Communications in Soil Science and Plant Analysis

ISSN: 0010-3624 (Print) 1532-2416 (Online) Journal homepage: www.tandfonline.com/journals/lcss20

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**To cite this article:** Kateryna Gubina , Tamara Gruzina , Olena Nikipelova , Denis Veliksar , Liudmyla Rieznichenko & Svitlana Dybkova (16 Jul 2026): Bioluminescent Test as an Effective Component in Agrochemical and Ecotoxicological Analysis of the Condition and Quality of Agricultural Soils, Communications in Soil Science and Plant Analysis, DOI: 10.1080/00103624.2026.2704180

**To link to this article:** https://doi.org/10.1080/00103624.2026.2704180

Published online: 16 Jul 2026.

COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS
https://doi.org/10.1080/00103624.2026.2704180





# Bioluminescent Test as an Effective Component in Agrochemical and Ecotoxicological Analysis of the Condition and Quality of Agricultural Soils

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**ABSTRACT**

This study evaluates the potential of the bioluminescent test as an effective component in the agrochemical and ecotoxicological assessment of agricultural soils. Thirteen soil samples, collected from different regions of Ukraine and representing different taxonomic soil types belonging to Mollisols and Entisols, were analyzed. The ecotoxicity level (E) was determined using bioluminescent sensor elements based on bacterial strains with natural luminescence (*Vibrio fischeri* F1, *Vibrio harveyi* Ms1, *Photobacterium phosphoreum* B7071) or induced (*Alcaligenes eutrophus* 1239, *Pseudomonas fragi* T2(5)) luminescence. Standard agrochemical characterization was conducted in accordance with the Recommended Chemical Soil Test Procedures for the North Central Region (NCR-221). The concentrations of heavy metals (Zn, Cu, Co, Ni, As, Cd, Hg, Pb, Cr) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Comparative analysis was performed between the results obtained by the bioluminescent test, diethylenetriaminepentaacetic acid (DTPA)-extraction method, and the quantification of heavy metals in aqueous soil extracts using ICP-MS. The results demonstrate that the bioluminescent test reliably reflects the contribution of the bioavailable fraction of toxicants to the overall ecotoxicity level. Thus, it can serve as a valuable component of integrated agrochemical and ecotoxicological diagnostics of soils. The combined application of classical soil analysis methods and bioluminescent tests provides a predictive and integrated framework for comprehensive soil quality and environmental risk assessment.

**ARTICLE HISTORY**
Received 20 November 2025
Accepted 12 July 2026

**KEYWORDS**
Agrochemical assessment; biosensor bioluminescent test; ecotoxicity; heavy metals; soil

## Introduction

Long-term anthropogenic influence has led to pronounced contamination of agricultural soils with various types of toxicants, in particular heavy metals (Rai et al. 2019; Hou et al. 2025). According to the latest data (Hou et al. 2025), up to 17% of agricultural land in the world is contaminated with one or more heavy metals. This has a significant impact on the state of ecosystems, the level of food security, as well as human and animal health, as many pollutants – especially heavy metals – are characterized by cumulative effects, are difficult to remediate, and are highly ecotoxic (Rai et al. 2019; Jomova et al. 2025).

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These considerations make it particularly important to control and monitor the condition and quality of soils in terms of their agrochemical and ecotoxicological parameters. The degree of potential ecotoxic impact of toxicants, in particular heavy metals, is determined by their bioavailability (Olaniran et al. 2013; Jomova et al. 2025). The bioavailability of heavy metals depends on many factors, including the chemical form of the metal, its concentration in the environment, the physical and chemical properties of the environment (pH, organic matter content, etc.), as well as the metabolic characteristics of a particular organism (Olaniran et al. 2013). Therefore, the use of bioassays (ecotoxicological biotests) for assessment of soil quality and the degree of soil contamination is particularly relevant (Wołejko et al. 2022; Albert and Bloem 2023).

The sensitive elements of such bioassays can be biological systems of different levels of organization: individual enzymes (Lee et al. 2020; Wang et al. 2023), microorganisms (Ghannem et al. 2024; Visca et al. 2025), higher plants (Wang and Zhang 2020; Cakaj et al. 2024), and invertebrates of the soil fauna (Santorufo et al. 2012; Ghannem et al. 2024). Within this variety of biological tests used for ecotoxicity monitoring, bacterial cells with natural luminescence attract special attention as sensitive elements (Fernández-Piñas et al. 2014). Such bacteria contain lux genes that encode the synthesis of the luciferase enzyme, which ensures the bioluminescence reaction in the cells of the specified test strains. Upon contact with toxicants, the intensity of luminescence of bacteria with natural luminescence is suppressed. The degree of inhibition correlates with the concentration of the toxicant (Zhang K et al. 2023). One of the most commonly test luminescent strains in ecotoxicity assessment is *Vibrio fischeri* (Abbas et al. 2018). For example, luminescent tests using such bacterial cells to assess water quality are being actively studied (Abbas et al. 2018; Ajuzieogu and Odokuma 2018; Mirjani et al. 2021; Dehghan et al. 2024). The biological test for bioluminescence inhibition of the bacterium *Vibrio fischeri* was first commercialized in 1978 as the Microtox test and has been studied and applied to assess the ecotoxicity of various classes of pollutants such as antibiotics, pesticides, heavy metals and even nanomaterials. Now commercial bioluminescent tests such as Microtox and LumiMARA are used not only for toxicological characterization of water quality, but also for monitoring soil ecotoxicity and for toxicological assessment of waste (Heinlaan et al. 2007; Weltens et al. 2014; Kowalik et al. 2024).

In addition to bacterial cells with natural luminescence, genetically modified strains that induce bioluminescence in response to the presence of certain metal ions in the determination medium attract considerable attention as sensitive elements in the practice of monitoring the ecotoxicity of soil contamination by heavy metals (Charrier et al. 2011; Chen et al. 2023).

The goal of the study was to evaluate the effectiveness and prospects of using the bioluminescent test as a component of the ecological, agrochemical, and ecotoxicological analysis of the state and quality of agricultural soils.

## Materials and methods

Thirteen soil samples from different regions of Ukraine (Odesa, Lviv and Kirovohrad regions) (Figure 1) were analyzed using bioluminescent tests, agrochemical analysis methods and by ICP-MS for the detection of heavy metals content in aqueous soil extracts.

Soil samples (Table 1) were collected according to standard agronomic protocols, ensuring representative sampling over the entire plot area. The depth of soil sampling was 0–25 cm.

### *Analysis of soil samples using bioluminescent test*

Soil samples in the bioluminescent test were characterized by the parameter "ecotoxicity level (*E*)" using sensor elements based on bacterial cells with natural or induced luminescence.

The bioluminescent test used to assess the level of ecotoxicity (E) of soil samples can be schematically represented as follows (Figure 2).


**Figure 1.** Map of Ukraine with soil sampling regions.

The specified method for determining ecotoxicity is based on the registration of changes in the intensity of bioluminescence of bacterial strains – components of the sensor element – under the influence of toxic substances present in water extracts from the analyzed soil samples, compared to the control.

Five sensor elements (SE) based on freeze-dried bioluminescent bacteria ($1{\cdot}10^9$ CFU/ml), prepared in accordance with the technical specifications of Ukraine TSU 21.2-05402714-005:2014 (TSU 2025), were used to analyze soil samples in the bioluminescent test:

SE1 – biosensor element based on the strain *Vibrio fischeri* F1 (natural luminescence). $EC_{50}$ (effective concentration, the concentration of a substance that causes a 50% decrease in bioluminescence of a bacterial suspension $5{\cdot}10^5$ cells/ml within 5 min) for the test strain is: 40 mg/L – under the action of potassium dichromate ($K_2Cr_2O_7$); 4.6 mg/L under the action of zinc sulfate ($ZnSO_4{\cdot}7\ H_2O$); 130 mg/L under the action of phenol ($C_6H_5OH$) and 60 mg/L under the action of sodium dodecyl sulfate (SDS);

SE2 – biosensor element based on the strain *Vibrio harveyi* Ms1 (natural luminescence). $EC_{50}$ for the test strain is: 60 mg/L – under the action of $K_2Cr_2O_7$; 1.6 mg/L under the action of $ZnSO_4{\cdot}7\ H_2O$; 280 mg/L under the action of $C_6H_5OH$ and 100 mg/L under the action of SDS;

SE3 – biosensor element based on the strain *Photobacterium phosphoreum* B7071 (natural luminescence). $EC_{50}$ for the test strain is: 100 mg/L – under the action of $K_2Cr_2O_7$; 1.3 mg/L under the action of $ZnSO_4{\cdot}7\ H_2O$; 100 mg/L under the action of $C_6H_5OH$ and 40 mg/L under the action of SDS;

SE4 – biosensor element based on the strain *Alcaligenes eutrophus* 1239 (highly sensitive to copper gene-modified strain – induction of bioluminescence in response to the presence of copper ions in the medium);

SE5 – biosensor element based on the strain *Pseudomonas fragi* T2(5) (highly sensitive to zinc gene-modified strain – induction of bioluminescence in response to the presence of zinc ions in the medium).

The strains of microorganisms with natural and induced luminescence used to create sensor elements were deposited in the collection of the State Scientific and Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine) (TSU. TSU 2025).

The aqueous extracts of the reference (control) and test soil samples were studied: the extraction was carried out with distilled water (1:20 ratio): 5 g of soil sample was placed in a 250 ml Erlenmeyer flask, 100 ml of distilled water was added, and kept for 1 hour at 30°C with constant shaking (shaker, ~50 rpm). After that, the suspensions were settled and cooled to room temperature (22–25°C), and the resulting extract was used for analysis (State Standard of Ukraine SSU 7534:2014 2015). The sensor elements were rehydrated in the obtained aqueous extract and the response of each sensor element was recorded using a bioluminometer (Triathler-Luminometer) (Hidex, Finland).

**Table 1.** Characteristics of the studied soil samples.

| Soil sample | Regions of Ukraine | Soil type according to WRB | USDA Soil Taxonomy | Current crop | Previous crop |
|---|---|---|---|---|---|
| 1 | Odesa region | Medium-humus Chernozem | **Mollisols → Ustolls** | Hazelnut tree | Perennial grasses |
| 2 | Lviv region | Technosol | **Entisols → Orthents** | Herbaceous phytocoenoses | – |
| 3 | Lviv region | Technosol | **Entisols → Orthents** | Herbaceous phytocoenoses | – |
| 4 | Lviv region | Technosol | **Entisols → Orthents** | Herbaceous phytocoenoses | – |
| 5 | Lviv region | Technosol | **Entisols → Orthents** | Herbaceous phytocoenoses | – |
| 6 | Lviv region | Low-humus Loamy Chernozem (on Loess) | **Mollisols → Udolls** | Sweet clover | Switchgrass (*Panicum virgatum*) |
| 7 | Kirovohrad region | Low-humus Loamy Chernozem (on Loess) | **Mollisols → Xerolls** | Under fallow | Winter rape, Corn, Sunflower |
| 8 | Kirovohrad region | Low-humus Loamy Chernozem (on Loess) | **Mollisols → Xerolls** | Sunflower | Soybean |
| 9 | Odesa region | Medium-humus Chernozem | **Mollisols → Ustolls** | Hazelnut tree | Perennial grasses |
| 10 | Odesa region | Medium-humus Chernozem | **Mollisols → Ustolls** | Hazelnut tree | Perennial grasses |
| 11 | Odesa region | Low-humus Loamy Chernozem (on Loess) | **Mollisols → Ustolls** | Peas of winter sowing | Winter wheat |
| 12 | Lviv region | Luvisol | **Alfisols → Udalfs** | Spring wheat | Fallow |
| 13 | Kirovohrad region | Low-humus Chernozem (on Loess) | **Mollisols → Xerolls** | Sunflower | Soybeans |

Note: WRB – World Reference Base for Soil Resources.
USDA – United States Department of Agriculture.

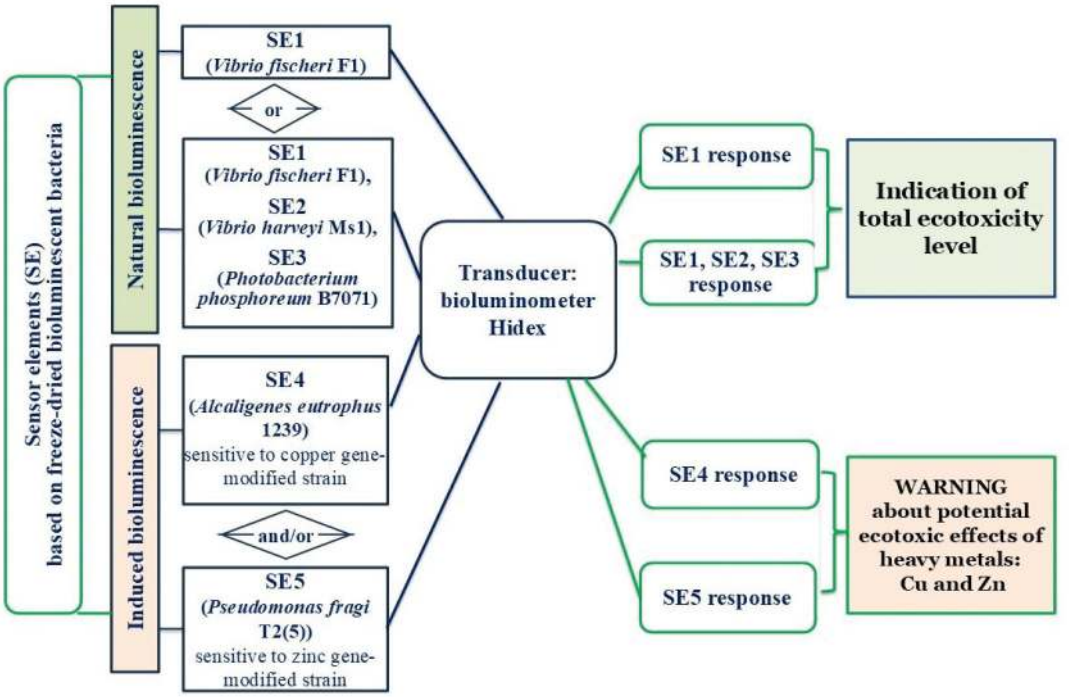


**Figure 2.** Scheme of the bioluminescent test.

The obtained luminescence intensity (QPS – quantity of photons per second) of the test sample was compared with the luminescence intensity of the control sample using the formula: $E = I_t / I_c$, where $I_t$ is the intensity of bioluminescence of the sensor element in contact with the test sample; $I_c$ is the intensity of bioluminescence of the sensor element in contact with the control sample; $E$ is the parameter "ecotoxicity level."

As a control for ecotoxicity assessment in the bioluminescence test, a reference natural sod-podzolic soil from Pushcha-Vodytsia, Ukraine, obtained from the laboratory collection of the F.D. Ovcharenko Institute of Biocolloidal Chemistry of the NAS of Ukraine, was used. The soil had the following characteristics: moisture content, 2.74%; pH, 5.6; total nitrogen, 0.2%; phosphorus ($P_2O_5$), 3.7 mg/g; organic matter, 2.3%; ammonium, 2.1%; Hg, <0.02 mg/kg; As, <1.00 mg/kg; and Cd, 0.02 mg/kg.

The ecotoxicity level ($E$) reflects the level of integral contamination of the object, in particular with heavy metals, and is expressed as the ratio of the intensity of bioluminescence of sensor elements based on bacterial cells in contact with the test sample ($I_t$) to the intensity of bioluminescence of sensor elements in contact with the reference (control) sample ($I_c$). Based on the results of the calculated parameter $E$ (relative units), a conclusion was made about the ecotoxicity level of the soil samples. Taking into account the nature and intensity of the microbial luminescent test strains' reaction on the presence of model toxicants, the risk ranges depending on the value of the calculated ecotoxicity level ($E$) were formed as follows: low – the value of the calculated parameter of ecotoxicity level ($E$, r.u.) is in the range (0.6–0.9) or (1.1–1.5); middle – the value of the calculated parameter $E$ (r.u.) is in the range (0.4–0.6) or (1.5–1.7); high – the value of the calculated ecotoxicity level ($E$, r.u.) is in the range (0.2–0.4) or (1.7–2.0). The revealed risk ranges correspond to the technical characteristics of the sensor elements (TSU 21.2-05402714-005:2014 TSU 2025; Methodical guidelines; 2015). Phenomenon of hormesis response of the test

luminescent bacteria under the influence of some pollutants (Methodical guidelines 2015; Agathokleous 2025; Si et al. 2024; Czyz, Plata, and Wegrzyn 2002) is taking into account in the risk ranges.

As shown in Figure 2, the ecotoxicity level is classified according to the ranges described above. The potential ecotoxic effects of heavy metals (Zn and Cu) are indicated based on the response levels of the sensor elements SE4 and SE5.

### Agrochemical analysis of soil samples

The agrochemical analysis of soil samples was performed in accordance with the Recommended chemical soil test procedures for the North Central Region (2015). Sample preparation for analysis was carried out by drying the samples at 37°C for 48 hours, carefully grinding, and sieving through a 2 mm mesh.

The measurement of pH and electrical conductivity was carried out using a conductometer (Denver Instrument Model 250).

Organic matter (OM) was determined using the loss-on-ignition (LOI) method at 360°C in a muffle furnace.

Determination of available forms of elements: available phosphorus (P) was extracted according to the Mehlich-3 procedure (Mehlich 1984) and determined spectrophotometrically in a flow cell (Lachat-QuikChem FIA +8000S). Nitrate nitrogen ($NO_3$-N) was extracted with a saturated $Ca(H_2PO_4)_2$ solution and analyzed spectrophotometrically by the cadmium reduction method (Huffman and Barbarick 1981). Sulfate sulfur ($SO_4$-S) was extracted with saturated $Ca(H_2PO_4)_2$ solution and determined turbidimetrically (Lachat-QuikChem FIA +8000S) (Tabatabai 1982). The cation exchange capacity was determined according to the standard protocols given in the Recommended chemical soil test procedures for the North Central Region (2015) (Warncke et al. 1980). Potassium (K), exchangeable cations of calcium, magnesium, and sodium were extracted with 1N $NH_4OAc$ (pH 7.0) (Thomas 1982) and analyzed by inductively coupled plasma optical emission spectrometry ICP-OES (Thermo ICP-OES 6000).

The content of available forms of trace elements was determined by DTPA extraction (10 g of soil: 10 ml of DTPA solution pH 7.3, extraction for 2 hours) (Lindsay and Norwell 1978; Whitney 2015) followed by atomic absorption spectrometry (AAS; Perkin Elmer Analyst 200/400) or ICP-OES (Thermo ICP-OES 6000).

### Analysis of heavy metals content in soil samples by ICP-MS

The content of heavy metals (Zn, Cu, Co, Ni, As, Cd, Hg, Pb, Cr) in aqueous soil extracts was determined according to DSTU EN 16171:2022 (EN 16171:2016, IDT) (2022) by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent ICP-MS 7500ce): a 1 g sample (accurate to 0.0005 g) was placed in a 250 ml Erlenmeyer flask and 100 ml of distilled water was added. After that, the flask was placed in a thermal shaker (50 rpm, 40°C) for 3 hours, and then kept in a water bath at 40°C for 1 hour (shaking gently every 10 minutes). After cooling to room temperature, the extract was filtered through a paper filter ("white tape"). Next, the internal elemental standard was added to the extract and measured by ICP-MS (Agilent ICP-MS 7500ce) according to DSTU EN 16171:2022 (EN 16171:2016, IDT) (2022).

### Statistical analysis

The measurement data of the bioluminescent test were presented as an arithmetic mean ± standard deviation (SD) of 3 replicates. The values of the ecotoxicity level ($E$, r.u.) were calculated using means of the corresponding numerical values. All calculations were performed using Microsoft Excel (Microsoft).

**Table 2.** Evaluation of ecotoxicity level of soil samples in the bioluminescent test using sensor element based on the strain *V. fischeri* F1 (sensor element SE1).

| Sample | Reference (Control) | | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Intensity of luminescence, QPS | 271 ± 4 | | 265 ± 4 | 466 ± 7 | 393 ± 4 |
| *E*, r.u. | 1.0 | | 0.98 | 1.72 | 1.45 |
| Ecotoxicity level | low | | low | medium | low |
| Sample | 4 | 5 | 6 | 7 | 8 |
| Intensity of luminescence, QPS | 475 ± 5 | 409 ± 5 | 254 ± 3 | 314 ± 5 | 270 ± 4 |
| *E*, r.u. | 1.75 | 1.51 | 0.94 | 1.16 | 1.00 |
| Ecotoxicity level | high | medium | low | low | low |
| Sample | 9 | 10 | 11 | 12 | 13 |
| Intensity of luminescence, QPS | 269 ± 4 | 272 ± 7 | 137 ± 2 | 84 ± 2 | 236 ± 5 |
| *E*, r.u. | 1.00 | 1.00 | 0.50 | 0.31 | 0.87 |
| Ecotoxicity level | low | low | medium | high | low |

Note: QPS – quantity of photons per second; r.u. - relative units. The measurement data of the bioluminescent test are presented as an arithmetic mean ± SD of 3 replicas in the same experimental series. The values of the ecotoxicity level (E, r.u.) were calculated using the arithmetic mean of the intensities of the test soil ($n = 3$) relative to the reference soil.

The means of three measurements and the relative standard deviation (RSD) were calculated when analyzing the samples by the agrochemical analysis methods and when obtaining the heavy metal concentrations in the samples by the ICP-MS method. The elemental data were processed using Thermo iTEVA software version 2.8.0.97 and Mass Hunter Workstation Software for ICP-MS Version B.01.01 Build 123.13Patch 6 from Agilent Technologies.

## Results and discussion

The biosensor bioluminescent analysis of the ecotoxicity level of 13 tested soil samples performed in this study using a sensor element based on the *Vibrio fischeri* F1 strain (SE1) is presented in Table 2.

Data showed the following: 8 soil samples were characterized by a "low" ecotoxicity level, 3 samples were classified as "medium" and 2 samples as "high" level of ecotoxicity.

In bioluminescent ecotoxicity tests based on bacteria with natural bioluminescence, other strains are used in addition to *Vibrio fischeri*, such as *Photobacterium phosphoreum*, *Vibrio harveyi*, and many others. This provides greater variety of sensor elements and expands the sensitivity range of this test. An example of such a solution is the LumiMARA ecotoxicity test (Kowalik et al. 2024), the sensitive elements of which are 11 different bacteria strains that demonstrate different sensitivity to chemical substances.

In our study, the ecotoxicity of soil sample 11 was assessed using three sensor elements based on bacterial strains with natural luminescence: *Vibrio fischeri* F1 (SE1), *Vibrio harveyi* Ms1 (SE2), and *Photobacterium phosphoreum* B7071 (SE3). The data is presented in Table 3. Based on the calculated ecotoxicity level (*E*), the sensor element based on the *V. fischeri* F1 strain (SE1) was more sensitive to the toxicants in the soil sample 11, while the sensor element based on the *P. phosphoreum* B7071 strain (SE3) was the least sensitive (TSU. TSU 2025). The response of the sensor element (SE2) based on *V. harveyi* Ms1 strain was similar to SE1. Despite the highest sensitivity of SE1 to the content of toxicants in the soil sample 11, the different response characteristics of the sensor elements (SE1, SE2,

**Table 3.** Comparative analysis of the response of sensor elements based on the strains *V. fischeri* F1 (SE1), *V. harveyi* Ms1 (SE2) and *P. phosphoreum* B7071 (SE3) in the evaluation of the ecotoxicity level of soil sample 11 by the bioluminescent test.

| | Bioluminescent test, *E*, r.u. | | | |
|---|---|---|---|---|
| Sample | SE 1 | SE 2 | SE 3 | Ecotoxicity level |
| 11 | 0.50 | 0.59 | 0.73 | medium |

Note: The values of the ecotoxicity level (E, r.u.) were calculated using the arithmetic mean of the intensities of the test soil ($n = 3$) relative to the reference soil.

SE3) may indicate the feasibility of their use in a bioluminescent test to provide a potentially wider range of sensitivity of the test to specific toxicants.

In the practice of ecological, agrochemical and ecotoxicological analysis of the state and quality of soils, special attention is paid to determining the content of heavy metals due to their high toxicity. Traditionally, the environmental risk caused by heavy metal pollution is assessed based on the analysis of their total content in the soil. However, the main factor determining the toxicity of metals in soil is their bioavailability. According to Alkorta et al. (2006) bioavailability is considered to be the part of the total metals in interstitial water and soil particles available to receptor organisms. Metals in soil have a negative effect on living organisms only when bioavailable. When unavailable, metals are only potentially toxic). The bioavailability of metals within soil ecosystems is not a fixed parameter; rather, it varies dynamically in response to changes in environmental conditions (Alkorta et al. 2006).

It is known that one of the approaches used to detect the bioavailable fraction of certain metals in soil is based on the use of genetically modified bacteria, in which the measured signal (bioluminescence) is induced upon contact with bioavailable metal ions (Liao et al. 2006).

In this study, four soil samples were analyzed using sensor elements based on genetically modified strains *Alcaligenes eutrophus* 1239 (SE 4) and *Pseudomonas fragi* T2 (5) (SE 5) which are sensitive to the presence of bioavailable copper and zinc ions, respectively (Table 4).

The analysis of the results obtained using bioluminescent sensor elements with induced luminescence (SE 4 (Cu) and SE 5 (Zn)) indicated a pronounced increase in the content of zinc ions in the soil samples 1, 9 and 10 compared to the control (the values of correspondent sensor elements response when measuring the reference (control) soil sample), with low levels of ecotoxicity (*E*) according to the measurement data received using sensor element SE1. Regarding the copper ion content, a slight increase in the response of the SE4 sensor element was noted for soil samples 1 and 9, in comparison with the data received for the control soil sample, while a slight suppression of the luminescence intensity was observed for sample 10 compared to the control (reference soil). For the soil sample 11, along with the suppression of the luminescence intensity of SE1, a pronounced suppression of the luminescence intensity of the sensor elements SE4 (Cu) and SE5 (Zn) was recorded. This may be due to the complex toxic effect of toxicants contained in the sample not only on the luminescent reaction, but also on the general physiological state of bacteria cells – the basis of sensor elements.

It is known that the bioavailability of toxicants, in particular heavy metals, strongly depends on the physical and chemical properties of the soil. Thus, soil pH is the main parameter that affects the bioavailability of most nutrients and toxic elements in the soil (Zhang Z et al. 2023). The pH value of the soil mediates the toxicity of metals, so at high pH, metals tend to form insoluble forms of minerals, while at low pH they are usually in the form of free ions or soluble organic complexes and are more bioavailable (Olaniran et al. 2013). For example, the bioavailability of cadmium (Cd) and its uptake by plants increases with decreasing soil pH (Bolan et al. 2003). A decrease in bioavailability with increasing soil pH and organic carbon (SOC) content was also shown for Pb and Cu (Cui et al. 2024).

Considering the above, along with the bioluminescence test, an agrochemical analysis (Table 5) of the studied soil samples and determination of the content of heavy metals by the ICP-MS method (Table 6) was performed.

**Table 4.** Induction of bioluminescence of sensor elements based on genetically modified strains *A. eutrophus* 1239 (SE 4 (Cu)) and *P. fragi* T2(5) (SE 5 (Zn)) in response to the presence of bioavailable copper and zinc ions in water extracts of soil samples.

| | *E*, r.u. | | | |
|---|---|---|---|---|
| Sample | SE 1 | SE 4 (Cu) | SE 5 (Zn) | Ecotoxicity level |
| 1 | 0.98 | 1.16 | 2.10 | low |
| 9 | 1.00 | 1.05 | 1.49 | low |
| 10 | 1.00 | 0.83 | 1.71 | low |
| 11 | 0.50 | 0.42 | 0.75 | medium |

Note: The values of the ecotoxicity level (E, r.u.) were calculated using the arithmetic mean of the intensities of the test soil ($n = 3$) relative to the reference soil.

**Table 5.** Agrochemical analysis of soil samples.

| | | | | | | $NH_4OAc$ | | | | CEC | | DTPA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | pH ($H_2O$) | Cond. mS/cm | O.M. % | $NO_3$-N mg/kg | P (M3)* mg/kg | Ca mg/kg | Mg mg/kg | K mg/kg | Na mg/kg | meq/ 100 g | $SO_4$-S mg/kg | Zn mg/kg | Mn mg/kg | Fe mg/kg | Cu mg/kg |
| 1 | 7.6 | 0.34 | 3.4 | 9.4 | 33 | 3678 | 501 | 400 | 71 | 23.9 | 9.6 | 1.56 | 13.9 | 12.2 | 0.75 |
| 2 | – | – | – | – | 17.1 | 2348 | 106.1 | 97.1 | 199.3 | – | – | – | – | – | – |
| 3 | 7.5 | 0.30 | 7.6 | – | 243 | 4101 | 204 | 203 | 30 | 22.8 | – | – | – | – | – |
| 4 | 7.7 | 0.42 | 2.2 | 3.8 | 8.5 | 4793 | 135 | 176 | 25 | 25.6 | 15.4 | 10.9 | 3.58 | 27.2 | 3.22 |
| 5 | 7.6 | 0.47 | 8.3 | 2.7 | 8.0 | 2101 | 81 | 88 | 139 | 12.0 | 14.8 | 2.67 | 10.2 | 45.5 | 7.17 |
| 6 | 7.5 | 0.43 | 3.3 | 27.4 | 244 | 4355 | 125 | 276 | 26 | 23.6 | 12.2 | 15.4 | 3.17 | 32.8 | 2.69 |
| 7 | 7.5 | 0.40 | – | – | 60 | 4058 | 311 | 279 | 25 | 23.7 | – | – | – | – | – |
| 8 | 7.0 | 0.25 | 3.1 | 6.7 | 25 | 3778 | 293 | 221 | 18 | 22.0 | 9.0 | 0.59 | 8.14 | 18.5 | 0.64 |
| 9 | 7.9 | 0.40 | 3.4 | 7.8 | 58 | 4012 | 456 | 463 | 67 | 25.3 | 8.3 | 1.72 | 7.78 | 11.4 | 0.84 |
| 10 | 7.8 | 0.40 | 3.4 | 9.3 | 38 | 3993 | 453 | 404 | 67 | 25.1 | 8.2 | 1.93 | 8.92 | 14.0 | 1.00 |
| 11 | 6.6 | 0.42 | 3.6 | 13.5 | 130 | 4431 | 464 | 409 | 29 | 27.2 | 9.3 | – | – | – | – |
| 12 | 7.2 | 0.51 | 32.7 | 9.4 | 76 | 3031 | 110 | 703 | 18 | 18.0 | 21.1 | 2.10 | 4.84 | 20.3 | – |
| 13 | 6.9 | 0.31 | 3.3 | – | 44 | 3867 | 270 | 216 | 21 | 22.2 | – | – | – | – | – |

Note: *M3-Mehlich III (Mehlich 1984). "-" - analysis was not performed. The mean of 3 replicates for each analyzed parameter are presented in the table.

**Table 6.** The content of heavy metals in soil samples (aqueous extracts[a]) by inductively coupled plasma mass spectrometry (ICP-MS).

| | Zn mg/kg | Cu mg/kg | Co mg/kg | Ni mg/kg | As mg/kg | Cd mg/kg | Hg mg/kg | Pb mg/kg | Cr mg/kg |
|---|---|---|---|---|---|---|---|---|---|
| Sample | [b]MPC 23.0 | [b]MPC 3.0 | [b]MPC 5.0 | [b]MPC 4.0 | [b]MPC 2.0 | [b]MPC 1.5 | [b]MPC 2.1 | [b]MPC 32.0 | [b]MPC 6.0 |
| 1 | 14.03 | 8.72 | 2.49 | 11.94 | <1.00 | 0.23 | <0.02 | 14.58 | 0.41 |
| 2 | 19.17 | 10.14 | 2.10 | 7.99 | <1.00 | 0.26 | <0.02 | 8.31 | 0.13 |
| 3 | 547.96 | 82.09 | 1.60 | 7.01 | <1.00 | 0.69 | <0.02 | 75.51 | 1.89 |
| 4 | 68.06 | 27.28 | 6.30 | 14.18 | <1.00 | 0.49 | <0.02 | 10.12 | 1.12 |
| 5 | 24.57 | 55.90 | 1.85 | 3.65 | <1.00 | 0.24 | <0.02 | 28.89 | 0.24 |
| 6 | 80.06 | 10.13 | 1.32 | 6.57 | <1.00 | 0.45 | <0.02 | 17.56 | 0.96 |
| 7 | 10.09 | 6.14 | 2.53 | 10.28 | <1.00 | 0.15 | <0.02 | 7.70 | 0.59 |
| 8 | 8.71 | 5.91 | 1.09 | 8.75 | <1.00 | 0.18 | <0.02 | 5.54 | 0.30 |
| 9 | 15.71 | 9.70 | 2.26 | 10.47 | <1.00 | 0.21 | <0.02 | 20.01 | 0.47 |
| 10 | 14.38 | 9.31 | 2.84 | 11.32 | <1.00 | 0.26 | <0.02 | 23.27 | 0.31 |
| 11 | 7.62 | 7.71 | 1.15 | 11.92 | <1.00 | 0.25 | <0.02 | 5.16 | 0.25 |
| 12 | 26.61 | 2.24 | 0.26 | 2.34 | <1.00 | 0.17 | <0.02 | 5.54 | 0.11 |
| 13 | 6.30 | 5.32 | 0.39 | 5.23 | <1.00 | 0.18 | <0.02 | 1.92 | 0.26 |

Note: The means of 3 replicates for each element concentration are presented in the table.
[a]Aqueous extracts of the soil samples were analyzed by ICP-MS.
[b]Order of 14.07.2020 No. 1595 "Hygienic regulations on the permissible content of chemical substances in soil: the value of the maximum permissible concentration (MPC), mg/kg, taking into account the background ("Clarke values")". (The Order regulates the permissible content of chemicals in the soil in Ukraine).

Table 5 shows the results of agrochemical analysis of the studied soil samples 1–13. According to the obtained data, samples 1–7, 9, and 10 exhibited slightly alkaline pH values, whereas the remaining samples were neutral. Elevated electrical conductivity values were consistent with the high concentrations of cations such as calcium, magnesium, potassium, and sodium in the ammonium acetate extracts across all samples. Nitrate nitrogen concentrations were generally within the normal range for most samples, with the exception of samples 6 and 11. The organic matter content was classified as medium to high in all samples, with an exceptionally high value recorded for sample 12. Available phosphorus was low in samples 4 and 5, medium to high in samples 1, 7, 8, 9, 10, 12, and 13, and extremely high in samples 3, 6, and 11. Sulfate sulfur content was consistently high in all analyzed samples. The content of available micronutrients (DTPA extract) indicated high to extremely high levels of zinc (Zn more 6 ppm) in samples 4 and 6, and of copper (more 2–5 ppm) in samples 4 and 5 (Lindsay and Norwell 1978; Whitney 2015).

In traditional agrochemical analysis, one of the main methods for assessing plant-available micronutrients or trace metals in soil is DTPA extraction. The DTPA extraction method was developed by Lindsay and Norwell (1978) to identify near-neutral and calcareous soils with insufficient amounts of available Zn, Fe, Mn, or Cu. This method is applicable to all soil types, but does not correlate with uptake by all plant species and varieties in all soil types. Although DTPA extraction is a widely used method for estimating plant-available micronutrients in soil, it has some limitations. These include sensitivity to temperature and soil properties, potential underestimation of available nutrients in certain soil types, and limitations in acidic soil conditions. In addition, DTPA extraction may not be suitable for all trace elements or for soils with a high organic content. To improve the interpretation of the availability of trace metals to plants in soils, it is recommended to use plant tissue analysis as an additional diagnostic tool (Liao et al. 2006; FAO 2022; Gürbüz 2025). Therefore, the main disadvantage of the DTPA extraction method is the issue of transferring the results to biological systems.

In this context, the results obtained by the bioluminescent test and the DTPA extraction method for the presence of bioavailable copper and zinc ions in three soil samples are presented in Figure 3. In the bioluminescent test the response of sensor elements SE 4 (Cu) and SE 5 (Zn) received for the reference soil were used as a control (Figure 3a). In the soil analysis (Figure 3b) mean standard values of medium amount in soil for DTPA-Cu (0.45 mg/kg) and DTPA-Zn (0.76 mg/kg) (Lindsay and Norwell 1978; Whitney 2015) were used as a control. The differences in the responses of biosensor test (Figure 3a) and the method of DTPA-extraction (Figure 3b) when

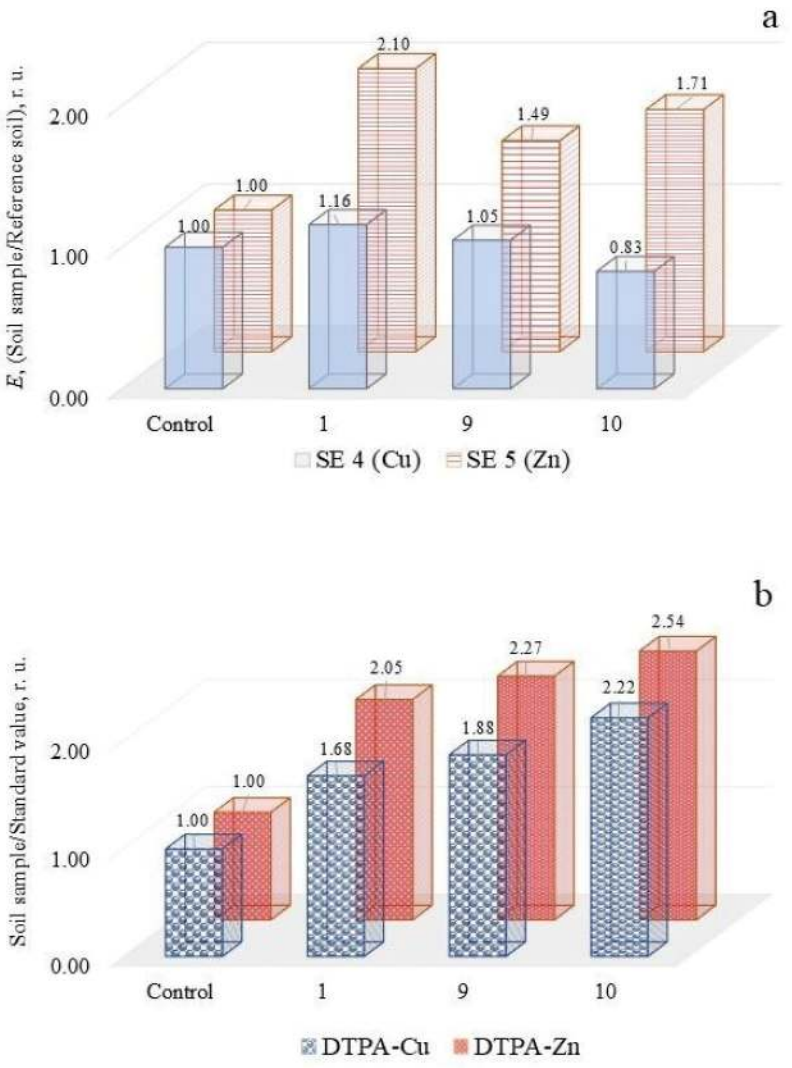


**Figure 3.** The response to the presence of bioavailable ions of copper and zinc of sensor elements SE 4 (Cu) and SE 5 (Zn) of the bioluminescent test (a) with parameters DTPA-Cu and DTPA-Zn of agrochemical analysis (b).

studying the bioavailable content of Cu and Zn content in the soil samples may be explained by the principal differences of the used approaches. The proposed bioluminescent test gives the opportunity to estimate the short-term ecotoxicity to the soil microbial community. It is proposed as a component of a test battery useful for soil ecotoxicity estimation together with the chemical analysis including agrochemical ones which allows predict soil fertility and potential for normal plants growth.

In view of the high sensitivity of living bacteria cells to determine the bioavailable part of heavy metals in soils, it can be assumed that biosensor bacteria can probably provide a more realistic picture of the level of their ecotoxic impact. However, the determination of bioavailable metals using whole-cell bacterial biosensors should not replace traditional chemical analysis, but such sensors can complement analytical chemical methods by distinguishing the bioavailable fraction of the metal from its total content in soil samples.

The ICP – MS analysis of heavy metals in the soil samples (Table 6) revealed exceedances of the maximum permissible concentrations (MPCs) established by Order No. 1595 of 14 July 2020 in several cases: zinc in samples 3, 4, 5, 6 and 12; copper in all samples except 12; cobalt in sample 4; nickel in all samples except 5 and 12; and lead in sample 3. In contrast, no exceedances of the MPCs were observed for the most toxic heavy metals, namely arsenic, cadmium, and mercury, in any of the studied soils.

Comparison of the results obtained on the level of heavy metal contamination of the studied soil samples (Table 6) with the results of the ecotoxicity level assessment in the bioluminescent test according to the response of the sensor element SE1 based on the *V. fischeri* F1 strain (Table 2) showed no correlation of the data. Thus, while soil samples characterized by "medium" (samples 2, 5, 11) and "high" (samples 4, 12) levels of ecotoxicity in the bioluminescent test revealed an excess of the MPC for Ni (sample 2 - by two times), zinc (samples 5 - by 1.57 mg/kg, 12 - by 3.61 mg/kg and 4 - by almost 3 times), cobalt (soil sample 4 - by 1.3 mg/kg) and copper (samples 2 - by 3.4 times, 5 - by 18.6 times, A – by 2.6 times, 4 - by 9 times), all other soil samples with Zn, Cu, Ni and Pb content above the MPC were characterized by a "low" level of ecotoxicity in the bioluminescent test.

No similarity was also observed when comparing the content of zinc and copper in the soil samples 1, 9, 10, 11 (Table 6) with the response of sensor elements based on genetically modified strains *A. eutrophus* 1239 (SE 4 (Cu)) and *P. fragi* T2 (5) (SE 5 (Zn)) (Table 4). Thus, the content of zinc determined by ICP-MS method in these samples was below the MPC, while the induction of bioluminescence of the sensor element SE 5 (Zn) in the bioluminescent test was more than twice the control values for soil sample 1, 1.5 times for soil sample 9, and 1.7 times for sample 10. For copper, the *E* (r. u.) of the SE 4 (Cu) sensor element in the bioluminescent test was 1.16, 1.05, 0.83 and 0.42 for soil samples 1, 9, 10, 11, respectively, while the copper content according to ICP-MS analysis exceeded the MPC by 2.9 times for sample 1, 3.2 times for sample 9, 3.1 times for sample 10 and 2.6 times for sample 11.

The results of the analysis of soil samples presented in Tables 5 and 6, as well as their comparison with the results of the bioluminescent test (Tables 2 and 4), bring us back to the issue of bioavailability of toxicants, in particular heavy metals. After all, it is the bioavailability, not the total content, that is the main factor determining their ecotoxicity (Alkorta et al. 2006; Olaniran et al. 2013; Zhang et al. 2024).

In addition to the obvious role of the bioavailability factor, in the traditional chemical analysis of the potential risks of heavy metals, the issue of their limits/maximum permissible concentrations according to standards deserves special attention. Table 7 shows, as an example, the MPCs in soil (mg/kg) according to the current Order No. 1595 of 14.07.2020 "Hygienic regulations on the permissible content of chemical substances in soil" in Ukraine, compared to the limits for heavy metals in (agricultural) soils set by the European Union (Tóth et al. 2016), Canada (CCME; Wan et al. 2024), and the United States (Wan et al. 2024).

**Table 7.** Limits of heavy metals in soil (mg/kg) according to the standard of Ukraine (MPC) compared to the limits of the EU, Canada, and the USA.

| Heavy metal | [a]Ukraine, MPC | [b]EU | [c,d]Canada | [d]USA |
|---|---|---|---|---|
| Cr | 6.0 | 100.0 | 64.0 | – |
| Co | 5.0 | 20.0 | 40.0 | – |
| Ni | 4.0 | 50.0 | 50.0 | 38.0 |
| Cu | 3.0 | 100.0 | 63.0 | 70.0 |
| Zn | 23.0 | 200.0 | 200.0 | – |
| As | 2.0 | 5.0 | 12.0 | 18.0 |
| Cd | 1.5 | 1.0 | 1.4 | 32.0 |
| Hg | 2.1 | 0.5 | 6.6 | – |
| Pb | 32.0 | 60.0 | 70.0 | 120.0 |

Note: [a]Order of 14.07.2020 No. 1595 Hygienic regulations on the permissible content of chemical substances in soil: the value of the maximum permissible concentration (MPC), mg/kg, taking into account the background ("Clarke values"); [b](Tóth et al. 2016); [c]CCME Summary Table: Chemical table (custom): data for agricultural soil; [d](Wan et al. 2024).

As shown by the data in Table 7, despite the fact that national standards for allowable heavy metal concentrations in soils often differ considerably among countries, the maximum permissible concentrations (MPCs) established in Ukraine for a number of metals are substantially lower. This is particularly evident for copper, zinc, and nickel. In contrast, the MPC for mercury is higher than the limit established in the European Union. Such discrepancies in maximum permissible concentrations of heavy metals may also introduce a degree of subjectivity when assessing soil toxicity.

These differences in MPC values, along with the essential need to account for bioavailability when evaluating the risks posed by potential toxicants, particularly heavy metals, clearly highlight the importance of actively implementing a battery of bioassays as an integral component together with agrochemical and ecotoxicological monitoring of the condition and quality of agricultural soils. In particular, biosensor-based bioluminescent test can serve as an effective component of such monitoring systems.

## Conclusions

According to the experimental data obtained, the biosensor bioluminescent test, through the determination of the ecotoxicity parameter, can be an effective component in the complex approach together with chemical, agrochemical and ecotoxicological analyses of the condition and quality of agricultural soils.

The biosensor bioluminescent test adequately reflects the contribution of the bioavailable component of the toxicant to the overall level of ecotoxicity. This is important for assessing the state of the soil microbial community, which is primarily affected by the negative impact.

The use of an integrated approach by combining agrochemical analysis with battery bioassays including biosensor assessment of the bioluminescent activity of the test microorganisms (bioluminescent test) may form a highly predictive methodology for soil biodiagnostics.

## Abbreviations

| | |
|---|---|
| AAS | atomic absorption spectrometry |
| DTPA | diethylenetriaminepentaacetic acid |
| DSTU | State Standard of Ukraine |
| DSTU EN 16,171:2016 (EN 16,171:2016, IDT) | Sludge, treated biowaste, and soil – Determination of elements using inductively coupled plasma mass spectrometry (ICP-MS) |
| E | ecotoxicity level parameter |
| $EC_{50}$ | effective concentration causing a 50% decrease in the bioluminescence intensity of a bacterial suspension containing $5\times10^5$cells $mL^{-1}$after 5min of exposure |
| FIA | flow injection analysis |
| ICP-MS | inductively coupled plasma mass spectrometry |
| ICP-OES | inductively coupled plasma optical emission spectrometry |
| $I_{(c)}$ | bioluminescence intensity of the sensor element in contact with the control sample |
| $I_{(t)}$ | bioluminescence intensity of the sensor element in contact with the test sample |
| LOI | loss-on-ignition method |
| LumiMARA | commercial microbial array for rapid assessment of sample toxicity based on changes in bacterial bioluminescence |
| Mehlich 3 | a multinutrient soil extraction method used to determine plant-available phosphorus, potassium, calcium, magnesium, sodium, and selected micronutrients |
| Microtox | commercial bioluminescence inhibition assay used for the rapid assessment of acute toxicity |
| MPC | maximum permissible concentration |
| NCR-221 | *Recommended Chemical Soil Test Procedures for the North Central Region* |

| | |
|---|---|
| OM | organic matter |
| QPS | quantity of photons emitted per second |
| RSD | relative standard deviation |
| SD | standard deviation |
| SDS | sodium dodecyl sulfate |
| SE1 | biosensor element based on the naturally bioluminescent strain *Vibrio fischeri* F1 |
| SE2 | biosensor element based on the naturally bioluminescent strain *Vibrio harveyi* Ms1 |
| SE3 | biosensor element based on the naturally bioluminescent strain *Photobacterium phosphoreum* B7071 |
| SE4 | biosensor element based on the genetically modified strain *Alcaligenes eutrophus* 1239, which is highly sensitive to copper and exhibits induced bioluminescence in the presence of copper ions |
| SE5 | biosensor element based on the genetically modified strain *Pseudomonas fragi* T2(5), which is highly sensitive to zinc and exhibits induced bioluminescence in the presence of zinc ions |
| TU U 21.2-05402714-005:2014 | Technical Specifications of Ukraine, registration number 21.2-05402714-005:2014 |

## Acknowledgements

The authors would like to acknowledge Analytical laboratory "Agrotest" (Kyiv, Ukraine) for support in performing agrochemical analysis of soil samples.

## Author contributions

CRediT: **Kateryna Gubina:** Conceptualization, Investigation, Resources, Writing – original draft; **Tamara Gruzina:** Conceptualization, Methodology, Validation, Writing – original draft; **Olena Nikipelova:** Project administration; **Denis Veliksar:** Investigation; **Liudmyla Rieznichenko:** Visualization, Writing – review & editing; **Svitlana Dybkova:** Data curation, Methodology, Supervision.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by the National Academy of Sciences of Ukraine under Project No. [0125U000364] "Biocolloidal approaches to "soil health" as a modern rationalisation of agriculture".

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## Data availability statement

Data will be available upon request.

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