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INTEGRATED ALGORITHM FOR TOXICITY ASSESSMENT OF MINE TAILINGS AND SURROUNDING SOILS BASED ON CHEMICAL AND GERMINATION ASSAYS

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ABSTRACT

The evaluation of the environmental impact arising from mine tailing toxicity can be accomplished by chemical and biological assays. Several research groups and environmental agencies established working protocols for heavy metal determination and risk assessment. Nevertheless, there is no standardized methodology that integrates chemical and biological approaches for the toxicity assessment of soils and waters contaminated with heavy metals and metalloids. This study presents a comprehensive algorithm designed to assess the environmental toxicity of mine tailings and surrounding soils by applying a germination assay. Therefore, wheat germination bioassays and evaluation of bioconcentration of As, Pb, Cu, Zn, Cd, Ag, Fe, Mn, Ni, and Cr in contaminated environmental samples are carried out. The germination index showed results greater than 80 % classified as "excellent" in soil samples P1 and P2, and less than 40 % in soil sample P3 due to high Pb toxicity. The capability of plant species to remediate soils was evaluated by the Bioconcentration factor (BCF) and the Translocation factor (TF). The average results for BCF, based on Pb, As, Zn, and Cu, were 0.25, and a low TF was obtained, with values ranging between 0.05 and 0.2.

Keywords: mine tailings, surrounding soils, germination, heavy metals, translocation and bioaccumulation factors.

INTRODUCTION

Flotation plants, metallurgical factories, mining enterprises [1], and automobile engines [2] are known as primary contributors to soil and environmental pollution. According to national and European regulatory authorities, a multi-step process is conducted to determine pollution levels and potential effects on the ecosystems related to them. The main stages typically involve data monitoring, laboratory analysis, and interpretation of the results, taking into consideration established national and/or international regulations. A particularly effective approach for evaluating the long-term accumulation effects of heavy metals on different plant species is based on the bioaccumulation properties in various plant parts, such as roots, stems, leaves, or plant species, including crops, trees, and weeds [3, 4]. The proposed methodologies are based on the calculation and classification of bioaccumulation and translocation capabilities, which refer to the plant uptake of metals from the soil and/or via atmospheric depositions. In this regard, bioaccumulation factor (BAF) and translocation factor (TF) are often used. The first one quantifies a particular metal content that is transferred from soil to plant. The second one shows the degree of metals translocated from plant roots toward stems and leaves [3, 5, 6]. Environmental conditions also play a key role in bioaccumulation, including soil physicochemical properties (pH, texture, organic matter, cation exchange capacity) and the nature of pollution sources (industrial activities, traffic, wastewater), which determine the presence and distribution of various metals such as Cd, Cr, Cu, Pb, Zn, Ni, Mn, and As [6 - 9]. The analysis of individual plant parts provides valuable insight and serves as an indicator for assessing pollution levels, as well as the type of contamination [9]. High concentrations in leaves indicate either atmospheric deposition or an efficient metal translocation mechanism within the plant [7], whereas high concentrations in roots suggest limited translocation and a lower risk to the above-ground plant parts. Some plant species accumulate metals mainly in shoots and leaves and are used for phytoremediation and removal of metals from soil. Those retaining metals mainly in the roots contribute to the stabilization of metals within the soil [11]. A comprehensive assessment of metal bioavailability involves comparing metal concentrations in soil and in plant parts. Hereby, short- or long-lasting programs are conducted. Changes in patterns over time can reveal accumulation specificity and predominantly extraction from different plant parts [8]. All models applied to assess the bioavailability of heavy metals and metalloids have certain limitations, as numerous influencing factors affect the development of toxicity protocols [12]. As an example, surface contamination from dust and particulate matter can increase metal concentrations in leaves [13]. In many cases, the total metal content in soils does not necessarily correlate with the bioavailable fraction, and the chemical composition of the soil becomes an important factor. The plant species are also of great significance, as different species vary substantially in their capacity to accumulate and to translocate metals from roots to other plant parts. Moreover, different metals exhibit varying behaviour with respect to their mobility within the plant.

A concise methodological framework for assessing environmental pollution in mining areas can be based on germination tests with *Triticum aestivum* (wheat), early seedling growth indicators, and the determination of metal and metalloid contents in roots, stems, and leaves [13 - 15]. The indices of interest are the Bioaccumulation Factor (BAF) or Bioconcentration Factor (BCF). A translocation factor (TF) greater than 1 indicates effective metal translocation from roots to shoots and leaves [11]. High metal concentrations in roots combined with a low TF value suggest that metals are largely retained in roots, indicating phytostabilization. Conversely, high concentrations in shoots or leaves (high TF) demonstrate active translocation. Higher concentrations detected in unwashed leaves compared to washed ones are due to atmospheric deposition [12]. In addition to bioaccumulation analyses, environmental matrices, including soils, waters, and air, are also evaluated. Key soil parameters include pH, organic matter, soil texture, cation exchange capacity, and extractable metal fractions. Comparisons should be made based on the plant available metal concentrations in soil, rather than total concentrations at all [13].

For human and animal risk assessment, the Estimated Daily Intake (EDI) and hazard indices are calculated when metals are detected in edible plant parts [16]. Some research groups and environmental agencies have developed integrated protocols that are well-developed and widely used in mining-related assessments. The main stages include (1) site characterization and sampling (collecting samples from tailings surfaces, surrounding soils, and sediments to determine basic physical parameters such as pH, redox potential, electrical conductivity, texture, organic matter, and moisture content); (2) chemical analyses-determining total metal content via acid digestion with aqua regia or HF/$HClO_4$ for determination of total metal content by oxidative acid digestion followed by spectrometric quantification; exchangeable or bioavailable metal fractions determined by extractions with DTPA, $CaCl_2$, or sequential extraction methods, along with measurements of sulphate, carbonate, and other relevant components [17]; (3) bioassays- conducting germination and phytotoxicity assays using various test species (e.g., *Lepidium sativum*, *Triticum aestivum*, *Lactuca sativa*, *Zea mays*). Wheat is often used for mine tailing studies [14] and parameters such as germination percentage (% GP), mean germination time (MGT), root and shoot length, seedling vigour index (SVI = GP × seedling length), biomass (fresh/dry weight) are reported. Toxicity and germination indices, as well as growth inhibition percentages, are then calculated [17, 18]. The results are compared with regulatory thresholds such as WHO standards, EU Soil Screening Values, and USEPA guidelines. The subsequent stage involves matrix risk evaluation and correlation studies on the phytotoxic effects. Soils and wastes are then classified as toxic, moderately toxic, or non-toxic based on germination or growth inhibition thresholds, followed by decisions on phytoremediation, stabilization, or safe disposal [19, 20]. Similar combined analytical and bioassay protocols have been applied: (1) in a Moroccan study on Pb-contaminated mining soils [15]; (2) tailings evaluation in Algeria for potential reuse as soil amendments [21]; (3) to wheat germination and growth tests in Pakistan under industrial wastewater irrigation [14], where germination index and seedling inhibition correlated with Pb and other heavy metals in soil and water. Comparable assessment protocols have also been implemented in China, India, and Eastern Europe to evaluate agricultural soils affected by smelting, metal casting, irrigation with urban wastewater, tailings, and slag deposits from copper, lead, and zinc mines [22, 23].

The study aims to contribute to the previously made toxicity assessment of mine tailing, surrounding soils, and waters. In addition to the main chemical and physicochemical characteristics, the application of sequential extraction procedures, the total heavy metal content, and the determination of different contamination indices are studied. Furthermore, the germination bioassay, bioconcentration factor, and translocation index of heavy metals are examined. Thus, combining the results from previous and current studies, an integrated algorithm for estimating the toxicity of mine tailings and surrounding soils and waters is proposed. The main contribution is based on chemical and germination bioassays, followed by the evaluation of germination index, bioconcentration, and translocation factors.

EXPERIMENTAL

Materials and methods

Sampling

The soil and water samples were collected from the surroundings of mine tailings in Tarniţa-Suceava, Romania. A detailed description of soil and water sampling is given in Table 1. The soil samples were taken from the surface horizons within the first 20 cm in depth. The water samples were stored in polyethylene bottles at 4°C until ICP-OES analysis. In our previous studies, detailed information regarding the sampling sites is provided [24].

Procedures

Germination tests

As an experimental plant culture, a *Triticum aestivum* (wheat) was chosen. Two different approaches were applied. The first one was based on the comparison of the studied samples with a control sample. The second one relies on the irrigation of a control soil sample with river samples, taken from the surrounding environment in a closed mine. The applied germination tests followed a previously validated analytical procedure [25]. For each experimental trial, two replicates consisting of 50 *Triticum aestivum* seeds were prepared. Seeds were pre-soaked in distilled water for 1 h to initiate germination. Polystyrene Petri dishes (100 mm × 20 mm) were lined with double-layered filter paper, onto which 2 g of test samples was evenly distributed using a minimal volume of water to ensure uniform moisture. Following pre-soaking, seeds were transferred to the dishes along with 5 mL of the soaking solution. Seeds were carefully arranged to maintain maximum spacing, with embryos oriented upward to promote consistent germination. The germination assay under controlled conditions lasts seven days. At the end of the germination period, the number of viable seedlings and non-germinated seeds was recorded. Seedlings were then separated, and their stems and roots were individually measured for length

Table 1. Description of soil and water sampling sites of the analysed samples.

Sample	№	Sampling Site
Soil	P1	2 m from the river; near tailing dump 1; between the main road and the river
	P2	100 m from the tailing dump 1 and about 1 m from the river, exactly from the bank
	P3	collected from the tailing dump 1 (100 m from the Tărnicioara river*)*
Water	W0	collected upstream (about 3 km) from the tailing dump
	W1	from the middle of the river (near tailing dump 1)
	W2	collected downstream (below) of tailing dump 2 (to the left of the road and upstream of tailing dump 1, near the river)
	W3	collected from the tailings pond (near tailing dump 1)

and fresh weight to evaluate growth performance and potential phytotoxic effects (Fig. 1)

Determination of heavy metals in plant tissues

Stems and roots from the planted experimental batches were harvested, pooled, and homogenized. Precisely weighed subsamples of 1.5 to 2.0 g (± 0.0001 g) fresh mass for leaves and 1.0 g (± 0.0001 g) for roots and seeds were transferred into glass beakers. Each sample was treated with 5 mL of distilled water, 5 mL of concentrated nitric acid (HNO_3), and 5 mL of hydrogen peroxide (H_2O_2). Digestion was conducted for 30 min on a hot plate without boiling until complete dissolution was achieved. The procedure was repeated until complete digestion was achieved. Upon cooling, the digested solutions were filtered and diluted in a measuring flask using distilled water. All samples were prepared in triplicate and processed for ICP-OES measurement.

Water samples

To evaluate the potential phytotoxic effects of the studied water samples, a second germination test was conducted. All the analysed samples were made in duplicate, and the mean values are presented in Table 3. The procedure mirrored the one applied to the soil samples, with the only modification that the seeds were sown in the control soil sample (P4). Instead of distilled water, irrigation was performed using the analysed water samples. A control group sample used the same soil sample and experimental conditions, but distilled water was used for irrigation.

Calculation of pollution indices

Bioconcentration and translocation factors were calculated using the following equations:

1. Bioconcentration factor (BCF, Eq. (1)):

$$BCF = \frac{C_{plant}}{C_{soil}}, \qquad (1)$$

where C_{plant} and C_{soil} represent the concentrations of heavy metals in the plant and the soil, respectively [26].

2. Translocation factor (TF, Eq. (2)):

$$TF = \frac{C_{leaves}}{C_{roots}}, \qquad (2)$$

where C_{leaves} and C_{roots} represent the concentrations of heavy metals in the leaves and the roots, respectively [26].

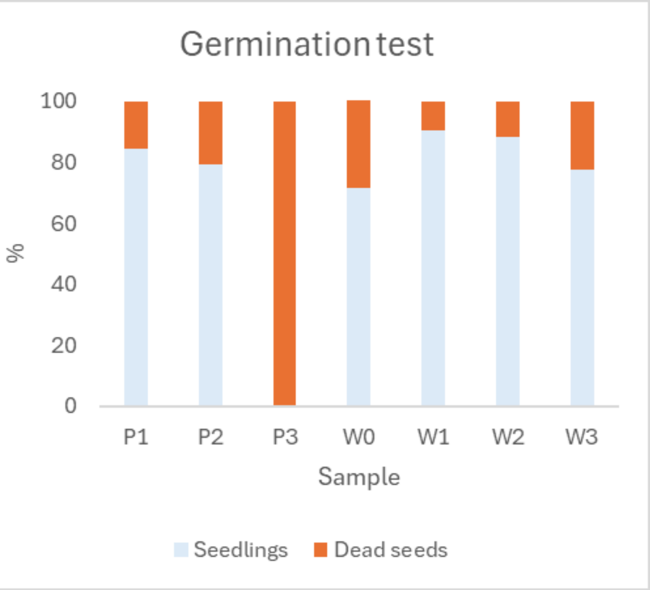

Fig. 1. Germination test of soil and water samples.

RESULTS AND DISCUSSION

Assessment of bioavailability of heavy metals

The toxicity of the collected soil samples was evaluated using a germination bioassay, following a previously validated protocol [27]. The results are presented in Fig. 1. Wheat *(Triticum aestivum),* selected for its rapid growth and capacity to accumulate heavy metals, served as a sensitive bioindicator of soil toxicity under controlled laboratory conditions. Among the tested conditions, water sample W2 and soil sample P2 yielded seedlings with the freshest, longest, and most vibrant green leaves, indicative of lower toxicity and enhanced growth potential. In contrast, no germination was observed in soil sample P3, classifying it as the most toxic among the tested samples. According to the data analysis of germination performance, the results for all water samples indicated no significant toxicity (Fig. 1). Sample W1 yielded the highest number of germinated grains, suggesting favourable conditions for germination. Conversely, sample W0 was associated with the highest seed mortality, though values remained within acceptable limits. Based on the criteria established in previous studies, the germination index exceeding 80 % is indicative of excellent germination performance, values between 60 % and 80 % represent good performance, those ranging from 40 - 60 % denote moderate performance, and those below 40 % indicate poor performance [17, 18, 28]. The results obtained in this study showed excellent performance for soil samples P1 and P2, and poor performance for sample P3 (less than 40 %), as no seedlings were observed. The germination assay based on the application of water samples showed that the seeds irrigated with water samples W0 and W3 have higher mortality, 33 and 22 %, respectively. Nevertheless, the samples can be classified as "a good performance". Samples W1 and W2 showed "excellent germination performance".

Comparable values were obtained for both seedlings' length (ranging from 8 to 13 cm, see Fig. 2) and biomass (between 2 and 3 g, see Fig. 3) across the studied samples. The classification as "excellent" or "good" germination performance indicated that the toxicity levels in soil samples P1 and P2, as well as in all water samples collected from different river locations, were comparable. The quantitative results of the bioaccumulation of heavy metals in the seedlings are detailed in Table 2 for soil samples and Table 3 for water samples. Each experimental measurement was evaluated in triplicate, and mean values along with the standard deviations are reported. In soil sample P3, no

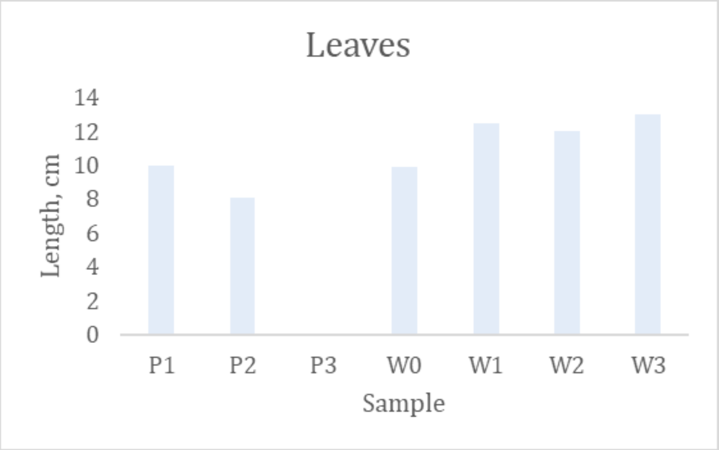

Fig. 2. Length of germinated seeds.

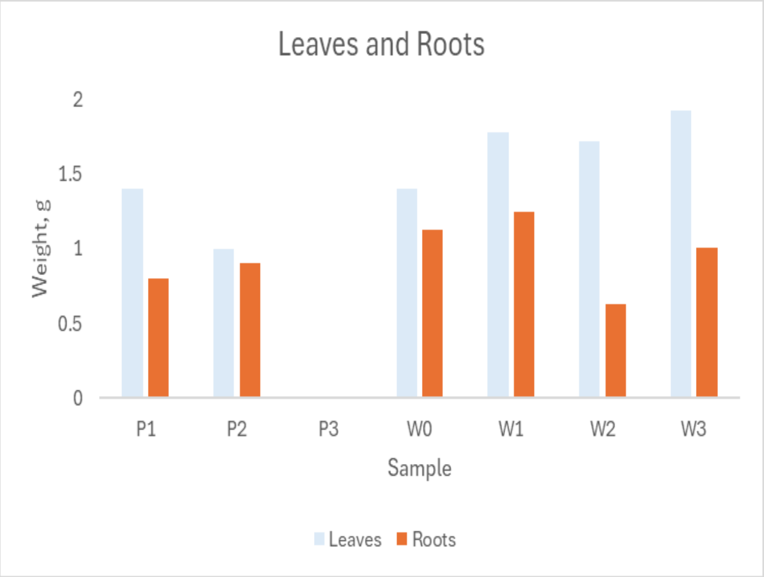

Fig. 3. Biomass of leaves and roots of germinated seeds.

Table 2. Heavy metal concentrations in plant tissues after germination in corresponding soil samples.

Heavy metal			Cu	Fe	Zn	Pb	Ni	As	Mn
Sample	Part of the plant		mg kg^{-1}						
P1	roots	1)	9	142	14	0.7	< 0.5	0.7	26
		2)	413 ± 67	84344 ± 4354	207 ± 14	354 ± 47	< 0.5	212 ± 0	139 ± 12
	leaves	1)	0.5	84	7	0.5	< 0.5	0.5	13
		2)	7.42 ± 1.79	847 ± 340	11.7 ± 3.8	0.59 ± 0.23	< 0.5	0.56 ± 0.23	16.0± 2.0
P2	roots	1)	0.5	175	8	0.5	0.5	0.5	16
		2)	362 ± 0	81217 ± 27444	169 ± 11	340 ± 95	< 0.5	189 ± 7	118 ± 36
	leaves	1)	0.7	336	10	0.8	< 0.5	0.7	14
		2)	11.6 ± 3.1	2040 ± 337	15.3 ± 1.0	0.75 ± 0.12	< 0.5	0.75 ± 0.11	17.9 ± 0.1
P3	roots	1)	0.7	258	9	0.7	< 0.5	0.7	23
		2)	0	0	0	0	< 0.5	0	0
	leaves	1)	6	330	13	0.5	< 0.5	0.5	14
		2)	0	0	0	0	< 0.5	0	0

**1) control sample; 2) analysed sample*

plant growth was detected, but P1 and P2 demonstrated some ability to support germination, both exhibiting high levels of heavy metal accumulation. Notably, the heavy metals were concentrated in the roots, with significantly lower levels detected in the leaves.

Based on the results of heavy metal bioaccumulation observed in the roots and leaves of *Triticum aestivum* seedlings, the samples can be ranked (excluding sample P3) as follows:

Sample P1 (roots and leaves): Fe > Cu > Pb > Zn > > As > Mn > Ni

Sample P2 (roots and leaves): Fe > Cu > Pb > As > > Zn > Mn > Ni

These distributions suggest iron and copper are the most prevalent metals accumulated by the seedlings, followed by lead, and a small difference in arsenic and zinc concentration between the two samples. The presence of lead in both samples showed a consistent uptake of toxic metals regardless of the sample origin, while the relatively lower concentrations of manganese and nickel indicate weak affinity in these conditions.

In all samples, higher concentrations are obtained in the roots than in the leaves. The bioconcentration factor is presented as the total content between roots and leaves for each element divided by the concentration, respectively, in the soil and/or water samples. The soil toxicity was examined in our previous work, and the following elements were found in the soil samples: As, Cu, Pb, Zn, Cd, Mn, Ni, and Cr. Cr and Ni content are below the Limit of Quantification of the measurement [24]. The highest Cd content is measured in sample P2 (52 mg kg^{-1}), in samples P1 and P3, it is 8 and 5 mg kg^{-1}, respectively. In biomass analysis of roots and leaves, Cd was not detected. Higher quantities are detected for Cu, Pb, and Zn (1322 mg kg^{-1}Cu; 1267 mg kg^{-1} Pb; 803 mg kg^{-1} Zn for sample P1; 1412 mg kg^{-1} Cu; 1552 mg kg^{-1} Pb; 817 mg kg^{-1} Zn in sample P2; 1222 mg kg^{-1} Cu; 4093 mg kg^{-1} Pb; 498 mg kg^{-1}Zn in sample P3). Fe content is approximately 400 g kg^{-1}. A significant amount of As is found in the analysed samples (748 mg kg^{-1}, 848 mg kg^{-1},

Table 3. Heavy metal concentrations in plant tissues after germination and irrigation with corresponding water samples.

Heavy metal			Cu	Fe	Zn	Pb	As	Mn
Sample	Part of the plant		mg kg^{-1}					
WO	roots	1)	13	3209	14	0.5	0.7	75
		2)	27 ±15	5418 ± 132	30 ± 11	6 ± 18	0.7 ± 0.6	129 ± 7
	leaves	1)	0.5	59	5	0.5	0.5	9
		2)	0.6 ± 0.05	66 ± 75	7 ±2	0.6 ± 0.05	0.6 ± 0.05	10 ± 3.4
W1	roots	1)	8	554	0.8	0.7	0.7	44
		2)	11 ± 4	747 ± 216	13 ± 2	0.6 ± 0.005	0.6 ± 0.005	51 ± 0.5
	leaves	1)	0.5	95	8	0.5	0.5	12
		2)	4 ± 0.2	72 ± 2	9 ± 0.9	0.4 ± 0.1	0.4 ± 0.08	10 ± 0.02
W2	roots	1)	54	5187	56	0.7	34	237
		2)	47 ± 5	9107 ± 3668	49 ± 2	1 ± 0.3	1 ± 0.3	222 ± 16
	leaves	1)	0.5	47	7	0.5	0.5	11
		2)	0.4 ± 0.2	42 ± 36	8 ± 7.7	0.4 ± 0.2	0.4 ± 0.15	11 ± 7
W3	roots	1)	8	1079	9	0.5	0.5	59
		2)	175 ± 178	6102 ± 769	46 ± 107	7 ± 22	8 ± 24	251 ± 256
	leaves	1)	0.2	49	5	0.2	0.2	8
		2)	22 ± 27	124 ± 197	22 ± 27	0.6 ± 0.4	0.6 ± 0.4	26 ± 28

935 mg kg^{-1} in samples P1, P2, and P3, respectively). The highest concentration of As is found in the roots of the germinated seedlings in samples P1 and P2 (Table 2). Arsenic content in sample P3 is in the same order, but a higher toxicity is noted, probably due to the Pb content.

The calculated results for soil-to-plant mobility indexes, presented by the Bioconcentration factor (BCF) of Pb, are 0.28 in sample P1 and 0.22 in sample P2 (Eq. (1)). The obtained results, compared with other research data and calculated BCF, based on analysis of Rice (0.37), wheat (0.0053 - 0.0069), *Ulva fasciata (0.48*), showed a significant transition of Pb [26]. The same tendency is observed in arsenic bioconcentration in samples P1 (BCF = 0.28) and P2 (BCF = 0.23). The bioconcentration factor of Zn for samples P1 and P2 is 0.27 and 0.23, respectively (Eq. (1)). In comparison to the literature data, the transmission of Zn in our study is significantly lower than experimental data for wheat, *Pisum sativum*, leaf lettuce, cabbage, and *Ulva fasciata* (with values ranging from 0.5 to 2), reported by Hussain et al. [26]. BCF represents the capability to accrue metals, and values greater than 1 illustrate the possibility of plant species to remediate soils. The obtained results for Fe concentration in the roots and leaves of wheat are greater than the other studied metals, P1 (85191 mg kg^{-1}) and P2 (83257 mg kg^{-1}) (Table 2). The obtained BCF values for soils P1 (0.56) and P2 (0.44), calculated by Eq. (1) are higher than the results cited by Hussain et al., for wheat, *Pisum sativum*, leaf lettuce, and cabbage. Only *Ulva facsciata* and rice showed high capability for copper accumulation. The values ranged from 1.1 to 4.6 [26].

Translocation factors from roots to leaves were calculated by applying Eq. (2) and ranged from 0.2 to 0.05 in samples P1 and P2 and are valid for all analysed elements. Thus, the phitostabilization is very low for wheat. The TF values, as well as BCF, greater than 1 represent a high bioaccumulation capability. The obtained results from this study showed low values for TF in wheat. For comparison, the capability for translocation of Cd, Cu, and Mo in Datura stramonium, rice, and Pisum sativum ranges from 1.1 to 8.36 [26].

Study of heavy metals in surface water samples

In addition to soil sample characterization, water samples collected from various locations along the river were analysed. Iron levels varied significantly across the samples, especially those near the riverbank, with concentrations ranging from 0.2 to 16.2 mg L^{-1}. Sample W0 showed no detectable heavy metal concentrations. In contrast, sample W3 revealed the presence of copper and lead, each at 0.25 mg L^{-1}, while manganese was found in samples W1 and W2 at 1.17 mg L^{-1} and 7.27 mg L^{-1}, respectively.

Low iron concentration was observed in water sample W0, remaining well below the drinking water threshold of 0.3 mg L^{-1}, thus the sample can be considered as uncontaminated. Iron oxides are recognized for their high ability to adsorb heavy metals, which aids natural filtration; consequently, the concentration of heavy metals in river water was significantly reduced compared to that in surrounding soils and mine tailings.

In Table 3, results are presented for wheat seeds, irrigated by samples W0 - W3 taken from the river passing by the mine tailing. The control soil sample was used for planting the wheat seeds. The BCF of all samples and all studied elements has low values due to the low heavy metal concentrations. TF has low values too, ranging from 0.001 to 0.2. The obtained values are based on the transition of heavy metals from the control sample.

Algorithm for estimating the environmental toxicity of mine waste and the surrounding environment

Drawing upon both relevant scientific literature and the experimental findings presented in this study, an algorithm has been proposed to evaluate the toxicity of heavy metals in mine waste, surrounding soils, and surface waters. This algorithm is based on the systematic and integrated analysis of chemical, physicochemical, and biological parameters of the investigated samples. The core structure of the algorithm comprises the following sequential steps:

1. Determination of the main characteristics of waste material, surrounding soils, and water.
2. Quantifying the heavy metal concentrations.
3. Determination and distribution of heavy metals in different geochemical phases through a sequential extraction procedure and calculation of toxicity indexes as the Individual contamination index and the Global contamination index.
4. Estimation of the potential toxicity of waste material through a biological test/a germination assay
5. Determination of heavy metal concentrations in a biological (plant) sample followed by estimation of Bioaccumulation and Translocation factors.
6. Interpretation of the results and risk assessment based on the regulatory limits and national thresholds.

CONCLUSIONS

The integrated algorithm developed in this study provides a systematic approach for assessing the environmental toxicity of mine tailings and surrounding soils by combining chemical analyses with germination bioassays. The results demonstrate that soil sample P3, collected from the tailing dump, 100 m away from the river, exhibited the highest toxicity, likely due to elevated lead concentrations, as evidenced by the complete absence of seed germination. In contrast, soil samples P1 and P2, as well as all tested water samples, supported germination classified as “good” to “excellent”, indicating lower toxicity levels. Across all studied samples, the bioaccumulation and translocation factors (BCF and TF) showed low values, suggesting limited transfer of heavy metals from soil to plant tissues and from roots to leaves. This indicates a low risk of heavy metal movement through the food chain under the studied conditions. The findings highlight the importance of integrating both chemical and biological indicators for a comprehensive environmental risk assessment in mining-impacted areas.

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Authors’ contributions

L.A.: Investigation, Methodology, Writing Original Draft, Data curation, Project administration; D.I.: Conceptualization, Methodology, Investigation, Data curation.

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