



© 2025. The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution-ShareAlike 4.0 International Public License (CC BY SA 4.0, <https://creativecommons.org/licenses/by-sa/4.0/legalcode>), which permits use, distribution, and reproduction in any medium, provided that the article is properly cited.

Analysis of variations in heavy metal levels and soil microorganism counts resulting from shelling incidents in Ukraine

Ewa Moliszewska^{1*}, Iryna Bida², Kacper Matik¹, Aleksandra Ślusarczyk¹, Dominik Pawliczek¹, Olesia Havryliuk^{2,3}, Vira Hovorukha^{1,2}, Oleksandr Tashyrev^{1,2}

¹Institute of Environmental Engineering and Biotechnology, University of Opole, Poland

²Department of Extremophilic Microorganisms Biology, D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

³Laboratory of Sanitary and Environmental Microbiology (MSMLab)-UNESCO Chair on Sustainability, Department of Chemical Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, Terrassa, Spain

* Corresponding author's e-mail: ewamoli@uni.opole.pl

Keywords: soil, heavy metals, soil contamination, microorganisms, shelling

Abstract: Military activities affect soil conditions through contamination with metal-containing debris, such as projectile and rocket fragments, as well as bullet remnants, leading to the release of heavy metals and subsequent environmental contamination. The goal of our study was to examine the concentration of heavy metals in areas affected by shelling and to assess their impact on the population of soil microorganisms, including those exhibiting heavy-metal resistance. Metal concentrations were analyzed via an XRF analyzer. The study involved examining both soil samples and missile fragments. Microorganisms were isolated using Koch's and Hungate's roll tube methods. The concentration of iron in soil was the highest, reaching $8,1991.3 \pm 132.8$ ppm. The concentration of other metals (Ni, Cu, Cr) varied between 407.5 ± 8.0 ppm and 4.6 ± 2.1 ppm. Cobalt compounds were not detected at the projectiles impact sites. The number of aerobic chemoorganotrophic bacteria in all soil samples was in the range of $(1.8 \pm 0.2) \times 10^5$ – $(3.7 \pm 0.2) \times 10^5$ CFU/g, while chromium-resistant bacteria were, on average, an order of magnitude fewer. The number of anaerobic microorganisms ranged from $(1.4 \pm 0.2) \times 10^5$ to $(2.6 \pm 0.2) \times 10^5$ CFU/g. A follow-up study conducted after three months indicated a tendency for an increase in both aerobic and anaerobic bacteria, including metal-resistant strains. Overall, the total number of microorganisms in all soil samples showed an upward trend. These results show that soil microbial communities may play a role in the detoxification of heavy metals in contaminated soils.

Introduction

Military operations in Ukraine result in environmental pollution, specifically soil contamination with heavy metals (Bonchkovskiy et al. 2023). This issue is particularly critical because Ukraine is one of the world's major exporters of agro-industrial raw materials. Pollution inevitably reduces the amount of cultivable land, and crops grown on contaminated soil may become unfit for consumption (Melnyk et al. 2023). The primary sources of agricultural soil pollution include the movement of heavy machinery, the construction of various fortifications, artillery shelling, contamination from the remnants of destroyed military equipment and explosives, and the poisoning of water reservoirs due to mine flooding (Tytykalo et al. 2022). Soil affected by military actions has been reported to be contaminated with toxic metals and metalloids, organic and inorganic explosives, oxidizable

secondary explosives, and their metallic derivatives (Tauqeer et al. 2021). These substances seep into the soil, potentially contaminating groundwater, entering food chains, and posing risks to both humans and animal health (Petrushka et al. 2024, Albrekhtienė-Plačakė & Paliulis 2024). In total, more than 30% of the territory of Ukraine has suffered environmental damage due to pollution, destruction, and bombings related to the war (Kholoshyn et al. 2023). In this regard, there is an urgent need to study the impact of heavy metal contamination on soil microorganisms in order to develop strategies for mitigating its negative consequences.

Each explosion of a projectile or rocket releases a chemical cocktail into the environment and causes the complete destruction of all animals, plants and microorganisms within the blast radius (Mitryasova et al. 2024). As a result, the ground cover is subjected to an excessively high environmental burden. The physical destruction of the soil due to projectile ruptures,



Figure 1. Sampling of soil samples from shelling sites (Hostomel, Kyiv region, Ukraine): (a) craters formed by shell explosions; (b) debris of missiles and military equipment left over from hostilities

leading to the formation of craters 10–12 m in diameter and up to 5–6 m deep, is further exacerbated by an excessive contamination with heavy metals (Mitryasova et al. 2024). The most common polluting metals are copper, lead, antimony, chromium, arsenic, mercury, nickel, zinc, cadmium, titanium, vanadium, strontium, and iron (Tytykalo et al. 2022, Lindh & Lemenkova 2022). Explosives contain large amounts of lead and mercury. Zinc, copper, nickel, lead, and chromium are used to coat bullets, missiles, gun barrels, and military vehicles. Barium, antimony, boron serve as charge compounds for weapons, while tungsten, due to its high density (19.3 g/cm^3), is commonly used for kinetic bombardment (Petrushka et al. 2024).

Metal fragments from projectiles pose significant environmental hazards. They are primarily composed of a mixture of cast iron and steel, with sulfur and copper additives (Petrushka et al. 2024). Fragments from mines and unexploded ordnance reduce soil productivity, causing long-term, cumulative damage through destruction and displacement of soil structure and increasing soil vulnerability to water and wind erosion, which negatively impacts ecosystems (Shekhunova et al. 2022; Al-Qadri and Alsaiair 2023). On the most active days, approximately 50,000 different shells are fired at military positions on Ukrainian territory. For example, a tank high

explosive projectile, which shatters into fragments, weighs 15.7 kg. The weight of a 122 mm artillery shell is 21.76 kg, while a 155 mm shell weighs 36.45 kg. However, the exact number of the shells fired each day is difficult to determine. For example, if 50,000 shells of 122 mm caliber were fired in a single day, this would result in approximately 1,080,000 kg of steel fragments littering Ukrainian soil (Shahini et al. 2024).

Millions of kilograms of metal, including remnants of ammunition and destroyed or abandoned equipment, saturate the soil with iron compounds, which can suppress plant growth and disrupt soil microbial activity for extended periods (Shahini et al. 2024). Microorganisms play a key role in maintaining soil fertility and participate in all aspects of nutrient cycling. Their population can significantly decline under stress conditions, leading to an accumulation of heavy metal residues (Shebanina et al. 2024). Despite this, ecosystems also harbor metal-resistant microorganisms, which form the basis for bioremediation strategies to restore contaminated soils (Liu et al. 2021, Huminilovych et al. 2023). Metal-resistant bacteria most commonly belong to the genera *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Ralstonia*, *Stenotrophomonas*, and *Desulfovibrio*. These bacteria demonstrate a high capacity to precipitate heavy metals into insoluble, non-toxic compounds

Table 1. The description of samples from Hostomel, Kyiv region, Ukraine

No	Sample description	Coordinates of sampling	Place of sampling
1	Soil from a crater 5.0 m in diameter and 1.5 m deep, field	50°63'64.8"N 30°26'77.4"E	Hostomel, Kyiv region, Ukraine
2	Soil from a crater 4.0 m in diameter and 2.0 m deep, field	50°63'38.2"N 30°26'85.6"E	
3	Sandy-clay soil from a crater 16.0 m in diameter and 6.0 m deep, forest	50°69'89.9N 30°34'39.8E	
4	Soil not affected by shelling, field	50°38'11.3"N 30°16'03.9"E	
5	Metal missile fragments (1)	3-5 m close to 50°63'64.8"N 30°26'77.4"E	
6	Metal missile fragments (2)	3-5 m close to 50°63'64.8"N 30°26'77.4"E	

(Margaryan 2021). Compared to conventional chemical and physical remediation methods (Saleh et al. 2022), the use of microorganisms resistant to high metal concentrations offer a promising, cost-effective, and efficient biotechnological approach for restoring metal-contaminated soils (Bukhari and Rehman 2023).

Therefore, the goal of our study was to examine the concentration of heavy metals in areas affected by shelling and assess their impact on soil microbial populations, with a particular focus on microorganisms resistant to heavy metals.

Materials and methods

Characteristics of soil samples

Sampled were collected near Hostomel, a city in the Kyiv region of Ukraine, where active hostilities took place in the spring of 2022 (Fig. 1). Sampling was carried out twice, in October 2023 and February 2024, at a depth of 5 to 10 cm. This approach allowed for the assessment of changes in metal concentrations and microbial populations within samples collected from identical locations over time. The selected sample series is described in the Table 1. Three soil samples were collected directly from different craters formed by missile explosions (samples 1, 2, and 3). Additionally, one sample of gray soil was taken from a field not affected by shelling (sample 4). To analyze the metal content directly in the fragments, two metal samples were collected (samples 5 and 6).

Determination of heavy metals concentrations

The concentrations of cobalt, nickel, iron, copper, chromium compounds in soil and missile fragment samples were analyzed using the Niton XL5 Plus handheld XRF analyzer (Thermo Scientific, Waltham, MA USA). The samples included three soil samples from projectile impact sites (samples 1, 2, and 3), one soil sample from an unaffected site (sample 4), and two metal missile fragments (samples 5 and 6).

Microbiological analysis of soils

Aerobic microorganisms in the soil samples were isolated using Koch's method (Havryliuk et al. 2020). The method is based on Koch's principle, which states that each colony originates from a single microbial cell. Determining microbial counts by this method involves three key steps: 1. preparation of tenfold serial dilutions of the microbial sample, 2. Plating on an agar medium in Petri dishes plates, and 3. counting the resulting colony-forming units (CFU).

For this purpose, nutrient agar (NA) was used. To prepare 300 mL of NA with a 2% agar concentration, 3.9 g of nutrient broth (NB) powder (HiMedia Laboratories Pvt. Ltd., India), 6 g of agar, and 290.1 mL of distilled water were added to a 500 mL vial, mixed thoroughly, and autoclaved at 1.5 atm for 30 minutes. The sterilized medium was poured into sterile Petri plates and left at room temperature for 4-5 days. To determine the presence of metal-resistant microorganisms in the soil samples, Cr(VI) solution was added to the medium, achieving a final concentration of 200 ppm of cations.

Microorganisms were isolated from tenfold dilutions of soil suspensions prepared in a sterile physiological solution (0.85% NaCl). For this, 1 g of soil was added to 100 ml of NaCl solution, suspended for 15 min, and shaken periodically.

Microorganisms were plated using the surface (striped plate) method with a Drygalsky spatula. 0.2 mL of each dilution was added to the surface of the agar medium in Petri dishes with a sterile pipette and distributed using a sterile glass Drygalsky spatula. A new sterile spatula was used for each dilution. Each dilution was plated in triplicate, and microorganisms were incubated at 30 °C for 7 days.

To determine the number of anaerobic microorganisms, Hungate's roll tube method was used (Hungate et al. 1969). Tenfold dilutions of the samples were inoculated into 120 mL flasks containing 10 mL of NA in the presence of low-potential redox buffer (Fe(II) citrate, -200 to -150 mV) Before inoculation, the flasks were flushed with argon gas to remove oxygen. After inoculation, the flasks were hermetically sealed with rubber stoppers. The NA was evenly spread along the inner surface of the flask, forming a cylindrical agar layer for microbial growth.

Preparation of Cr(VI) solution

To prepare 250 mL of a chromate solution with a Cr(VI) concentration of 30,000 ppm, 28 g of K_2CrO_4 salt was added to a measuring cup and dissolved in 200 mL of distilled water. After complete dissolution, the solution was transferred to a 250 mL volumetric flask and diluted to the mark with distilled water. The solution was then poured into a sterile bottle and sealed. Usually, chromate solutions do not require additional sterilization (Havryliuk et al. 2020).

Statistical analysis of the results

The number of CFUs isolated from Hostomel soils on nutrient agar in 1 mL of suspension was calculated according to the formula:

$$M = a \cdot 10^n / V \quad (1)$$

where: M is the number of cells in 1 mL; a is the average number of colonies; 10^n is the dilution factor; and V is the volume of the inoculum (mL).

The number of microorganisms in the samples was converted to the total number of microorganisms per 1 g of completely dry sample using the formula:

$$X = a \times k \quad (2)$$

where: X is the number of CFUs per 1 g of completely dry sample; a is the number of cells in 1 mL of the microorganism suspension; and k is the humidity coefficient of the sample.

The humidity coefficient was determined using the formula:

$$k = 100 / (100 - H) \quad (3)$$

where: H is the moisture content of the sample.

The moisture content (H) of the samples was determined using the gravimetric method. The samples were dried in an oven at 105 °C until a constant weight was achieved. Humidity was calculated using the formula:

$$H = A / (m - A) \cdot 100\% \quad (4)$$

where: A is the weight of evaporated moisture; m is the total weight of the sample (Havryliuk et al. 2020).

Each experiment was performed in triplicate. Data analysis was carried out using Microsoft Excel 2013 and Origin 2017.

Table 2. The concentration of metals in missile fragments.

Sample description	Metal concentration [ppm]				
	Co(II)	Ni(II)	Cu(II)	Fe(III)	Cr(VI)
Metal missile fragments (1)	588.7 ± 33.8	110.2 ± 14.6	38.9 ± 8.8	1870.4 ± 71.1	9212.0 ± 95.8
Metal missile fragments (2)	72.9 ± 22.1	576.6 ± 21.1	152.9 ± 11.1	1758.6 ± 60.4	332.9 ± 30.5

Means and standard deviations (SDs) were determined with a 95% confidence level. Values were presented as mean±SD.

Results and Discussion

Investigation of soil contaminated with heavy metals due to shelling

The study involved measuring the concentration of the following heavy metals in missile fragments: iron, chromium, zinc, cobalt, and nickel. The concentrations of these metals in the fragments are presented in Table 2.

In metal fragments, the concentration of cobalt ranged from 72.9 ± 22.1 to 589 ± 34 ppm. The nickel content in rocket fragments was 110.2 ± 14.6 – 576.6 ± 21.1 ppm. Copper had the lowest concentration among the tested metals, ranging from 38.9 ± 8.8 to 152.9 ± 11.1 ppm, while iron had the highest concentration, ranging from 1758.6 ± 60.4 to 1870.4 ± 71.1 ppm (Table 2). The concentration of chromium varied widely (332.9 ± 30.5 – 9212.0 ± 95.8 ppm), which may indicate differences in the chemical composition of the missiles or their fragments.

The measurements results show that metal missile fragments left in the soil after shelling pose a potential environmental hazard. The detected metal concentrations reach hundreds or even thousands of ppm. Large fragments can be collected, but small fragments remain in the soil. Given that many of these small fragments range in size from 1 to 10 cm,

it is evident that metal mobilization from these fragments is a potential pathway for soil contamination.

The investigation of heavy metal concentrations in soil samples was conducted twice, with a three-month interval. Three soil samples were collected from craters formed by explosions near the city of Hostomel (Kyiv region, Ukraine) and one sample was taken from an area unaffected by shelling (Table 3). Though the study showed that cobalt was present in relatively high concentrations in missile fragments, cobalt compounds were not detected in either the compact sites or the soil unaffected by shelling. Nickel compounds, reaching up to 407.5 ± 8.0 ppm, were observed in two samples of affected soil. Additionally, small amounts (36.1 ± 4.2 ppm) were found in soil not exposed to shelling, indicating the natural distribution of nickel in ecosystems. Notably, during the second measurement, nickel concentrations were significantly lower, ranging from 6.0 ± 3.3 to 50.6 ± 6.6 ppm, which may suggest its transformation over time. Copper was not detected in the control soil, whereas its concentration in the affected soil samples varied between 4.6 ± 2.1 and 97.6 ± 3.9 ppm (Table 3). Considering the concentrations of nickel and copper in both the metal fragments and the affected soils, it can be assumed that these metals were likely introduced into the soil during projectile detonation, whereas no such pattern was observed for cobalt. Chromium concentrations in all soil samples remained relatively stable across both measurement periods, ranging from 17.1 ± 3.5 to 36.7 ± 3.5 ppm (Table 3). This level

Table 3. The concentration of metals in samples of soils.

Sample description	Measurement	Concentration [ppm]				
		Ni(II)	Co(II)	Cu(II)	Fe(III)	Cr(VI)
Soil from a crater 5.0 m in diameter and 1.5 m deep, field	1	<LOD ¹	<LOD	<LOD	2577.9 ± 39.6	<LOD
	2	6.0 ± 3.3	<LOD	4.6 ± 2.1	9067,8 ± 56.9	27.1 ± 3.7
Soil from a crater 4.0 m in diameter and 2.0 m deep, field	1	302.7 ± 9.1	<LOD	<LOD	15725.4 ± 86.6	31.0 ± 4.9
	2	50.6 ± 6.6	<LOD	4.7 ± 2.8	81991.3 ± 132.8	34.3 ± 7.5
Sandy-clay soil from a crater 16.0 m in diameter and 6.0 m deep, forest	1	407.5 ± 8.0	<LOD	97.6 ± 3.9	3256.8 ± 32.9	34.5 ± 4.5
	2	8.0 ± 3.1	<LOD	59.9 ± 3.2	2731.4 ± 30.1	36.7 ± 3.5
Soil not affected by shelling, field	1	36.1 ± 4.2	<LOD	<LOD	9783.2 ± 60.5	29.4 ± 3.9
	2	<LOD	<LOD	<LOD	5495.0 ± 46.7	17.1 ± 3.5

¹ <LOD – represents the value lower than limits of detection (limits of detection in the soil of Ni(II) – 11 ppm, of Co(II) – 5 ppm, of Cr(VI) – 4 ppm, of Cu(II) – 3 ppm)

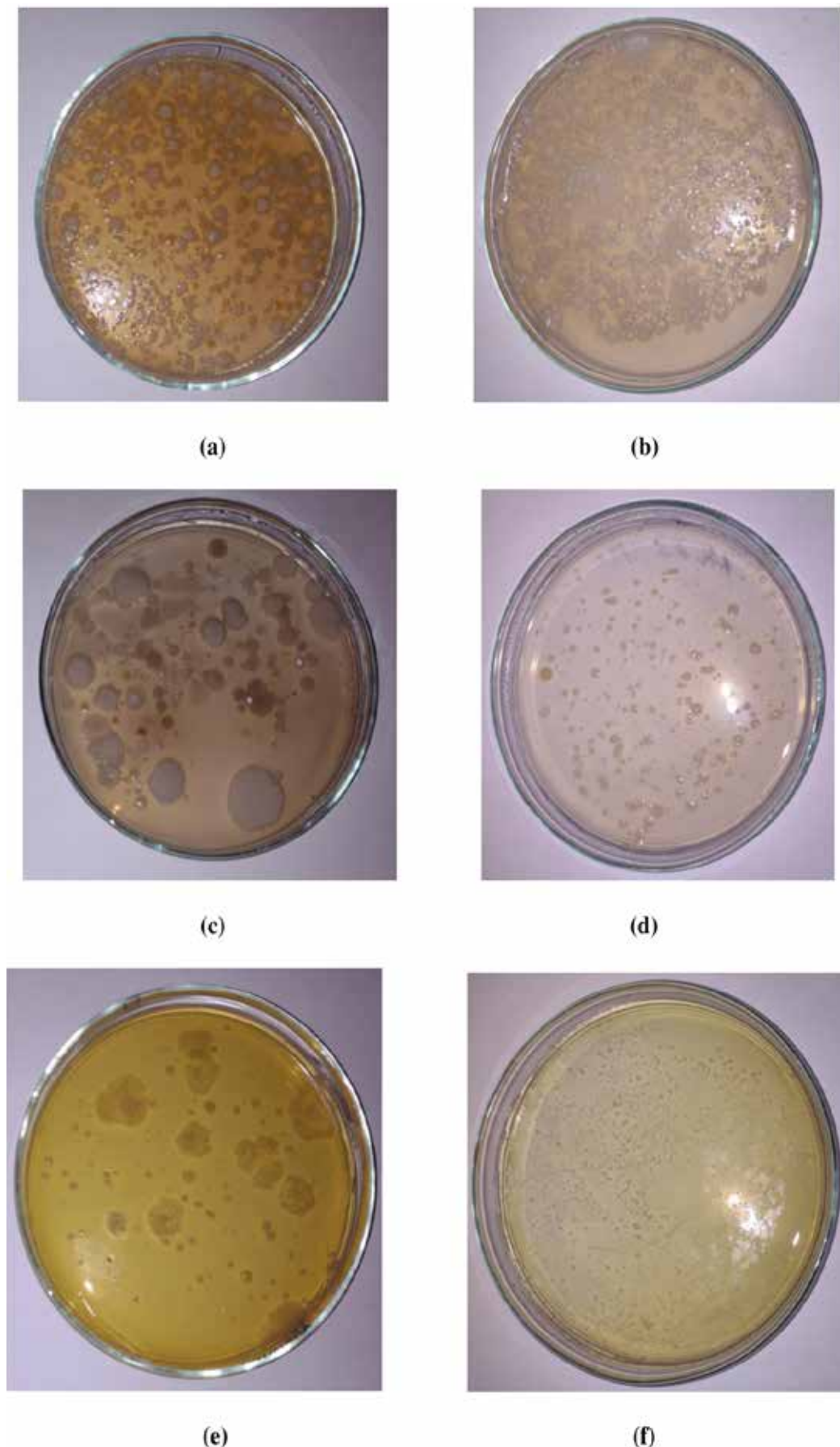


Figure 2. Microorganisms were isolated from the studied soil samples on nutrient agar medium: (a) microorganisms were isolated from sample 4 in October 2023 and (b) in February 2024; (c) microorganisms isolated from sample 1 in October 2023 and (d) in February 2024; (e) microorganisms isolated from sample 1 on medium with 200 ppm Cr(VI) in October 2023 and (f) in February 2024

of chromium contamination should be considered potentially hazardous to the environment.

The concentration of iron was the highest, which is inevitable considering that iron is one of the most prevalent metals in soil. Samples obtained from shelling sites exhibited an increase in iron concentration in the range of 3 to 5 times (up to $81,991.3 \pm 132.8$ ppm) during the research period (Table 3). This increase can be attributed to the mobilization of iron from missile debris left in the craters after the explosion. Due to the capability of soil microorganisms to produce organic acids, they can chelate iron compounds, converting them into soluble forms. Regarding other metals, their concentrations remained relatively stable throughout the study period.

Thus, it should be noted that the concentrations of chromium, copper, cobalt, nickel were similar in the studied samples, whereas the amount of iron differed significantly. Although the iron levels in soil are generally high, its increase observed after shelling suggests the mobilization of iron from missile debris, posing a potential environmental hazard. The quantity of toxic metals that can be released from these fragments is substantial. Therefore, developing new approaches

for the in-situ detoxification of metals in soil is both important and necessary.

Determination of the number of microorganisms in soils affected by shelling

To assess the impact of heavy metal pollution caused by hostilities on soil microorganisms, their number in the samples was determined. It is known that a significant portion of microorganisms have resistance mechanisms to heavy metals, regardless of whether they inhabit polluted or unpolluted areas with heavy metals. Therefore, we also examined the presence of metal-resistant microorganisms, specifically chromium-resistant ones (Figure 2).

According to the obtained results, the number of aerobic chemoorganotrophic bacteria in all samples during the initial study ranged from $(1.8 \pm 0.2) \times 10^5$ to $(3.7 \pm 0.2) \times 10^5$ CFU/g (Figure 3). The number of aerobic microorganisms resistant to chromium in samples 1, 2, and 3 was an order of magnitude lower, ranging from $(1.8 \pm 0.2) \times 10^4$ to $(2.3 \pm 0.2) \times 10^4$ CFU/g (Figure 3a, b, c). At the same time, microbial diversity also decreased. In sample 4, the number of chromium-resistant

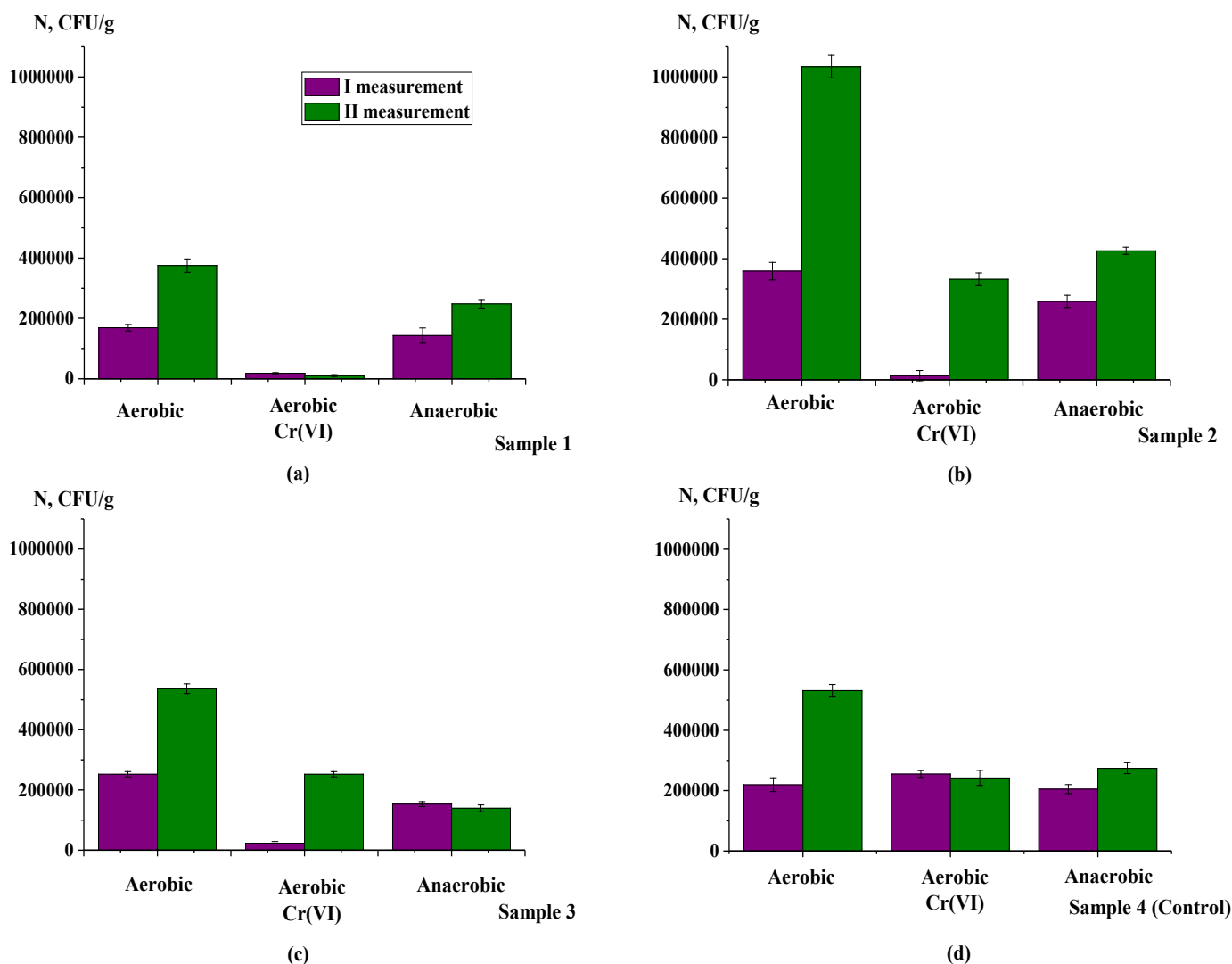


Figure 3. The number of microorganisms in samples of soils. Research options: (a) number of microorganisms of soil from sample 1, (b) number of microorganisms of soil from sample 2, (c) number of microorganisms of soil from sample 3, (d) number of microorganisms of soil from sample 4

microorganisms was comparable to that in the control medium without metals, reaching $(2.6 \pm 0.2) \times 10^5$ CFU/g. Thus, the presence of Cr(VI) at a concentration of 200 ppm in the agar nutrient medium did not inhibit microbial growth in the control soil sample 4 (Figure 3d). The number of anaerobic microorganisms in all samples turned out to be lower than that of aerobic ones, ranging from $(1.4 \pm 0.2) \times 10^5$ to $(2.6 \pm 0.2) \times 10^5$ CFU/g.

A follow-up study conducted after 3 months showed a general increase in the number of microorganisms across all soil samples. In particular, the number of aerobic chemoorganotrophic bacteria in samples 1, 3 and 4 approximately doubled, reaching $(3.8 \pm 0.2) \times 10^5$ – $(5.4 \pm 0.2) \times 10^5$ CFU/g (Figure 3a, c, d), while in sample 2, it tripled to $(1.0 \pm 0.2) \times 10^6$ CFU/g (Figure 3b). The number of aerobic chromium-resistant microorganisms increased by an order of magnitude in samples 2 and 3 (Figure 3b, c) but remained almost constant in soil samples 1 and 4 (Fig. 3a, d). The number of anaerobic microorganisms in samples 1, 2, and 4 increased to the values of $(2.5 \pm 0.2) \times 10^5$ – $(4.3 \pm 0.2) \times 10^5$ CFU/g (Figure 3a, b, d), while in sample 3, it remained almost unchanged (Figure 3c).

Thus, the study showed that soil samples contained between several dozen and several hundred thousand CFU/g. This suggests that the soil microbial community can interact with metals, either facilitating metal mobilization and increasing contamination or aiding in immobilization and detoxification. Notably, during microbial growth on a medium with 200 ppm Cr(VI), a darkening ring formed around some colonies, which indicates bacterial interaction with the studied metal and its deposition. These findings are crucial for understanding soil conditions after shelling.

Military actions pose a significant environmental threat due to the spread of various metal-containing materials in the soil, such as projectile and rocket fragments, bullet remnants, and other debris. Along with the release of pollutants during explosions, toxic metals enter the soil as parts of shells and missiles corrode over time. Soil contamination with toxic elements is a global issue affecting human health and food security (Khan et al. 2021). Heavy metals can accumulate in soils through natural processes, such as volcanic emissions, dust transport, and weathering of metal-rich rocks, or through anthropogenic activities, including mining, metallurgy, and the use of metal-containing chemicals (Ahmad et al. 2021). While industrial metal emissions cause significant environmental damage, preventive measures, forecasting, and treatment facilities can often mitigate these effects (Agboola et al. 2020). In contrast, military operations lead to severe and unpredictable soil pollution with far-reaching consequences (Broomandi et al. 2020).

Over two years of war in Ukraine, environmental pollution has reached an unprecedented scale, with soil contamination damages estimated at 18 billion dollars. According to the United Nations (UN), nearly 15% of Ukraine's land area is heavily mined, making it one of the most mined countries in the world. The demining process and detonation of mines will further contribute to soil and groundwater contamination. Explosions release projectile fragments containing cast iron, iron, carbon, sulfur, and copper, which can pollute both soil and underground water sources (Havryliuk et al. 2024). In addition, the large-scale disaster caused by the Kakhovka Dam

breach led to massive contamination of surrounding areas with toxic organic and inorganic compounds immobilized in sludge (Parakhnenko et al. 2023). Without proper bioremediation, such contamination will render the soil unsuitable for agriculture (Butu et al. 2020). In a military environment, complex multi-stage remediation methods, such as excavating contaminated soil, treating it, and returning to the site, are impractical. Therefore, rapid and effective strategies are needed to neutralize toxic metal compounds directly in the soil.

Biological methods are the most promising for neutralizing heavy metals in soils, especially given the scale of pollution. In particular, utilizing metal-resistant microorganisms from the same soils has proven effective. However, the composition and ratio of heavy metals in contaminated environments can vary significantly. Our research aimed to determine the concentration of the most representative metals, in particular iron, chromium, cobalt, nickel, zinc, and copper, in soil samples affected by shelling, as well as to assess the impact of military actions on soil microorganisms. The metals were distributed in decreasing concentration as follows: Fe(III) > Cr(VI) > Ni(II) > Cu(II) > Co(II). Moreover, the examined soil samples contained microorganisms ranging from several dozen to several hundred thousand CFU/g, indicating that microorganisms can survive in such environments and interact with metals through their metabolic activity. Thus, we hypothesize that the soil microbial community plays a role in the detoxification of heavy metals in contaminated soils.

According to the literature, several groups of metal-resistant bacteria have been previously isolated from contaminated soils. For example, Pb- and Cd-tolerant root bacteria were isolated from *Helianthus petiolaris* grown on Pb/Cd contaminated soil. A total of 105 microelement-tolerant rhizosphere and endophytic bacterial strains, belonging to eight different genera, have been reported. Most of these strains demonstrated the ability to immobilize trace elements on their cell wall (Saran et al. 2020). The microbial diversity of soils contaminated with varying concentrations of lead (Pb) and zinc (Zn) has also been investigated. The ten most common bacterial genera found across all samples were *Solirubrobacter* (Actinobacteria), *Geobacter* (Proteobacteria), *Edaphobacter* (Acidobacteria), *Pseudomonas* (Proteobacteria), *Gemmatimonas* (Gemmatimonadetes), *Nitrosomonas*, *Xanthobacter* and *Sphingomonas* (Proteobacteria), *Pedobacter* (Bacterioides) and *Ktedonobacter* (Chloroflexi) (Hemmat-Jou et al. 2018). Additionally, microbial groups isolated from Cd-, Pb-, and Zn-contaminated soils include both heavy metal-sensitive (*Ralstonia*, *Gemmatimona*, *Rhodanobacter*, and *Mizugakiibacter*) and tolerant (unidentified *Nitrospiraceae*, *Blastocatella*, and unidentified *Acidobacteria*) species (Guo et al. 2017).

Thus, heavy metal contamination of soil due to military operations is a critical environmental issue. Contamination can occur both from the direct explosion of projectiles and the gradual release of metals from rocket fragments left in the soil. The accumulation of toxic metals poses a threat to the environment.

Conclusions

The studies have examined the distribution of heavy metals in contaminated soils and metal projectile fragments. To further develop methods for neutralizing these metals, it is necessary

to investigate the impact of pollution on soil microorganisms and the interactions between microorganisms and metals. Soil samples affected by shelling showed microbial populations ranging from several dozen to several hundred thousand of CFU/g, including aerobic and anaerobic bacteria, as well as chromium-resistant bacteria. This finding highlights the potential for developing new ecological biotechnologies for cleaning up contaminated ecosystems. We recommend continuing such research to monitor factors influencing the natural soil environment.

Acknowledgements

This work was carried out at MCBR UO (International Research and Development Center of the University of Opole), established as part of a project co-financed by the European Union through the European Regional Development Fund, RPO WO 2014-2020, Action 1.2 Infrastructure for R&D. Agreement No. RPOP.01.02.00-16-0001/17-00 dated January 31, 2018.

The authors express their gratitude to Professor Volodymyr Bakhmutov (Institute of Geophysics of the National Academy of Sciences of Ukraine) for his assistance with soil sampling in the Kyiv region, Ukraine.

Funding

This research was funded by the Polish National Agency for Academic Exchange (NAWA), grant number BPN/GIN/2023/1/00028/U/00001, the FEMS Ukraine Support Grant, grant number 1836, and the project “Supporting the cooperation of the University of Opole with Ukrainian universities within the FORTHEM Alliance 2024”. The publication of this paper was financed by the Polish Agency for Academic Exchange, agreement No. BNI-UE-2023-9.

References

- Agboola, O., Babatunde, D. E., Isaac Fayomi, O. S., Sadiku, E. R., Popoola, P., Moropeng, L., Yahaya, A. & Mamudu, O. A. (2020). A review on the impact of mining operation: Monitoring, assessment and management. *Results in Engineering*, 8, 100181. DOI:10.1016/j.rineng.2020.100181
- Ahmad, W., Alharthy, R. D., Zubair, M., Ahmed, M., Hameed, A. & Rafique, S. (2021). Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. *Scientific Reports*, 11(1), 17006. DOI:10.1038/s41598-021-94616-4
- Albrekzienė-Plačakė, R. & Paliulis, D. (2024). Investigation on applying spropel for removal of heavy metals (cadmium, chromium, copper, and zinc) from aqueous solutions. *Archives of Environmental Protection*, 50, 2, pp. 55-64. DOI:10.24425/aep.2024.150552
- Al-Qadri, F. A. & Alsaier, R. (2023). Silica ash from waste palm fronds used as an eco-friendly, sustainable adsorbent for the removal of copper (II). *Archives of Environmental Protection*, 49(2), pp. 30-39. DOI:10.24425/aep.2023.145894
- Bonchkovskyi, O. S., Ostapenko, P. O., Shvaiko, V. M. & Bonchkovskyi, A. S. (2023). Remote sensing as a key tool for assessing war-induced damage to soil cover in Ukraine (the case study of Kyivska territorial hromada). *Journal of Geology, Geography and Geoecology*, 32(3), 474-487. DOI:10.15421/11234
- Broomandi, P., Guney, M., Kim, J. R. & Karaca, F. (2020). Soil Contamination in Areas Impacted by Military Activities: A Critical Review. *Sustainability*, 12(21), 9002. DOI:10.3390/su12219002
- Bukhari, D. A. & Rehman, A. (2023). Metal-resistant bacteria as a green bioresource for arsenic remediation in wastewaters. *Current Opinion in Green and Sustainable Chemistry*, 40, 100785. DOI:10.1016/j.cogsc.2023.100785
- Butu, A., Grozea, I., Sarac, I. & Butnariu, M. (2020). Global Scenario of Remediation Techniques to Combat Pesticide Pollution. [In] R. A. Bhat, K. R. Hakeem, & M. A. Dervash (Eds.), *Bioremediation and Biotechnology*, Vol 2 (pp. 47-72). Springer International Publishing. DOI:10.1007/978-3-030-40333-1_4
- Guo, H., Nasir, M., Lv, J., Dai, Y. & Gao, J. (2017). Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. *Ecotoxicology and Environmental Safety*, 144, pp. 300-306. DOI:10.1016/j.ecoenv.2017.06.048
- Havryliuk, O., Bida, I., Hovorukha, V., Bielaieva, Y., Liubinska, A., Gladka, G., Kalinichenko, A., Zaimenko, N., Tashyrev, O. & Dziuba, O. (2024). Application of Granular Microbial Preparation and Silicon Dioxide Analcime for Bioremediation of Ecocide Areas. *Sustainability*, 16(3), 1097. DOI:10.3390/su16031097
- Havryliuk, O. A., Bida, I. O., Hovorukha, V. M., Danko, Y. P., Gladka, G. V., Sachko, A. V., Yastremska, L. S., Tashyrev, O. B. & Muchnyk, P. V. (2020). Metal-resistant microorganisms of tap water: theoretical justification and biotechnological application. *Problems of Environmental Biotechnology*. 1-2. DOI:10.18372/2306-6407.1-2.16059
- Hemmat-Jou, M. H., Safari-Sinegani, A. A., Mirzaie-Asl, A. & Tahmourespour, A. (2018). Analysis of microbial communities in heavy metals-contaminated soils using the metagenomic approach. *Ecotoxicology*, 27(9), pp. 1281-1291. DOI:10.1007/s10646-018-1981-x
- Huminiłowych, R., Stadnik, V., Sozanskyi, M., Pidlisnyuk, V. & Ivaniuk, A. (2023). Monitoring of Soils Contaminated by Military Activities During Phytoremediation Using *Miscanthus X Giganteus*. *International Conference of Young Professionals «GeoTerrace-2023»*, 1-5. DOI:10.3997/2214-4609.2023510113
- Hungate, R. E. (1969). Chapter IV A Roll Tube Method for Cultivation of Strict Anaerobes. [In] *Methods in Microbiology*, 3, pp. 117-132. Elsevier. DOI:10.1016/S0580-9517(08)70503-8
- Khan, S., Naushad, Mu., Lima, E. C., Zhang, S., Shaheen, S. M. & Rinklebe, J. (2021). Global soil pollution by toxic elements: Current status and future perspectives on the risk assessment and remediation strategies – A review. *Journal of Hazardous Materials*, 417, 126039. DOI:10.1016/j.jhazmat.2021.126039
- Kholoshyn, I. V., Syvyj, M. J., Mantulenko, S. V., Shevchenko, O. L., Sherick, D. & Mantulenko, K. M. (2023). Assessment of military destruction in Ukraine and its consequences using remote sensing. *IOP Conference Series: Earth and Environmental Science*, 1254, 1, 012132. DOI:10.1088/1755-1315/1254/1/012132
- Lindh, P. & Lemenkova, P. (2022). Soil contamination from heavy metals and persistent organic pollutants (PAH, PCB and HCB) in the coastal area of Västernorrland, Sweden. *Gospodarka Surowcami Mineralnymi–Mineral Resources Management*, 38, 2, pp. 147-168. DOI:10.24425/gsm.2022.141662

- Liu, L., Xia, M., Hao, J., Xu, H. & Song, W. (2021). Biosorption of Pb (II) by the resistant *Enterobacter* sp.: Investigated by kinetics, equilibrium and thermodynamics. *Archives of Environmental Protection*, 47, 3, pp.28-36. DOI:10.24425/aep.2021.138461
- Margaryan, A. (2021). Diversity and Application of Heavy-Metal Resistant Microbes. [In] Singh, R.P., Manchanda, G., Bhattacharjee, K. & Panosyan, H. (Eds.), *Microbes in Microbial Communities*, pp. 153–174) Springer Singapore. DOI:10.1007/978-981-16-5617-0_7
- Melnyk, O., Shevchenko, O., Kuzmin, O. & Niemirich, O. (2023). Risks of toxic environmental pollution from military operations. *Food security: modern challenges and mechanisms to ensure*, 25. DOI:10.5281/zenodo.7859027
- Mitryasova, O., Smyrnov, V., Koszelnik, P., Salamon, I., Smyrnova, S. & Mats, A. (2024). Geochemical Anomalies of the Heavy Metals in the Industrial and Urban Agglomeration Soils. *Ecological Engineering & Environmental Technology*, 25, 3, pp. 165–177. DOI:10.12912/27197050/177838
- Parakhnenko, V. H., Zadorozhna, O. M., Liakhovska, N. O. & Blahopoluchna, A. H. (2023). Environmental assessment of chemical pollution of soils as a result of the war. *Taurian Scientific Herald*, 131, pp. 367–373. DOI:10.32782/2226-0099.2023.131.46
- Petrushka, K., Malovanyy, M. S., Skrzypczak, D., Chojnacka, K. & Warchoń, J. (2024a). Risks of Soil Pollution with Toxic Elements During Military Actions in Lviv. *Journal of Ecological Engineering*, 25, 1, pp.195–208. DOI:10.12911/22998993/175136
- Saleh, T. A., Mustaqeem, M. & Khaled, M. (2022). Water treatment technologies in removing heavy metal ions from wastewater: A review. *Environmental Nanotechnology, Monitoring & Management*, 17, 100617. DOI:10.1016/j.enmm.2021.100617
- Saran, A., Imperato, V., Fernandez, L., Gkorezis, P., d'Haen, J., Merini, L. J., Vangronsveld, J. & Thijs, S. (2020). Phytostabilization of Polluted Military Soil Supported by Bioaugmentation with PGP-Trace Element Tolerant Bacteria Isolated from *Helianthus petiolaris*. *Agronomy*, 10, 2, 204. DOI:10.3390/agronomy10020204
- Shahini, E., Shebanina, O., Kormyshkin, I., Drobitko, A. & Chernyavskaya, N. (2024). Environmental consequences for the world of Russia's war against Ukraine. *International Journal of Environmental Studies*, 81, 1, pp. 463–474. DOI:10.1080/00207233.2024.2302745
- Shebanina, O., Kormyshkin, I., Bondar, A., Bulba, I. & Ualkhanov, B. (2024). Ukrainian soil pollution before and after the Russian invasion. *International Journal of Environmental Studies*, 81, 1, pp. 208–215. DOI:10.1080/00207233.2023.2245288
- Shekhunova, S. B., Stadnichenko, S. M. & Siumar, N. P. (2022). The Issue of Assessing Environmental Risks and Economic Losses of Ukraine's Subsoil as a Result of Russian Military Aggression Against Ukraine. *16th International Conference Monitoring of Geological Processes and Ecological Condition of the Environment*, 1–5. DOI:10.3997/2214-4609.2022580249
- Tauqeer, H. M., Karczewska, A., Lewińska, K., Fatima, M., Khan, S. A., Farhad, M., Turan, V., Ramzani, P. M. A. & Iqbal, M. (2021). Environmental concerns associated with explosives (HMX, TNT, and RDX), heavy metals and metalloids from shooting range soils: Prevailing issues, leading management practices, and future perspectives. [In] *Handbook of Bioremediation* (pp. 569–590). Elsevier. DOI:10.1016/B978-0-12-819382-2.00036-3
- Tytykalo, R., Pavlovska, N. & Andriiets, M. (2022). Economic and administrative methods of restoration by local governments of the environment of Ukraine destroyed as a result of military operations. *Baltic Journal of Economic Studies*, 8, 5, pp. 184–190. DOI:10.30525/2256-0742/2022-8-5-184-190

Analiza zmian w poziomach metali ciężkich i liczbie mikroorganizmów glebowych w wyniku ostrzałów na Ukrainie

Streszczenie. Działania militarne negatywnie wpływają na warunki glebowe poprzez zanieczyszczenie odpadami zawierającymi metale, takimi jak fragmenty pocisków i rakiet, a także pozostałości pocisków. Materiały te ulegają korozji w glebie, co prowadzi do uwolnienia metali ciężkich i skażenia środowiska. Celem naszych badań było zbadanie stężenia metali ciężkich na obszarach dotkniętych ostrzałami i ocena wpływu tego zanieczyszczenia na populację mikroorganizmów glebowych, z uwzględnieniem mikroorganizmów odpornych na metale ciężkie. Stężenie metali (żelaza, chromu, miedzi, kobaltu i niklu) analizowano za pomocą przenośnego analizatora XRF Niton XL5 Plus. Badanie obejmowało zarówno próbki gleby, jak i fragmenty pocisków. Mikroorganizmy tlenowe z badanych próbek gleby izolowano metodą Kocha, natomiast beztlenowe oznaczano metodą rurkową Hungate'a. Stężenie żelaza okazało się najwyższe w glebie, do $81991,3 \pm 132,8$ ppm. Stężenie innych metali (Ni, Cu, Cr) wahało się w granicach $407,5 \pm 8,0$ - $4,6 \pm 2,1$ ppm, w zależności od próbki. Związków kobaltu nie wykryto w miejscach trafień pocisków. Liczba tlenowych bakterii chemoorganotroficznych we wszystkich próbkach gleby mieściła się w zakresie $(1,8 \pm 0,2) \times 10^5$ - $(3,7 \pm 0,2) \times 10^5$ jtk/g, podczas gdy bakterii odpornych na chrom było średnio o rząd wielkości mniej. Liczba mikroorganizmów beztlenowych w próbkach mieściła się w zakresie $(1,4 \pm 0,2) \times 10^5$ - $(2,6 \pm 0,2) \times 10^5$ jtk/g próbki. Badanie kontrolne przeprowadzone po trzech miesiącach wykazało tendencję do wzrostu zarówno bakterii tlenowych, w tym odpornych na metale, jak i beztlenowych. W szczególności liczba tlenowych bakterii chemoorganotroficznych wzrosła do $(1,0 \pm 0,2) \times 10^6$ jtk/g. Wyniki badań wskazują, że społeczności mikroorganizmów glebowych mogą odgrywać rolę w detoksykacji metali ciężkich w zanieczyszczonych glebach.