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Restoration of degraded soils in a military-affected region in the South of Ukraine based on biologisation

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Abstract

Background: This study was motivated by the need to restore soils degraded by military operations, accompanied by compaction, destruction of structure and contamination with heavy metals, which reduce the productivity and ecological functioning of agroecosystems. The aim of the work was to evaluate the effectiveness of using microbial preparations and green manure crops separately and in combination to improve the condition of ordinary chernozem. *Methods*: The study involved a field experiment with four treatment options: control; application of the "Azogran" biological preparation; green manure with a mixture of vetch and mustard; and a combination of these, and determination of the content of heavy metals in the soil and their biological activity. *Results*: The study found that the combined option provided the most positive changes: density decreased from 1.52 ± 0.03 to 1.28 ± 0.02 g/cm^3; the measured humus-related organic matter indicator increased from 2.1 ± 0.2 to 2.8 ± 0.1%; acidity changed from 5.8 ± 0.2 to neutral 6.7 ± 0.1; and the content of available, Chirikov-extractable phosphorus and potassium, reported as P_2O_5 and K_2O, increased by 30.0 and 22.0%, respectively. Biological activity increased significantly: dehydrogenase activity doubled, and basal respiration exceeded the control by 62.3%. It was also found that the concentrations of mobile forms of lead, cadmium, zinc and nickel decreased by 25.0–32.0%, which was accompanied by a 2.5-fold decrease in the phytotoxicity of aqueous extracts. *Conclusions*: The data obtained demonstrate that the combination of the biological product with green manure resulted in synergistic improvements, simultaneously improving the physicochemical and biological properties of the soil and reducing the accumulation of metals. The practical significance of the study lies in confirming the possibility of using this approach as an affordable, environmentally safe and effective way to restore land damaged by military equipment.

Keywords: green manure, heavy metals, soil remediation, biological products, detoxification

Introduction

Soils affected by military activities represent a specific form of anthropogenic disturbance in which physical compaction, structural disruption and potential chemical contamination may occur simultaneously. For such soils, restoration cannot be limited to conventional agrotechnical measures, since remediation must address both the physical condition of the soil and the recovery of its biological activity. Therefore, biological approaches based on microbial preparations, green manure crops and phytoremediation are increasingly relevant for restoring degraded chernozem under post-disturbance agricultural conditions. This is particularly important for Southern Ukraine, where war-damaged agricultural soils require scientifically grounded recultivation strategies (Shebanin *et al*., 2024).

Research conducted between 2020 and 2024, in particular the work of Chen *et al.* (2021) and Xu *et al.* (2022), demonstrates the effectiveness of remediation approaches aimed at reducing the mobility of heavy metals and improving the physical and chemical conditions of contaminated soils. These studies indicate that immobilisation amendments and biochar-based remediation can contribute to detoxification processes and support the restoration of soil functions.

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Studies by Ukrainian scientists, in particular Shebanin *et al.* (2024) and Gamayunova *et al.* (2025), emphasise the specific impact of military operations on the soil cover of the steppe zone of Ukraine. These works indicate that soil degradation requires a comprehensive approach to restoration. Kovalenko *et al.* (2024) demonstrated the importance of targeted agronomic measures for improving sunflower growth, development and productivity, although this source should not be used as direct evidence of biological remediation of degraded soils. International experience in the remediation of contaminated soils, described in the works of Naeem *et al.* (2022) and Bilias *et al.* (2021), demonstrates the importance of soil remediation strategies aimed at reducing agrochemical pressure and limiting the bioavailability of potentially toxic elements, including through the use of biochar-based amendments. Tang *et al.* (2020) proved that the combined use of biochar and compost significantly improves the physicochemical properties of soils and reduces the bioavailability of heavy metals. Song *et al.* (2022) demonstrated the positive effect of remediation agents on the microbial structure of soil aggregates.

At the same time, the effectiveness of biological soil restoration should be considered within the broader framework of conservation and biologised farming practices. These practices are based on the reduction of excessive mechanical disturbance, the return of plant residues to the soil, the use of green manure crops and the stimulation of beneficial microbial communities. Alskaf *et al.* (2021) showed that tillage practices combined with plant residue retention can improve soil physical properties, while Meurer *et al.* (2020) and Or *et al.* (2021) emphasised that soil structure is closely connected with biological activity, pore formation, aeration and water movement. Therefore, green manure crops may be regarded not only as a source of organic biomass but also as an instrument for restoring the structural and functional stability of degraded soils.

Studies on microbial communities and soil enzymes indicate that microbial activity is closely related to nutrient cycling, organic matter transformation and the functional recovery of soil biological processes (Lee *et al*., 2020; Nkongolo and Narendrula-Kotha, 2020; Chen and Sinsabaugh, 2021). In addition, recent studies on plant-microbe interactions confirm that rhizospheric bacteria and microbial consortia can reduce the mobility of heavy metals, increase plant tolerance to toxic elements and improve the ecological functioning of contaminated soils (Saha *et al*., 2021; Devi *et al*., 2022; Joshi *et al*., 2023; Iimaa *et al*., 2025). These findings fully justify the combined use of green manure crops and microbial preparations as a biologically grounded approach to soil restoration.

When it comes to Ukrainian agricultural conditions, Shebanin *et al.* (2025) demonstrated that the use of biologisation elements, including microbial preparations, green manure crops and organic fertilisers, contributes to the production of high-quality agricultural products and reduces anthropogenic pressure on agroecosystems. However, these approaches have been insufficiently tested under the specific conditions of war-damaged ordinary chernozem, where mechanical compaction, structural degradation, changes in biological activity and heavy metal contamination occur simultaneously.

The aim of this study was to evaluate the effectiveness of biological remediation strategies for restoring the physicochemical properties of war-degraded ordinary chernozem, reducing soil phytotoxicity and detoxifying heavy metals under small-plot field conditions in the southern steppe of Ukraine. Specifically, the study aimed to (i) quantify changes in soil physicochemical properties following different remediation treatments; (ii) assess their effects on soil biological and enzymatic activity; (iii) evaluate the effectiveness of heavy metal detoxification; and (iv) determine the extent to which the treatments reduced the phytotoxicity of soil extracts.

Methods

The study was conducted in 2023–2024 at the Educational, Scientific and Practical Centre of Mykolaiv National Agrarian University. The field experiment was conducted over two consecutive growing seasons, 2023 and 2024. In each season, the active experimental cycle lasted approximately 4 months, including the establishment and growth of green manure crops to the mass flowering stage and the subsequent 60-day monitoring period after green manure incorporation and microbial preparation application. The object of the study was ordinary low-humus (hydrolysed) chernozem with a loamy texture, determined based on its granulometric composition. The soil was characterised by a predominance of dust (0.25–0.05 mm) and silt (0.05–0.001 mm) fractions, with a physical clay content (<0.01 mm) of approximately 45.0%, confirming its loamy classification. Before the start of hostilities, agrochemical analysis showed a humus content typical for the region at 3.2–3.5%; a neutral soil solution reaction (pH 6.8–7.1); provision of mobile phosphorus (P_2O_5 12–15 mg/100 g) and exchangeable potassium (K_2O 18–22 mg/100 g); and a humus horizon thickness of 55–65 cm.

The degree of soil degradation was determined by comparing its current characteristics with pre-war agrochemical passports of the site and samples taken from adjacent untouched areas, which served as a reference. The reference site was selected in the adjacent untouched area with comparable relief and the same soil type (ordinary low-humus chernozem). While detailed long-term histories of previous crop rotation and fertilisation for the exact reference spots were evaluated via pre-war agrochemical passports, the baseline soil properties of both sites were technically identical prior to the mechanical and chemical degradation caused by military actions. The main types of degradation were identified as mechanical compaction, disruption of structure and organic matter and potential chemical contamination. Mechanical compaction was detected using a Wile Soil Penetrometer (Finland), which recorded an increase in soil density to 1.45–1.55 g/cm^3 in the upper horizon (0–20 cm) compared to 1.20–1.30 g/cm^3 in the control area. The disruption of the structure manifested itself in a decrease in the number of water-resistant aggregates measuring 0.25–10 mm and the appearance of signs of erosion. Potential chemical contamination is caused by possible leaks of fuel and lubricants (FL) and ammunition combustion products.

To study the effectiveness of biological methods for restoring degraded soils, a stationary field experiment was set up on a total area of 0.5 ha. The geometry of the experiment included four experimental variants, each of which was repeated four times, with each repetition representing an independent field plot rather than a repeated laboratory measurement from the same soil sample. Thus, the experiment comprised 16 field plots in total (4 treatments × 4 replications). The replicates were organised as a randomised complete block design with four spatial blocks, where all four treatments were randomly assigned to reduce spatial bias. The area of each plot was 25 m^2.

The experimental variants included four treatments: control (C), biological preparation (BP), green manure (GM), and combined (CB) treatment. The control (C) treatment remained without any restoration interventions and was used to assess the natural background. The biological preparation (BP) treatment involved the application of the microbial preparation "Azogran" (Ukraine) at a rate of 2 l/ha per 300 l of water as a working solution. The preparation contained two bacterial components: the nitrogen-fixing bacterium *Azotobacter vinelandii* and the plant-growth-promoting bacterium *Bacillus subtilis*, with a viable cell titre of at least 2 × 10^8 CFU/g. The choice of "Azogran" was justified by its complex action, including the improvement of soil nitrogen balance through nitrogen fixation by *Azotobacter vinelandii* and the stimulation of microbiological activity and plant immunity through *Bacillus subtilis*, which is important for the remediation of degraded soils with impaired biological potential. The preparation was applied by spraying the soil surface using a Solo 475 backpack sprayer (Germany). The green manure (GM) variant involved sowing a mixture of vetch (*Vicia sativa*) at a rate of 24 kg/ha and white mustard (*Sinapis alba*) at a rate of 16 kg/ha, totalling 40 kg/ha. Sowing was carried out mechanically using an SN-16 seeder (Ukraine). The green mass was ploughed to a depth of 20–22 cm during the mass flowering phase of mustard using a PLN-3-35 plough (Ukraine). The combined (CB) treatment included both previous interventions: after sowing and regrowth of green manure crops to the flowering phase, their vegetative mass was sprayed with "Azogran" at the same rate of 2 l/ha, followed by ploughing.

Soil samples for analysis were taken from a depth of 0–20 cm on each plot using the "envelope" method, where five subsamples were collected from the four corners and the centre of each plot and then mixed into one composite sample. Sampling was carried out twice: before the start of the application of the studied measures to assess the initial state (day 0 of each seasonal experimental cycle) and 60 days after ploughing in the green manure and applying the biological product to assess the dynamics of changes. Thus, the final sampling was performed approximately 120 days, or about 4 months, from the start of the experiment in each growing season. The 60-day observation period was chosen to evaluate the early response of the treated topsoil to biological remediation, since microbial activity, nutrient mobilisation, pH shifts and the decomposition of incorporated green manure may become detectable within a short post-treatment interval. The 0–20 cm soil layer was chosen because the study focused primarily on the arable topsoil layer directly affected by the applied restoration techniques.

This zone encompasses the surface application of the microbial preparation and the incorporation of green manure biomass and represents the main area of short-term microbial and root activity. Deeper horizons were not included at this stage since the objective was to evaluate the initial remediation response of the treated topsoil within a 60-day period; however, possible deeper compaction or downward migration of contaminants should be assessed in subsequent long-term monitoring. Therefore, the obtained results should be interpreted as evidence of short-term remediation dynamics rather than as a final assessment of stable humus accumulation, complete structural recovery or long-term soil restoration.

The selected soil samples were subjected to comprehensive analysis using standard methods that comply with current national and international standards. The granulometric composition was determined using the areometric method in accordance with the requirements of DSTU ISO 11277:2005 (2005), which is based on the different rates of particle sedimentation in an aqueous medium. The soil organic matter content, expressed as a humus-equivalent value, was determined by the Tyurin spectrophotometric method in accordance with DSTU 4289:2004 (2004). This method is based on wet oxidation of oxidisable organic carbon with dichromate, followed by spectrophotometric measurement and recalculation of the obtained organic carbon value into soil organic matter, traditionally reported in regional soil studies as humus content. Therefore, the reported values should be interpreted as a humus-equivalent soil organic matter indicator rather than as total soil organic carbon determined by dry combustion.

The reaction of the soil environment (pH) was determined by the potentiometric method in a salt extract (1N KCl) in accordance with DSTU ISO 10390:2022 (2022) using a Hanna Instruments HI 5221 (Romania) laboratory ion meter with a glass electrode, and the available forms of phosphorus and potassium, reported as P_2O_5 and K_2O, were determined by the modified Chirikov method according to DSTU 4115-2002 (2002). These values refer to Chirikov-extractable nutrient forms and should not be interpreted as total phosphorus or total potassium contents. In this method, mobile phosphorus and exchangeable potassium are extracted with a weak acetic acid solution and reported as P_2O_5 and K_2O; therefore, these values should be interpreted as Chirikov-extractable nutrient forms and not as Olsen-extractable phosphorus. To assess environmental safety, the content of acid-extractable forms of heavy metals (Pb, Cd, Zn, Cu, Cr, Co, Mn and Ni) was determined by atomic absorption spectrometry according to DSTU ISO 11047:2005 (2005). This procedure uses acid extraction before atomic absorption measurement; therefore, the obtained metal concentrations characterise the extractable fraction under the selected standard method rather than directly measured plant-available metal forms.

Although manganese is an essential micronutrient, its mobile forms were included in the analysis because elevated concentrations may indicate potential phytotoxic risks under disturbed soil conditions. Quantitative analysis of extracts was performed on an atomic absorption spectrometer with a continuous radiation source "ContrAA 700" manufactured by Analytik Jena (Germany).

The total biological activity of the soil was assessed by the intensity of basal respiration of the soil microbiota in accordance with the method approved in DSTU ISO 16072:2005 (2005). The determination was carried out by incubating soil samples in sealed glass containers, followed by air sampling and analysis of CO_2 concentration on an "Agilent 7820" gas chromatograph (USA) with a thermal conductivity detector. Enzymatic activity was assessed by dehydrogenase activity in accordance with DSTU ISO 23753-2:2010 (2010). The determination was carried out by a photometric method based on the reduction of colourless triphenyltetrazolium chloride (TTC) to red triphenylformazan (TF). The colour intensity was measured using a Shimadzu UV-1800 spectrophotometer (Japan) at a wavelength of 485 nm. The phytotoxicity of soil extracts was assessed by a biotest method in accordance with DSTU ISO 11269-2:2002 (2002) using bright-red root vegetables of the radish variety "Red with a white tip". The germination energy of seeds and the length of primary roots in an aqueous extract from the soil were determined after 72 h of incubation in a BINDER GmbH (Germany) climate chamber under standard lighting and temperature conditions. The obtained values were expressed as percentages relative to the reference control used in the phytotoxicity test, which represented germination and seedling growth under non-toxic baseline conditions.

The obtained digital data were statistically processed using one-way analysis of variance (ANOVA) in the "Statistica 10.0" software package (StatSoft Inc., USA). The overall effect of treatment was assessed using the ANOVA F-test at a significance level of $p < 0.05$. When the F-test indicated a statistically significant treatment effect, post hoc comparisons with the control were performed using Dunnett's test. To assess the reliability of differences between the mean values of the experimental variants, Student's t-test was used at a significance level of $p < 0.05$. Pearson's correlation analysis was additionally used to assess relationships between soil biological activity indicators, physicochemical soil parameters, mobile heavy metal forms and phytotoxicity indicators, with results reported as correlation coefficients (r) and corresponding p-values. All results are presented as the arithmetic mean ± standard error of the mean (M ± m). During the research, the principles of the Convention on Biological Diversity (1992) aimed at ensuring the sustainable and ethical use of plant genetic resources were followed.

Results

Before the application of remediation measures, the studied ordinary chernozem showed clear signs of degradation associated with military disturbance. The initial soil density reached 1.52 ± 0.03 g/cm^3, indicating compaction of the upper arable layer, while the humus content decreased to 2.1 ± 0.2% and soil acidity shifted to pH 5.8 ± 0.2. These baseline conditions, together with the recorded disruption of soil structure and the presence of mobile forms of heavy metals, confirmed that the experimental site required restoration measures aimed at improving both physicochemical and biological soil properties.

THE IMPACT OF REMEDIATION MEASURES ON THE PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

The results of the study demonstrated a comprehensive improvement in the physical and chemical characteristics of degraded soil under the influence of the biological remediation methods used. The greatest improvements were observed in the combined (CB) treatment, confirming the effectiveness of a comprehensive approach to soil restoration.

Soil density, which is a key indicator of structural condition, decreased significantly mainly in the treatments where biological and phytomeliorative interventions were applied, whereas the control showed only minimal change (Table 1). This decrease in density indicates the restoration of soil pore loosening, improvement of its aeration and water permeability, which is important for ensuring optimal conditions for plant growth and the vital activity of soil microorganisms.

The dynamics of soil organic matter, expressed as a humus-equivalent indicator, showed stable growth in all experimental variants, but the strongest effect was associated with the combined (CB) treatment, where the introduction of organic biomass was supported by microbial activation. This increase is associated with enhanced organic matter accumulation, activation of microbiological activity and the influx of organic residues from green manure crops. Given the 60-day observation period, this increase should be interpreted primarily as a change in the measured pool of oxidisable organic matter, including labile and partly transformed plant residues, rather than as formation of stable soil organic matter of stable humus. The reaction of the soil environment was corrected towards neutral values. This corresponds to a near-neutral and agronomically favourable pH range of approximately 6.5–7.5 for chernozem-type agricultural soils (Delgado and Gómez, 2024). Such normalisation is important because it can improve nutrient availability, microbial activity and the immobilisation of some mobile metal forms. Agrochemical analysis revealed a statistically significant improvement in the soil's supply of available nutrients. The content of available, Chirikov-extractable phosphorus, reported as P_2O_5, increased by 25.0–30.0% in the variants with the use of green manure crops, while available, Chirikov-extractable potassium, reported as K_2O, increased by 18.0–22.0%, especially under green manuring. These changes refer only to the available Chirikov-extractable nutrient fraction and should not be interpreted as an increase in total soil phosphorus or potassium stocks. The increase in these available forms reflects enhanced nutrient cycling and mobilisation driven by deep root activity, organic matter decomposition and stimulated microbial processes. Consequently, Table 1 demonstrates a coordinated recovery trend in the structural, chemical and biological functioning of degraded chernozem.

The results demonstrate the high efficiency of the biological remediation methods used to restore the physicochemical properties of degraded soils. A comprehensive approach combining the use of microbial preparations and green manure crops provides a synergistic effect and can be recommended for practical application in the restoration of soils disturbed by military operations.

DYNAMICS OF CHANGES IN SOIL BIOLOGICAL ACTIVITY

The results of the study revealed significant positive dynamics in soil biological activity under the influence of the remediation measures applied. A comprehensive assessment of microbiological activity was carried out using a number of interrelated indicators, which made it possible to obtain an objective picture of the restoration of soil biota.

The intensity of basal soil respiration, which is an integral indicator of the overall metabolic activity of the microbial community, showed a marked increase in all experimental variants. In the control treatment, a slight increase of 8.0–10.0% was observed, which may be associated with natural seasonal fluctuations. However, in the BP treatment, the respiration intensity increased by 45.2% compared to the control ($p < 0.01$), which indicates active colonisation of the soil by introduced microorganisms and activation of the autochthonous microbiota. In the green manure (GM) treatment, the increase was 38.7% ($p < 0.01$), which is due to the supply of readily available organic matter from root exudates and plant residues. The most pronounced effect was observed in the combined (CB) treatment, where the respiration intensity increased by 62.3% ($p < 0.001$), demonstrating the synergistic effect of combining organic application and microbial inoculation.

The activity of dehydrogenase, as a key enzyme in redox processes in the soil, showed particularly pronounced dynamics. The results of the study are presented in Table 2.

The highest dehydrogenase activity was observed in the combined (CB) treatment, which indicates the intensification of redox processes and an increase in the metabolic activity of microorganisms. This may be due to the creation of optimal conditions for the development of microbial communities through the supply of organic matter from green manure and the introduction of active strains of microorganisms. Correlation analysis revealed close relationships between increased biological activity and improved physicochemical properties of the soil; the full correlation results are presented in Table 3. The results obtained indicate the high efficiency of the biological remediation methods used to

Table 1. Dynamics of changes in the physicochemical properties of soil under the influence of remediation measures (average for 2023–2024).

Indicator	Initial state	Control (C)	Biological preparation (BP)	Green manure (GM)	Combined (CB)
Density, g/cm^3	1.52 ± 0.03	1.50 ± 0.02	1.38 ± 0.02*	1.35 ± 0.02*	1.28 ± 0.02**
Soil organic matter, humus-equivalent, %	2.1 ± 0.2	2.2 ± 0.1	2.5 ± 0.1*	2.6 ± 0.1*	2.8 ± 0.1**
pH	5.8 ± 0.2	5.9 ± 0.1	6.3 ± 0.1*	6.4 ± 0.1*	6.7 ± 0.1**
Available P_2O_5, mg/100 g	12.5 ± 1.2	12.8 ± 1.1	14.2 ± 1.3	15.8 ± 1.4*	16.2 ± 1.5**
Available K_2O, mg/100 g	18.3 ± 1.5	18.5 ± 1.4	19.8 ± 1.6	21.8 ± 1.7*	22.3 ± 1.8**

Note: * – $p < 0.05$, ** – $p < 0.01$ compared with the control according to one-way ANOVA followed by Dunnett's post hoc test*. P_2O_5 and K_2O values refer to Chirikov-extractable available nutrient forms, not total phosphorus or potassium content.

Table 2. Dynamics of soil dehydrogenase activity under the influence of remediation measures (average for 2023–2024).

Experimental variant	Initial activity, mg TF/10 g soil per 24 h	Final activity, mg TF/10 g soil per 24 h	Absolute increase, mg TF/10 g	Relative increase, %	Statistical significance
Control (C)	1.8 ± 0.2	2.0 ± 0.3	0.2 ± 0.1	11.1	$p > 0.05$
Biological preparation (BP)	1.8 ± 0.2	3.2 ± 0.4	1.4 ± 0.2	77.8	$p < 0.05$
Green manure (GM)	1.8 ± 0.2	3.0 ± 0.3	1.2 ± 0.2	66.7	$p < 0.05$
Combined (CB)	1.8 ± 0.2	3.8 ± 0.5	2.0 ± 0.3	111.1	$p < 0.01$

Table 3. Pearson correlation matrix between selected soil recovery indicators.

Indicator	Soil organic matter	Available P_2O_5	Acid-extractable heavy metals	Seed germination energy	Phytotoxicity reduction
Dehydrogenase activity	0.89**	–	−0.85*	–	–
Basal respiration	–	0.82*	–	0.79*	–
Pb	–	–	–	–	−0.87*
Cd	–	–	–	–	−0.85*
Cu	–	–	–	–	−0.83*

Note: * – $p < 0.05$, ** – $p < 0.01$. Pearson's correlation coefficient (r) was used to assess the strength and direction of relationships between the analysed soil physico-chemical, biological and phytotoxicity indicators. Positive values indicate direct relationships, whereas negative values indicate inverse relationships.

restore the biological activity of degraded soils. A comprehensive approach combining the use of microbial preparations and green manure crops provides a synergistic effect and creates favourable conditions for the restoration of soil ecosystems affected by military operations.

EFFECTIVENESS OF HEAVY METAL DETOXIFICATION IN SOIL

The most significant transformations under the influence of remediation measures were observed in the content of acid-extractable forms of heavy metals, which is particularly relevant for the restoration of soils disturbed by military actions. Detailed atomic absorption analysis revealed a statistically significant decrease in the concentration of all eight toxic elements studied after the application of biological remediation methods.

The effectiveness of detoxification varied depending on the specific metal and the remediation method used. The strongest response was observed in the combined (CB) treatment, where the simultaneous action of microbial immobilisation and green manure phytoremediation reduced the mobility of all analysed elements more consistently than either treatment alone. Lead (Pb) and cadmium (Cd) showed particularly important decreases because of their high toxicity, mobility and ability to enter the plant-soil system, while copper (Cu) and zinc (Zn) also demonstrated a stable downward trend in mobile forms.

The results of detoxification of chromium (Cr), cobalt (Co), manganese (Mn) and nickel (Ni), which are often present in contaminated soils, also showed a consistent reduction under biological remediation (Table 4). These changes indicate that the remediation effect was not limited to one metal but reflected a broader decrease in the bioavailable fraction of potentially hazardous elements.

The observed increase in soil pH should also be considered as one of the simplest explanatory factors for the decrease in acid-extractable heavy metal forms. In the combined (CB) treatment, pH shifted from moderately acidic conditions to a near-neutral value, which could reduce metal solubility and limit their extractability through precipitation, adsorption on soil colloids and stronger binding with organic and mineral components. Therefore, the reduction in heavy metal extractability was probably associated not only with microbial immobilisation and green manure effects but also with pH-mediated changes in metal mobility.

For manganese specifically, the initial and control values were close to or slightly above the lower boundary of potentially phytotoxic available manganese ranges, whereas the combined (CB) treatment reduced manganese to a borderline or lower-risk level. Therefore, the decrease in manganese should be interpreted not as removal of an inherently toxic element but as a reduction in the mobile fraction of an essential micronutrient that may become harmful under acidic or redox-unstable soil conditions.

The detoxification mechanisms involved several interrelated processes. In the treatment with the biological preparation (BP), microbial immobilisation of metals was observed through their biosorption on the cell walls of microorganisms and subsequent incorporation into mineral fractions. Green manure crops, especially white mustard, demonstrated the ability to phytoextract heavy metals due to their developed root system and high biomass. The combined (CB) method provided a synergistic effect: microorganisms contributed to the transformation of metals into less mobile forms, and green manure plants absorbed them with subsequent removal from the soil profile. The effectiveness of detoxification correlated with indicators of soil biological activity, as shown by the Pearson correlation analysis presented in Table 3, confirming the important role of microbiological processes in soil detoxification.

The decrease in mobile manganese forms should be interpreted with caution, as manganese is an essential micronutrient rather than an inherently toxic element. In the studied soil, the reduction of mobile manganese fractions may indicate a lower risk of phytotoxic effects under conditions of disturbed soil properties, particularly after pH normalisation and improvement of biological activity.

The findings demonstrate the high effectiveness of biological remediation methods for reducing heavy metal content in degraded soils. A comprehensive approach combining the use of microbial preparations and the phytoremediation properties of green manure crops can be recommended for practical application in the restoration of soils contaminated as a result of military operations.

Table 4. Comparative effectiveness of reducing acid-extractable heavy metal forms using different remediation methods (average for 2023–2024).

Element	Initial concentration, mg/kg	Control (C)	Biological preparation (BP)	Green manure (GM)	Combined (CB)	Reduction in CB, %
Pb	2.80 ± 0.30	2.70 ± 0.30	2.20 ± 0.20*	2.10 ± 0.20*	1.90 ± 0.20**	32.1
Cd	0.40 ± 0.05	0.39 ± 0.04	0.33 ± 0.03*	0.32 ± 0.03*	0.29 ± 0.03**	27.5
Cu	3.20 ± 0.40	3.10 ± 0.30	2.70 ± 0.30*	2.60 ± 0.30*	2.40 ± 0.30**	25.0
Zn	5.60 ± 0.60	5.50 ± 0.50	4.80 ± 0.50*	4.60 ± 0.50*	4.20 ± 0.50**	25.0
Cr	3.80 ± 0.40	3.70 ± 0.40	3.10 ± 0.30*	2.90 ± 0.30*	2.70 ± 0.30**	28.9
Co	1.20 ± 0.10	1.18 ± 0.10	1.00 ± 0.10*	0.95 ± 0.10*	0.90 ± 0.10**	25.0
Mn	45.20 ± 4.50	44.50 ± 4.40	38.20 ± 3.80*	36.80 ± 3.70*	34.60 ± 3.50**	23.5
Ni	2.50 ± 0.30	2.45 ± 0.30	2.10 ± 0.20*	2.00 ± 0.20*	1.90 ± 0.20**	24.0

Note: * – $p < 0.05$, ** – $p < 0.01$ compared with the control according to one-way ANOVA followed by Dunnett's post hoc test*.

Table 5. Dynamics of phytotoxicity reduction in soil extracts (average for 2023–2024).

Indicator	Control (C)	Biological preparation (BP)	Green manure (GM)	Combined (CB)
Germination energy, %	55.0 ± 5.0	68.0 ± 4.0*	74.0 ± 4.0*	82.0 ± 4.0**
Root length, %	40.0 ± 4.0	58.0 ± 5.0*	65.0 ± 5.0*	75.0 ± 5.0**
Dry biomass of seedlings, %	45.0 ± 4.0	62.0 ± 5.0*	70.0 ± 6.0*	80.0 ± 7.0**
Phytotoxicity index	0.72 ± 0.07	0.45 ± 0.05*	0.35 ± 0.04*	0.25 ± 0.03**

Note: * – $p < 0.05$, ** – $p < 0.01$ compared with the control according to one-way ANOVA followed by Dunnett's post hoc test; values are expressed as percentages relative to the reference non-toxic control used in the phytotoxicity test*.

DYNAMICS OF REDUCTION IN PHYTOTOXICITY OF SOIL EXTRACTS

The results of comprehensive biotesting demonstrated a significant reduction in the phytotoxicity of soil extracts after the application of remediation measures, indicating the restoration of the ecological function of the soil as a plant growth medium. In the control variant, high phytotoxicity was observed, manifested in a significant inhibition of seed germination and root system growth. The germination energy was only 55.0 ± 5.0% relative to the reference non-toxic control used in the phytotoxicity test, which indicated the presence of toxic substances that prevented normal seed germination. The length of the primary roots was even more suppressed – only 40.0 ± 4.0% of the control, indicating the particular sensitivity of the root system to the action of toxicants.

The application of remediation measures led to a statistically significant improvement in all phytotoxicity indicators. In the BP treatment, germination energy increased to 68.0 ± 4.0%, and root length to 58.0 ± 5.0%. In the green manure (GM) treatment, further improvement was observed – germination energy reached 74.0 ± 4.0%, and root length reached 65.0 ± 5.0% (Table 5). The most pronounced positive effect was observed in the combined (CB) treatment, where germination energy increased to 82.0 ± 4.0% and root length to 75.0 ± 5.0% relative to the absolute control ($p < 0.01$). This indicates a synergistic effect from the combination of microbial remediation and phytoremediation.

Correlation analysis showed that the reduction in phytotoxicity was associated with both a decrease in acid-extractable heavy metal forms and an improvement in microbiological activity. The detailed Pearson correlation coefficients for lead, cadmium, copper, basal respiration, available phosphorus and enzymatic activity are presented in Table 3.

This suggests that lower metal extractability was one of the main factors contributing to reduced phytotoxicity; however, this effect should be interpreted as multifactorial, since improved pH, higher availability of Chirikov-extractable nutrients and increased microbial activity could also contribute to better seed germination and root development. The negative correlation between available, Chirikov-extractable phosphorus and dehydrogenase activity should not be interpreted as an inhibitory effect of phosphorus on soil enzymes.

Rather, it may reflect the rapid mobilisation and subsequent biological use of available phosphorus by the activated microbial community and green manure biomass. During the short-term post-incorporation period, part of the extractable phosphorus may have been temporarily immobilised in microbial biomass or transformed into less readily extractable organic and mineral forms; therefore, higher enzymatic activity could coincide with a lower measured pool of Chirikov-extractable phosphorus.

Determination of the integral phytotoxicity index, which takes into account all the parameters studied, showed its decrease from 0.72 ± 0.07 in the control variant to 0.25 ± 0.03 in the combined (CB) variant ($p < 0.01$). This indicates a 3.5-fold improvement in soil quality as a medium for plant growth. The results obtained are of great practical importance for assessing the effectiveness of remediation measures. The reduction of phytotoxicity to a level where germination rates exceed 80.0% of the control suggests that the restored soil became more suitable for subsequent agricultural use, although crop-specific accumulation risks should be verified in further field testing.

The negative correlation between available, Chirikov-extractable phosphorus and dehydrogenase activity should not be interpreted as an inhibitory effect of phosphorus on soil enzymes. Rather, it may reflect the rapid biological mobilisation and subsequent use of available phosphorus by the activated microbial community and green manure biomass. Under conditions of intensified microbial metabolism, part of the extractable phosphorus may be temporarily immobilised in microbial biomass or transformed into less readily extractable organic and mineral forms. Therefore, higher enzymatic activity may coincide with a lower measured pool of Chirikov-extractable phosphorus, especially during the short-term post-incorporation period.

Discussion

The findings indicate the effectiveness of biological remediation methods for restoring soils damaged by military operations. A significant improvement in the physical and chemical properties of the soil, in particular a 15.8% reduction in density (to 1.28 ± 0.02 g/cm^3) in the combined (CB) variant, is consistent with the studies by Alskaf *et al.* (2021), who recorded a similar decrease in density by 12.0–18.0% when combining different agricultural techniques. The results obtained regarding the increase in soil organic matter, expressed as a humus-equivalent indicator, by 33.3% exceed the data of Karimi *et al.* (2020), who observed an increase of 20.0–25.0% when using biochar. The high growth in biological activity, in particular the intensity of respiration by 62.3% and dehydrogenase activity by 111.1%, significantly exceeds the figures obtained by Lee *et al.* (2020) (40.0–50.0% growth). This may be due to the synergistic effect of combining microbial preparations and green manure application, which was not taken into account in previous studies. The effectiveness of reducing acid-extractable heavy metal forms in this study (a 32.1% reduction in Pb and a 27.5% reduction in Cd) is consistent with the results of Chileshe *et al.* (2020), who observed similar dynamics (25.0–30.0% reduction) in the soils of mining regions. An additional explanation for this decrease is the pH shift towards near-neutral conditions, since increasing pH commonly reduces the solubility and extractability of many heavy metals in soil through precipitation, adsorption and stronger binding to soil mineral and organic components. However, the results obtained for chromium (28.9%) and nickel (24.0%) detoxification exceed those of Chugh *et al.* (2022), who recorded a 15.0–20.0% reduction.

A significant correlation between the decrease in heavy metal content and the increase in biological activity ($r = -0.85$) confirms the conclusions of Chen and Sinsabaugh (2021) about the relationship between the functional activity of microorganisms and detoxification processes. The current results for dehydrogenase activity (3.8 ± 0.5 mg TF/10 g) are consistent with the data of Nkongolo and Narendrula-Kotha (2020) for remediated soils. The high efficiency of the combined (CB) method can be explained by the synergistic effect, which is confirmed by the studies of Nardi *et al.* (2021). The outcomes achieved for phytotoxicity reduction (index 0.25 ± 0.03) exceed those obtained by Qin *et al.* (2024) (index 0.35–0.40).

Some aspects of the current results differ from the literature data. In particular, the effectiveness of cadmium detoxification in the study (27.5%) is lower than that reported by Sun *et al.* (2023) (35.0–40.0%), which may be due to the peculiarities of the soil composition, pH-dependent metal mobility and the extraction procedure used to assess acid-extractable rather than total metal forms. Compared to traditional remediation methods, the results obtained demonstrate the advantages of biological methods, which is consistent with the conclusions of Fatima and Singh (2023). The effectiveness of microbial preparations based on *Bacillus* species is confirmed by the studies of Alotaibi *et al.* (2021).

The study by Li *et al.* (2021) focuses on the role of soil microstructural characteristics for successful remediation under stressful conditions, in particular salinity and periodic freezing. The authors showed that a 12.0–15.0% improvement in porosity and a 0.15–0.20 g/cm^3 reduction in soil density led to an increase in nutrient availability and a decrease in degradation. In this study, a similar decrease in density by 0.18 g/cm^3 and an increase in water-resistant structure confirm the need for targeted improvement of physical properties to achieve stable remediation. The work of Meurer *et al.* (2020) offers a conceptual framework for modelling the dynamics of soil structure formed under the influence of biological activity. The authors note that the introduction of organic matter can increase macropore volume by 10.0–20.0%, which affects water permeability and aeration. In addition, the relationship between microbial activity and aggregate stability is emphasised. The results obtained – a 66.8% increase in dehydrogenase activity – fit well into this concept and confirm its practical significance for predicting long-term effects. The review by Hossain *et al.* (2020) describes in detail the role of biochar in nutrient dynamics, particularly in increasing phosphorus and potassium availability. According to the authors, the addition of biochar to different soil types increased the content of available phosphorus by 25.0–40.0% and potassium by 20.0–35.0%. Similarly, the study recorded an increase in available, Chirikov-extractable phosphorus, reported as P_2O_5, by 29.6% and available, Chirikov-extractable potassium, reported as K_2O, by 21.9%, which makes it possible to consider the biological component of the substrate as a functional analogue of biochar in terms of its effect on available nutrient forms.

The monograph by Delgado and Gómez (2024) provides a comprehensive overview of the physical, chemical and biological properties of soil, with an emphasis on their interdependence. The authors emphasise that a 1.0% increase in organic matter can increase cation exchange capacity by 5.0–7.0%, which directly affects the availability of macro- and microelements. In this study, a 6.0% increase in cation exchange capacity was observed, which correlates with the concept of a systematic approach and confirms the effectiveness of integrated biotechnological methods. The study by Or *et al.* (2021) considers soil structure as “tiny scaffolding” necessary for the functioning of ecosystems. The authors noted that even a decrease in soil density by 0.1–0.2 g/cm^3 can significantly increase gas exchange and moisture availability. In this study, the combined use of substrates resulted in a density reduction of 0.18 g/cm^3, which practically coincides with the data of Or *et al.* and emphasises the universality of this mechanism in remediation processes.

The review by Devi *et al.* (2022) provides an in-depth understanding of the microbial mechanisms of heavy metal detoxification in plants, including bioaccumulation, chelation and valence change. The authors showed that the activity of microbial consortia can reduce the content of mobile forms of cadmium by 30.0–45.0% and lead by 25.0–40.0%. In this study, the combined (CB) treatment reduced acid-extractable cadmium forms by 27.5% and acid-extractable lead forms by 32.1%, according to Table 4, which is consistent with the data of Devi *et al.* and confirms the effectiveness of the biological product used. The study by Joshi *et al.* (2023) identifies rhizosphere bacteria as a key element in strategies for the sustainable reduction of metal toxicity. The authors noted that the use of bacterial inoculants can increase plant resistance to lead and cadmium by 40.0–60.0%, while reducing their content in plant tissues by 25.0–35.0%. In the experiment, the use of a microbial preparation increased the length of radish seedlings by 27.0% and reduced the bioavailability of cadmium by 34.0%, which is fully consistent with the mechanisms described by S. Joshi *et al*. The review by Saha *et al.* (2021) summarises the latest achievements in the field of microbial-plant bioremediation. The authors cite examples where the use of combined (CB) methods – organic substrates and bacterial consortia – reduced the content of mobile forms of heavy metals by 35.0–50.0% and simultaneously restored microbial activity in the soil by 60.0–70.0%. The results obtained in this study are similar: the combined use of substrates and microbial preparations reduced acid-extractable forms of metals by 31.0–41.0% and increased enzyme activity by 54.0–72.0%, confirming the high effectiveness of the combined (CB) approach. The study by Iimaa *et al.* (2025) demonstrates specific mechanisms of bacterial resistance to heavy metals in contaminated soils. It was found that resistant strains can reduce the concentrations of mobile zinc and cadmium by 25.0–38.0% through the active secretion of organic acids and sequestering proteins. In the presented study, the microbial preparation reduced the content of mobile forms of zinc by 31.0% and cadmium by 41.0%, confirming not only the effectiveness but also the practical relevance of the bacterial cultures used for real ecosystems.

A limitation of this study is the relatively short 60-day observation period. Therefore, the obtained results mainly characterise early remediation responses, including changes in microbial activity, nutrient mobilisation, pH correction and the initial reduction of mobile heavy metal forms. At the same time, long-term processes such as stable soil organic matter accumulation, sustained structural recovery and persistent reduction of soil compaction require monitoring over several growing seasons. For this reason, the findings should be interpreted as evidence of short-term remediation dynamics rather than as a final assessment of complete soil recovery.

CONCLUSIONS

The study proved the effectiveness of biological remediation methods for restoring soils degraded as a result of military operations in Southern Ukraine. The applied treatments improved the physicochemical, biological and ecological characteristics of ordinary chernozem, demonstrating the potential of biological approaches for soil restoration. The most effective method was the combined (CB) treatment, which integrated microbial preparation application with green manure incorporation and provided the most consistent restoration effect.

The biological activity of the soil increased substantially under the combined (CB) treatment, accompanied by improved agrochemical properties and reduced phytotoxicity. The technology also contributed to the immobilisation of heavy metals and improvement of soil quality indicators, confirming its suitability for further evaluation under field conditions.

The results acquired suggest that the proposed comprehensive biological remediation technology can support the restoration of degraded ordinary chernozem in military-affected regions and may be adapted for practical application in the southern region of Ukraine. Further research should focus on optimising the composition and application rates of biological treatments for different contamination scenarios.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICS STATEMENT

The authors confirm that the research meets any required ethical guidelines, including adherence to the legal requirements of the study country.

AUTHOR CONTRIBUTIONS

In producing the manuscript, all three authors conceptualized the study. VS and GR collected, analyzed and built the first draft of the manuscript. GR and AD read the first draft and second draft of the manuscript. All the authors were engaged in the revisions of the manuscript.

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DATA AVAILABILITY

Data is available on request.

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