraine: Forming and efficiency of realization. *TEM Journal*, 10(3), 1228-1238. doi: 10.18421/TEM103-29.

[15] Ilderbayeva, G., Rakhyzhanova, S., Utegenova, A., Salkhozhayeva, G., & Ilderbayev, O. (2024). Combined effect of gamma radiation and heavy metals on some living organisms. *Biological Trace Element Research*, 203(3), 1764-1775. doi: 10.1007/s12011-024-04272-8.

[16] Jiang, W., Yao, G., Jing, X., Liu, X., Liu, D., & Zhou, Z. (2021). Effects of Cd^{2+} and Pb^{2+} on enantioselective degradation behavior of α-cypermethrin in soils and their combined effect on activities of soil enzymes. *Environmental Science and Pollution Research*, 28(34), 47099-47106. doi: 10.1007/s11356-021-13929-z.

[17] Khassanova, G., *et al.* (2024). Zinc finger knuckle genes are associated with tolerance to drought and dehydration in chickpea (*Cicer arietinum* L.). *Frontiers in Plant Science*, 15, article number 1354413. doi: 10.3389/fpls.2024.1354413.

[18] Kim, D., *et al.* (2021). Selecting bioassay test species at the screening level of soil ecological risk assessments. *Applied Sciences*, 11(9), article number 4314. doi: 10.3390/app11094314.

[19] Lyubchyk, S., Shapovalova, O., Lygina, O., Oliveira, M.C., Appazov, N., Lyubchyk, A., Charmier, A.J., Lyubchik, S., & Pombeiro, A.J.L. (2019). Integrated green chemical approach to the medicinal plant *Carpobrotus edulis* processing. *Scientific Reports*, 9(1), article number 18171. doi: 10.1038/s41598-019-53817-8.

[20] Malik, N., Gawdiya, S., Kumar, R., & Mishra, V. (2023). Comparative analysis of soil toxicity and microbial content in organic vs conventional farming fields. *Ecology, Environment and Conservation,* 29, 302-305. doi: 10.53550/eec.2023.v29i02s.049.

[21] Mesnage, R., *et al.* (2022). Comparative toxicogenomics of glyphosate and roundup herbicides by mammalian stem cell-based genotoxicity assays and molecular profiling in sprague-dawley rats. *Toxicological Sciences*, 186(1), 83-101. doi: 10.1093/toxsci/kfab143.

[22] Mustafayeva, K., Mahiou-Leddet, V., Suleymanov, T., Kerimov, Y., Ollivier, E., & Elias, R. (2011). Chemical constituents from the roots of Cephalaria kotschyi. *Chemistry of Natural Compounds*, 47(5), 839-842. doi: 10.1007/s10600-011-0079-y.

[23] Odunjo, T., & Thomas, E. (2021). Status of accumulated heavy metals in soils from organic and conventional farms in Akinyele Local Government, Ibadan, Oyo State, Nigeria. *Journal of Agricultural Research and Development,* 20(1), 29-40. doi: 10.4314/jard.v20i1.4.

[24] Olowoyo, J.O., & Mugivhisa, L.L. (2019). Evidence of uptake of different pollutants in plants harvested from soil treated and fertilized with organic materials as source of soil nutrients from developing countries. *Chemical and Biological Technologies in Agriculture*, 6, article number 28. doi: 10.1186/s40538-019-0165-0.

[25] Osyczka, P., Chowaniec, K., & Skubała, K. (2023). Membrane lipid peroxidation in lichens determined by the TBARS assay as a suitable biomarker for the prediction of elevated level of potentially toxic trace elements in soil. *Ecological Indicators*, 146, article number 109910. doi: 10.1016/j.ecolind.2023.109910.

[26] Regulation of the European Parliament and of the Council No. 396/2005 "On Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC Text with EEA Relevance". (2005, February). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32005R0396.

[27] Rizwan, M., Nawaz, A., Irshad, S., & Manoharadas, S. (2024). Exogenously applied melatonin enhanced chromium tolerance in pepper by up-regulating the photosynthetic apparatus and antioxidant machinery. *Horticultural Science*, 323, article number 112468. doi: 10.1016/j.scienta.2023.112468.

[28] Shabatura, O.V., Tyutyunnyk, Y.H., & Blum, O.B. (2023). Biogeochemical lichen indication study of formation and temporal changes of Oleksandriia *Arboretum* atmospheric pollution. *Ukrainian Hydrometeorological Journal*, 32, 115-129. doi: 10.31481/uhmj.32.2023.08.

[29] Shahini, S., Mustafaj, S., Sula, U., Shahini, E., Skura, E., & Sallaku, F. (2023). Biological control of greenhouse whitefly *Trialeurodes vaporariorum* with *Encarsia formosa*: Special case developed in Albania. *Evergreen*, 10(4), 2084-2091. doi: 10.5109/7160868.

[30] Shuaib, M., Azam, N., Bahadur, S., Romman, M., Yu, Q., & Xuexiu, C. (2021). Variation and succession of microbial communities under the conditions of persistent heavy metal and their survival mechanism. *Microbial Pathogenesis*, 150, article number 104713. doi: 10.1016/j.micpath.2020.104713.

[31] Shumyhai, I., Konishchuk, V., & Dushko, P. (2022). Biogeochemical characteristics of heavy metals in agroecosystems of Forest-Steppe of Ukraine. *Agroecological Journal*, 4. doi: 10.33730/2077-4893.4.2022.273256.

[32] Shuvar, I., Korpita, H., Shuvar, A., Shuvar, B., Balkovskyi, V., Kosylovych, H., & Dudar, I. (2022). Relationship of potato yield and factors of influence on the background of herbological protection. *Open Agriculture*, 7(1), 920-925. doi: 10.1515/opag-2022-0153.

[33] Splodytel, A.O. (2019). Landscape and geochemical assessment of ecological condition of environmental protection territories. *Visnyk of V.N. Karazin Kharkiv National University. Series Geology. Geography. Ecology,* 51, 234-242. doi: 10.26565/2410-7360-2019-51-17.

[34] Velychko, V.O. (2020). Physiological and environmental monitoring of technogenic xenobiotics in the food-animal-production system. *Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology*, 21(1), 46-51. doi: 10.36359/scivp.2020-21-1.04.

[35] Verderame, M., & Scudiero, R. (2019). Health status of the lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) subject to different anthropogenic pressures. *Reports. Biologies*, 342(3-4), 81-89. doi: 10.1016/j.crvi.2019.04.001.

[36] Wan, Y., Liu, J., Mai, Y., Hong, Y., Jia, Z., Tian, G., Liu, Y., Liang, H., & Liu, J. (2024). Current advances and future trends of hormesis in disease. *NPJ Aging,* 10, article number 26. doi: 10.1038/s41514-024-00155-3.

[37] Wei, C., Jiao, Q., Agathokleous, E., Liu, H., Li, G., Zhang, J., Fahad, S., & Jiang, Y. (2022). Hormetic effects of zinc on growth and antioxidant defense system of wheat plants. *Science of the Total Environment*, 807(2), article number 150992. doi: 10.1016/j.scitotenv.2021.150992.

[38] Yalçin, E., & Çavuşoğlu, K. (2022). Spectroscopic contribution to glyphosate toxicity profile and the remedial effects of *Momordica charantia*. *Scientific Reports*, 12, article number 20020. doi: 10.1038/s41598-022-24692-7.

[39] Zakharchuk, O.V., Lupenko, Y.O., Hutorov, A.O., & Dorokhov, O.V. (2019). Economics of development of the seed-growing in Ukraine. *Bulletin of the Transilvania University of Brasov Series II Forestry Wood Industry Agricultural Food Engineering*, 12(2), 127-136. doi: 10.31926/but.fwiafe.2019.12.61.2.11.

[40] Ziarati, P., Moradi, D., & Vambol, V. (2020). Bioadsorption of heavy metals from the pharmaceutical effluents, contaminated soils and water by food and agricultural waste: A short review. *Labour Protection Problems in Ukraine*, 36(2), 3-7. doi: 10.36804/nndipbop.36-2.2020.3-7.

Оцінка забруднення сільськогосподарських культур важкими металами та пестицидами з використанням *Allium cepa*

Олександр Бабич

Магістр, асистент
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0002-4271-3629

Любов Хоненко

Кандидат сільськогосподарських наук, доцент
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0002-5365-8768

Валентина Гамаюнова

Доктор сільськогосподарських наук, професор
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0002-4151-0299

Ігор Бульба

Кандидат сільськогосподарських наук, старший викладач
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0009-0004-9545-8475

Анна Салнікова

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0001-6706-2140

Анотація. Зростання вимог до екологічної безпеки аграрних систем зумовлює необхідність застосування надійних біоіндикаторних методів для оцінювання токсичності ґрунтових забруднювачів для рослин. Метою цього дослідження було порівняння фіто-, цито- та генотоксичних ефектів бінарних комбінацій важких металів (Cd+Pb, Cu+Zn) і гліфосату з використанням тест-системи *Allium cepa*. Проведено контрольований лабораторний експеримент із 20 цибулинами в кожній групі, п'ятьма повтореннями, а також однією контрольною та трьома дослідними групами. Для оцінювання застосовано морфометричний аналіз росту коренів (довжина коренів і свіжа маса), візуальну оцінку морфологічних ушкоджень, цитогенетичний аналіз клітин апікальної меристеми (мітотичний індекс і частота та спектр хромосомних аберацій), а також t критерій Стьюдента. Значення EC50 обчислювали за допомогою нелінійної регресії, та порівнювали з нормативами гранично допустимих концентрацій. Найвищу фітотоксичність виявлено для комбінації Cd+Pb, яка зменшувала ріст коренів на 55,1 %, далі – Cu+Zn (47,2 %), тоді як гліфосат проявляв найнижчий ефект (39,0 %). Цитогенетичний аналіз показав суттєве пригнічення мітотичної активності (відповідно 54,2 %, 38,5 % і 26,3 %) та зростання частоти хромосомних аберацій, причому важкі метали переважно проявляли кластогенну дію, а гліфосат характеризувався вищою часткою хромосомних містків. Експериментально визначені значення EC50 для Cu, Zn, Pb і Cd були у 3-25 разів нижчими за чинні гранично допустимі концентрації, що свідчить про біологічно значущі ефекти за концентрацій, які вважаються допустимими. Отримані результати демонструють високу чутливість тест-системи *Allium cepa* та вказують на те, що наявні екологічні нормативи щодо важких металів можуть недооцінювати ризики для рослинних організмів, особливо в аграрних системах, де дозволене застосування мідьвмісних препаратів

Ключові слова: фітотоксичність; генотоксичність; цитотоксичність; цитогенетичний аналіз; апікальна меристема