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ENVIRONMENTAL TOXICITY ASSESSMENT OF MINING WASTE FROM AN ABANDONED Zn-Pb MINE

Purpose. To assess the impact of mining waste on the heavy metal content of water surfaces, plants, and topsoil near the tailings dam of a Zn-Pb mine using both biotests and analytical methods.

Methodology. A battery of microbiotests on different animal and plant species was carried out, making it possible to evaluate the toxic effect of residues and surrounding soils on living organisms. Furthermore, the possible relationship between the observed toxicity and the results of the physicochemical analysis of the samples was studied.

Findings. The tests showed that the topsoil in contact with the tailings dam is slightly toxic to the living organisms used while the mining tailings are toxic or even very toxic. The heavy metal content of the samples is particularly high for Fe, Zn, Pb and Cu. The correlation of physic-chemical parameters and the results of microbiotests using the principal components analysis (PCA) and the multiple correspondence factor analysis (MCFA) indicate that the toxicity of tailings and the surrounding topsoil can be associated with anthropogenic mining activity.

Originality. The study aimed to assess the impact of mining waste on the heavy metal content using biotests and analytical methods. The evaluation considers the concentrations of the samples (highly concentrated samples and samples after dilution) and the different phases of exposure (solid, liquid) for a more detailed assessment of the potential toxicity of the samples.

Practical value. It is important to conduct a comprehensive assessment of mining waste and the risks it may pose to humans and the environment in order to develop an adequate rehabilitation plan.

Keywords: *toxicity, mine waste, lead-zinc (Zn-Pb) mine, microbiotests, tailings, soil, heavy metals, mining activity*

Introduction. The El Abed mine (Tlemcen, Algeria) was opened in 1952 for the exploitation of Zn-Pb and it was closed in 2002 without any rehabilitation plan. The processing of the Zn-Pb ore has been carried out in the El Abed mine since 1972 with a capacity of 2,000 tons per day. The discharges after treatment are transported and stored in a tailings pond located two kilometers to the south of the mining installation; they are deposited in the open air and exposed to climatic factors.

The tailings pond has a dike that no longer seems to be able to ensure the stability (neither physical nor chemical) of the mining wastes. The fine fraction of mining residues is particularly subject to wind erosion at the slightest gust of wind. This can negatively affect air, water, and local agriculture because tailings erosion caused by the weathering can result in earth sediments appearing in nearby soils, waters, and drainage systems [1, 2]. The earth sediments resulting from tailings erosion inherently contain heavy metals, which can be harmful to organisms living in contact with these sediments.

Heavy metals are some of the most important components of environmental pollution, exerting dangerous long-term effects on soil ecosystems [3]. Heavy metals such as antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc are present in the environment and living beings are very exposed to them. In small doses, they are necessary for the proper functioning of the metabolism, but they can become toxic or even very toxic in large doses. Some heavy metals have harmful effects on health, even at low concentrations [4]. Indeed, heavy metals present a risk to human health through ingestion of contaminated water and bio-organisms [5]. Exposure to heavy metals can cause physical and neurological health disorders, in the long term it can cause more serious diseases like Alzheimer's disease, Parkinson's disease, multiple sclerosis, and cancer [6–9]. Therefore, proper assessment of the degree of heavy metal contamination is very important to determine what measures should be taken to mitigate the extent of heavy metal exposure to protect the environment and human health.

The aims of this study are:

1. To assess the environmental toxicity of El Abed mine tailings, the surrounding topsoil, and their leachates using three bioassays: Phytotestkit, Ostracodtoxkit F and Daphtoxkit F Magna.

2. To correlate the chemical composition of the tailings and the surrounding topsoil, the enrichment factor (EF) and the geo-accumulation index (IGEO) of the heavy metals using a principal component analysis (PCA) to determine the possible source of these heavy metals. A multiple correspondence factor analysis (MCFA) was also performed to account for the toxicity variable.

Microbiotests are an effective tool in assessing the risk associated with the presence of chemical substances in soil and water. Microbiotests have proven to be a sensitive and inexpensive means of routine screening of aquatic environments and soil samples, they are recommended by the implementation of the Water Framework Directive. The impact on living test organisms is measured as the result of the effect of all potential pollutants contained in the samples [8, 9].

Principal component analysis (PCA) is one of the statistical methods used to evaluate the relationship between different concentrations of heavy metals and samples. PCA is widely used to reduce data sizes to analyze relationships between observed variables. The enrichment factor (EF) and the geo-accumulation index (IGEO) are mainly used to assess the ecological risks presented by heavy metal concentrations in relation to the value of the geochemistry background [10].

This study will contribute to the knowledge necessary to choose the most appropriate soil and water remediation techniques to resolve the environmental problems caused by metal contamination in this mining area and which can harm crops, animal and human health.

Materials and methods. ***Site description.*** The El-Abed mine is located in the mining village of El-Abed, in the commune of El Bouihi, in the wilaya of Tlemcen, on the Algerian-Moroccan border. It is located 94 kilometers southwest of Tlemcen and 60 kilometers south of Maghnia (Fig. 1). The El Abed deposit represents the eastern part of one of the largest lead-zinc mining fields in North Africa, Sidi Boubekeur and Touissit in Morocco represent the western part of it. The exploitation of the deposit allowed the extraction of more than 12 million tons of ore with average grades of 1.04 % for lead and 4.90 % for zinc.

The region presents high plateau lands with mountainous relief to the north and flattened topography to the south. According to the numerous works that have been carried out in the region since 1852, our study area, El Abed, belongs to the Ghar Rouban mountains, which are part of the Tlemcenian domain. The Tlemcenian domain is the northern margin of the Algerian-Moroccan Highlands. From a stratigraphic point of view, the Ghar Roubane mountains are made up of a Paleozoic base made up of metamorphic, sedimentary, and volcanic formations intersected by magmatic intrusions. An essentially discordant and transgressive Mesozoic carbonate cover on the Paleozoic basement. The El Abed deposit is characterized by metallic mineralization (Galena, Blend, Pyrite, Chalchopyrite, Marcasite, Hematite, Gray Copper, Cerussite, etc.) and a non-metallic gangue mineralization (Quartz, Dolomite, Kaolinite, Muscovite, Tourmaline, Apatite, Epidote, etc.).

Fig. 1. Localization of the studied area and sampling sites distribution (Map source: Google Earth Pro)

The studied area is characterized by a continental climate with harsh winters (–5 °C, minimum recorded) and very hot summers (+45 °C, maximum recorded). Precipitation is relatively low, with an interannual average of 300 mm, it is very unevenly distributed throughout the year. For vegetation, there are generally holm oaks, pines, and lots of brush. There are also large areas of fields and meadows used by the local population for agro-pastoral activities. Pastoralism is considered the main economic activity of the region.

Currently, the mining village of El Abed has a few hundred inhabitants. The population is low but shows positive development. The child population under four years old represents more than 9 % of the population. A public mining school was created in 2004 based on the infrastructure of the former zinc and lead mining complex of El Abed; it is responsible for carrying out training activities in professions linked to mining activity.

Physico-chemical analyzes. Field observations and sampling campaigns to collect samples were carried out in May 2018 and October 2019. We focused our study on the tailings deposit as well as the topsoil surrounding it. Sampling is done to cover the entire tailings basin as well as the topsoil in contact with these tailings. The samples were taken using an auger (10 to 20 cm deep), and they were stored in transparent plastic bags. The collected samples were dried, homogenized then passed through a 1 mm sieve before analysis.

The samples were analyzed to determine heavy metals concentrations (Cd, Cu, Fe, Mn, Pb, and Zn) in the tailings and the topsoil samples. Metals were extracted from the samples with 2M HNO_3: 10 g of soil sample with 100 ml of 2M HNO_3 were shaken for 1h [11]. Metals concentration in the filtered extracts was measured by inductively coupled plasma atomic emission spectroscopy instruments (ICP-AAS). The pH (hydrogen potential) is also measured for all the samples using a pH meter with a ratio (1:2.5) distilled water/simple.

To quantify the contamination induced by the tailings, two indicators were used: enrichment factor and geo-accumulation index. EF and IGEO indexes are calculated to correlate the concentrations of heavy metals in the tailings and in the surrounding topsoil with their concentrations in the geochemical background measured far from the tailings pond to estimate the involvement of the anthropogenic source in the pollution by heavy metals compared to the involvement of the natural source [12].

The Enrichment Factor (EF) was calculated as follows: EF = (CX/CFe)Sample/(CX/CFe)Shale where CX is the concentration of the metal X, CFe the concentration of iron. $EF < 1.5$ indicates no enrichment, $1.5 < EF < 3$ minor enrichment, $3 < EF < 5$ moderate enrichment, $5 < EF < 10$ high-moderate enrichment, $10 < EF < 25$ high enrichment, $25 < EF < 50$ very high enrichment, and $EF > 50$ extremely high enrichment.

The geo-accumulation index (IGEO) formula is: $Igeo = \log 2\,(Cn/1.5\,Bn)$ where Cn is the concentration of the concerned element in the soil sample and Bn is the concentration of the concerned element in the shale. The geo-accumulation index (*Igeo*) indicate several classes of metal pollution: $Igeo < 0$ indicates uncontaminated, $0 < Igeo < 1$ uncontaminated to moderately contaminated, $1 < Igeo < 2$ moderately contaminated, $2 < Igeo < 3$ moderately to heavily contaminated, $3 < Igeo < 4$ heavily contaminated, $4 < Igeo < 5$ heavily to extremely contaminated, $Igeo \geq 5$ extremely contaminated.

To combine all the obtained results, principal component analysis (PCA) is applied to assess the levels of heavy metal contamination in the studied area. The main components providing information on the indices evaluated are concentrations of heavy metals, pH, enrichment factor, and the geo-accumulation index. To add information on toxicity, which is a qualitative variable, multiple correspondence factor analysis (MCFA) was also performed [13].

Microbiotests. The toxicity tests were carried out using direct contact microbiotests: phytotoxkit (plants/sediments) [14], Ostracodtoxkit F (crustaceans/sediments) [15] and Daphtoxkit F magna (crustaceans/water) [16]. Toxkits are microbiotests containing all the material necessary to perform simple, rapid, sensitive, and reproducible toxicity tests at low cost. They are particularly suitable for testing the toxicity of chemicals and wastes released in aquatic as well as terrestrial environments. They were carried out following the procedures recommended by the developer (Professor Dr. G. Person in association with the Laboratory of Environmental Toxicology and Aquatic Ecology of the University of Ghent in Belgium). The advantage of the Toxkits microbiotests is that the test organisms are incorporated in the kits in an immobilized form from which they can be hatched at any time before the toxicity tests are performed.

Phytotestkit.The test was carried out to assess the toxicity of the mine tailings, the surrounding topsoil, and their leaching water using three plants: Sorghum saccharatum (sorghum), Lepidium sativum (watercress), and Sinapsis alba (mustard). Inhibition of seed germination and inhibition of root elongation were measured.

The procedure consists of using propylene plates (25 × × 15.5 × 0.8 cm) with two compartments, the lower compartment of the test plate was filled with a foam pad and filter paper soaked in leaching water (20 cm^3), and the seeds were placed in a row 1cm below the midline of the plate. The plates, once prepared, are incubated in a horizontal position at 25 °C, in the dark for 72 hours. Then they were scanned, and germination and root length were measured using ImageJ software.

Ostracodtoxkit. It is a simple, rapid, sensitive, and low-cost toxicity test. It is developed to detect and quantify the toxicity of freshwater sediments and also soils contaminated by organic and inorganic pollutants. The experiments were carried out with larvae of the freshwater ostracod Heterocypris incongruens. The principle of the test consists of putting an ostracod larva in direct contact with the sediments for six days to then determine the percentage of mortality and the growth of the crustaceans and compare them with the results obtained in reference sediment (not toxic).

The steps followed during the test can be summarized as follows: the H. Incongruens eggs are incubated in standard fresh water at 25 °C under continuous lighting, and the larvae are prefed 48 hours after the start of incubation to be collected 52 hours after the start of incubation. Assays are performed in 20-cell polystyrene multicell plates with ten organisms in each cell in three replicates, each cell to contain 1ml sediment and 2 ml standard fresh water. The plates are incubated in the dark at 25 °C for six days. Ostracod mortality and growth are determined at the end of the incubation period and compared to the control test.

Daphtoxkit. The Daphtoxkit uses the eggs (ephippia) of the crustaceans Daphnia magna or Ceriodaphnia dubia, when these eggs are placed in specific environmental conditions they develop after about three days into newborns which can then be used immediately for the toxicity tests.

To activate the ephippia, they must be transferred to a petri dish containing 15 ml of standard fresh water and incubated for 72 hours at a temperature of 20 to 22 °C under continuous light. 48 hours after the start of incubation, pre-feeding with a suspension of spirulina microalgae is necessary to prevent mortality from starvation of newborns. At the end of incubation, the daphnids are transferred to multi-cell polycarbonate plates, each plate contains four test cells for controls (standard freshwater) and four test cells for each concentration of leachate diluted with fresh water standard to obtain a series of dilutions (100; –50; –25; –12.5; –6.25 %). Five live daphnia were transferred to each cell. Then the plates were incubated in the dark at 20 °C. After 24 and 48 hours of incubation, the number of dead daphnia in each cell was recorded.

Results and discussion. *Physico-chemical characterization.* The pH in both sample types is alkaline; It varies from 7.3 to 7.8 for the mine tailings and from 7.7 to 7.9 for the topsoil (Table 1).

The concentrations of heavy metals in the tailings samples are very high for Fe, Zn and Pb followed by Mn, Cu, and Cd. The concentrations of heavy metals in the topsoil samples are also relatively very high for Fe, Zn and Pb followed by Mn, Cu, and Cd (Table 2).

The enrichment factor results show a moderate to high enrichment of Pb and Zn, a moderate enrichment of Cd and no enrichment of Cu and Mn. The geo-accumulation index results indicate moderate to heavy contamination with Pb and Zn, moderate contamination with Cd, and no contamination with Cu and Mn (Table 3)

Table 1

pH measurement

Sample	T1	T2	T3	T4	T5	T6	T7	S1	S2	S3	S4	S5
pH	7.6	7.6	7.3	7.8	7.6	7.3	7.4	7.7	7.9	7.8	7.8	7.7

Note: T: Tailings samples; S: Soil samples

Table 2

Concentration of heavy metals extracted with 10 % HNO_3

HNO_3	Cd(mg/l)	Pb(mg/l)	Cu(mg/l)	Zn(mg/l)	Mn(mg/l)	Fe(mg/l)
S1	5.86	526.62	21.91	1,150.84	314.09	1,506.74
S2	5.84	500.38	20.89	1,276.43	316.54	1,354.65
S3	5.88	468.23	16 .67	1,167 .43	320 .98	1,552.12
S4	5.83	412.87	18.25	1,326 .23	322.43	1,443.54
S5	5.82	400.26	15.86	1,338.15	323.73	1,499.47
T1	6.24	1,812.91	44.027	1,553.743	393.16	5,039.05
T2	6.81	1,371.40	17.701	1,565.861	441.94	2,438.08
T3	5.96	1,054.93	16.82	1,278.126	438.01	2,044.29
T4	7.514	758.87	19.48	1,550.778	455.74	2,740.84
T5	11.08	1,969.90	29.71	3,038.919	411.68	2,865.11
T6	9.75	1,126.28	21.22	2,080.57	427.80	2,812.48
T7	5.04	985.53	9.088	1,256.34	467.64	2,363.41

Table 3

Enrichment Factor (EF) and Geo-accumulation Index (IGEO) values

	Cd IGEO	Cd FE	Pb IGEO	Pb EF	Cu IGEO	Cu EF	Zn IGEO	Zn EF	Mn IGEO	Mn EF	Fe IGEO
S1	2.66	4.04	3.17	5.76	–0.39	0.49	3.09	5.47	0.19	0.73	0.64
S2	2.65	4.48	3.09	6.09	–0.46	0.52	3.24	6.75	0.20	0.82	0.49
S3	2.66	3.94	3.00	4.97	–0.79	0.36	3.11	5.39	0.22	0.73	0.68
S4	2.65	4.20	2.82	4.71	–0.66	0.42	3.30	6.58	0.23	0.78	0.58
S5	2.65	4.03	2.77	4.40	–0.86	0.36	3.31	6.39	0.23	0.76	0.63
T1	2.75	1.29	4.95	5.93	0.61	0.29	3.53	2.21	0.51	0.27	2.38
T2	2.87	2.90	4.55	9.27	–0.70	0.24	3.54	4.60	0.68	0.64	1.34
T3	2.68	3.03	4.17	8.51	–0.77	0.28	3.24	4.48	0.67	0.75	1.08
T4	3.01	2.85	3.69	4.56	–0.56	0.24	3.52	4.05	0.73	0.58	1.50
T5	3.57	4.02	5.07	11.33	0.05	0.35	4.49	7.60	0.58	0.50	1.57
T6	3.39	3.60	4.26	6.60	–0.44	0.25	3.95	5.30	0.64	0.53	1.54
T7	2.44	2.22	4.07	6.87	–1.66	0.13	3.22	3.81	0.76	0.69	1.29

The PCA results showed the presence of two principal components: COM1 at 64.28 % and COM2 at 13.98 %. The concentration of Pb, Zn, Cd, Mn, and Fe were strongly correlated with COM1 and formed an individualized group. The pH was strongly negatively correlated with COM1. Cu concentration was also negatively correlated with COM1 (Fig. 2).

The MCFA results showed that toxicity (qualitative parameter) is correlated with the heavy metals concentration (Fig. 3).

Microbiotestes. *Phytotestkit.* With the tailings, the inhibition of seed germination in test plants was 0.00 % for all three species (S. Saccharatum, L. Sativum and S. Alba). Root growth inhibition is 07.96 % for S. Saccharatum species, 11.59 % for S. Alba species and 17.58 % for L. Sativum species. The three species of plants showed different reactions to the pollutants present in the sediment. L. Sativum is the most sensitive plant to pollutants with 17.58 % inhibition, followed by S. Alba with 11.59 %, S. Saccharatum is the least sensitive with 7.96 %. The germination index is between 81 and 93 %, so tailings can be classified as growth inhibitors and, therefore toxic.

For the soil, the inhibition of seed germination in test plants is 0.00 % for all three species (S. Saccharatum, L. Sativum and S. Alba). Root growth inhibition is –29.8 % for S. Saccharatum species, 9.79 % for S. Alba species and 25.88 % for L. Sativum species. The three plant species showed different responses to pollutants in the topsoil. L. Sativum is the most sensitive plant to pollutants with 25.88 % inhibition, followed by S. Alba with 9.79 %, S. Saccharatum is the least sensitive with –29.8 %. So for the species S. Alba and L. Sativum the topsoil is inhibiting; on the other hand, for the species S. Saccharatum it is stimulating. The germination index is between 73 and 129 %; so the topsoil can be classified as being little inhibiting to stimulating (Table 4).

Ostracodtoxkit. The percentage of mortality = B/(T) · 100, where B is the number of dead ostracods and T is the total number of ostracods. The percentage of mortality is 100 % in the tailings and 0.00 % in the topsoil.

Growth inhibition is determined by comparing the length of ostracods surviving in the test sediment with those in the reference sediment at the end of the test. Percentage of growth inhibition = 100 – [(growth in test sediment/growth in reference sediment)] · 100.

The percentage of growth inhibition in tailings is 100 % and the percentage of growth inhibition in topsoil is 57 %. The mortality of ostracods in the tailings and the growth inhibition are both 100 % which, leads us to say that the tailings are very toxic for these organisms. Mortality in topsoil is 0.00 % and growth inhibition is 57 %; so this soil can be considered slightly toxic (Table 5).

Daphtoxkit. In the tailings, the toxicity effect recorded is 5 to 30 % and the mortality is 100 % for the 50 and 100 % concentrations, the tailings therefore exhibit a high level of toxicity for these organisms. For the topsoil, the toxicity effect is 5 to 15 % and the mortality is 10–15 %. So the soil can be considered slightly toxic to these organisms.

Conclusion. This study demonstrates that the tailings and the surrounding topsoil present relatively high concentrations of Fe, Zn, Pb, Mn, Cu, and Cd. The enrichment factor results show a moderate to high enrichment of Pb and Zn and the Geo-accumulation index shows a moderate to heavy contamination of soil samples with Pb and Zn. The microbiotests show that the tailings are toxic, and the topsoil is slightly toxic to the

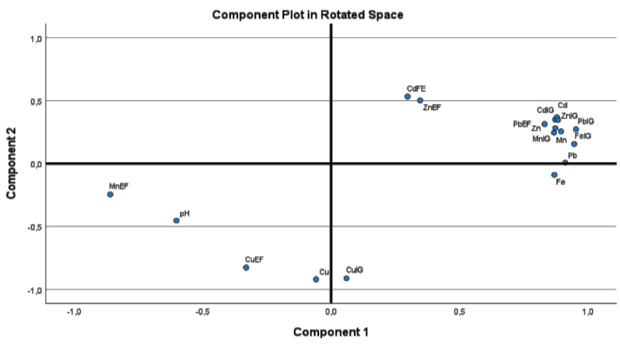

Fig. 2. Plot of COM1 and COM2 data in PCA

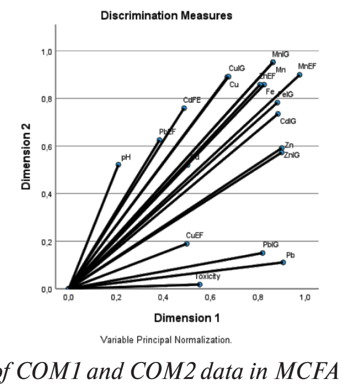

Fig. 3. Plot of COM1 and COM2 data in MCFA

Table 4

Results of phytotestkit microbiotest

	Phytotestkit					
	Sorghum Saccharatum		Lepidium Sativum		Sinapsis Alba	
	Tailings	Topsoil	Tailings	Topsoil	Tailings	Topsoil
Inhibition of seed germination (%)	0.00	0.00	0.00	0.00	0.00	0.00
Root growth inhibition (%)	7.96	–29.8	17.58	25.88	11.59	9.79
Germination index (%)	93	129	81	73	89	89

Table 5

Results of ostracodtoxkit and daphtoxkit microbiotests

	Ostracodtoxkit		Daphtoxkit	
	Mortality, %	Growth inhibition, %	Mortality, %	Toxicity effect, %
Tailing	100	100	100	5–30
Soil	0.00	57	10–15	5–15

living organisms. According to the results of the ACP and MCFA, the toxicity of the tailings and the surrounding topsoil is caused by heavy metals associated with anthropogenic activity related to the mining processing of the El Abed lead-zinc deposit.

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Оцінка токсичності відходів виробництва покинутої цинк-свинцеворудної (Zn-Pb) шахти для навколишнього середовища

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Мета. Оцінка впливу відходів гірничодобувної промисловості на вміст важких металів у воді, рослинах і верхньому шарі ґрунту поблизу дамби хвостосховища цинк-свинцеворудної (Zn-Pb) шахти із застосуванням як біотестів, так і аналітичних методів.

Методика. Була проведена низка мікробіологічних випробувань на різних видах тварин і рослин, що дозволило оцінити токсичний вплив відходів і прилеглих ґрунтів на живі організми. Крім того, був вивчений можливий зв’язок між виявленою токсичністю й результатами фізико-хімічного аналізу зразків.

Результати. Випробування показали, що верхній шар ґрунту, який контактує з дамбою хвостосховища, є слаботоксичним для живих організмів, тоді як хвости (відходи) видобутку є токсичними або навіть дуже токсичними. Вміст важких металів у зразках особливо високий для Fe (заліза), Zn (цинку), Pb (свинцю) і Cu (міді). Взаємозв’язок фізико-хімічних параметрів і результатів мікробіологічних випробувань із використанням аналізу головних компонент (PCA) і факторного аналізу множинної кореляції (MCFA) вказує на те, що токсичність хвостосховищ і оточуючого верхнього шару ґрунту може бути пов›язана з техногенною гірничодобувною діяльністю.

Наукова новизна. Метою цієї роботи є оцінка впливу відходів гірничодобувної промисловості на вміст важких металів за допомогою біотестів і аналітичних методів. Оцінка враховує концентрацію проб (висококонцентровані проби та проби після розчинення), а також різні фази присутності (тверда, рідка) з метою більш детальної оцінки потенційної токсичності зразків.

Практична значимість. Для розробки відповідного плану заходів з реабілітації важливо було провести комплексну оцінку відходів гірничодобувної промисловості й ризиків, які вони можуть становити для людей і довкілля.

Ключові слова: *токсичність, цинк-свинцеворудна* (*Zn-Pb*) *шахта, мікробіотести, хвостосховища, ґрунт, важкі метали, гірничодобувна діяльність*

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