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## Estimation of soil and tailing dump toxicity: development and validation of a protocol based on bioindicators and ICP-OES

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# Estimation of soil and tailing dump toxicity: development and validation of a protocol based on bioindicators and ICP-OES

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**Abstract.** A protocol for estimation of soil toxicity based on germination test, open acid sample digestion and ICP-OES determination of heavy metals and metalloids was proposed. *Triticum aestivum* was used as a bioindicator and germinated on contaminated soils. After estimation of the state of the plantlets, the accumulated heavy metals and metalloids content were determined. The sample digestion procedure and ICP-OES measurement were validated by analysing plant certified reference material. Additionally, standard addition before sample pretreatment was applied for estimation of method recovery at the levels of heavy metals found in plants from contaminated region. The recovery obtained was between 97 and 105 %. The precisions of the complete protocol (RSD), which included germination tests, sample digestion and ICP-OES measurements, ranged between 4.7% for Mn and 11 % for As. The expanded uncertainty was estimated. The proposed protocol was applied for estimation of soil toxicity and heavy metals bioavailability of tailing dump material and surrounding soils from an abandoned barite mine in Tarnița, Suceava, Romania. The coltsfoot used for remediation of the soil in the studied region was analysed. Cu, Ni, Zn, Mn, Pb, Fe and Al were found in the sample harvested nearby the tailing dump.

## 1. Introduction

Ore-processing activities have been generated a high quantity of solid and liquid wastes. Their environmental fate, state of the surrounding environment, efficiency of remediation procedures and the impact on human health have been intensively studied [1-5]. Regular monitoring of mining sites has been based on different approaches for analysis of environmental samples: soils, waters, plants, food, biological fluids, etc. [5-10]. Some studies showed that total heavy metal content could overestimate the toxicity of soils [11,12]. Study of bioavailability of heavy metals and use of bioindicators have been proposed for estimation of soil toxicity effects [6, 11-14]. Such an approach has required development and validation of protocols for determination of heavy metals using bioindicators for their accumulation. Nowadays, intensive research has been made in attempt to developed and validate analytical methods capable of providing comprehensive information on heavy metals and metalloids content in different environmental and biological samples [7,10, 14-18]. Large concentration variation in the environmental samples, high sensitivity, selectivity, determination of total heavy metals content and speciation analysis are only small part of current requirements for analytical methods. The



complex matrix and appropriate sample pretreatment are crucial points in determination of inorganic pollutants.

This paper presents the results from a study on development of a protocol for determination of bioavailability of heavy metals and metalloids in soils based on bioindicators capable of accumulating heavy metals and metalloids, open acid digestion of sample and ICP-OES measurements.

## 2. Materials and Methods

### 2.1 Germination test

*Triticum aestivum* (wheat) seeds were used as bioindicators in this study. Three batches of 50 wheat seeds were prepared for each test. The seeds were transferred in test tubes and 5 mL distilled water were added. 60-min swelling of the seeds stimulate their germination. The Petri dishes were covered with 2 filter paper discs and 1 g treated soil was uniformly dispersed on it. Seeds were added and carefully arranged on the wet soil material. The seeds were transferred with soaking solution (5 mL). Wheat seeds were evenly distributed to assure a maximum distance between them, with embryo above. Petri dishes were covered with a lid for 3 days, after that uncovered and soaked with distilled water. The total time of germination was 7 days. The plantlets were removed from Petri dishes and number of plantlets (length greater than 0.5 cm) and dead seeds were counted. The seedlings were separated from seeds. The stems and roots were separated and their weight and length were measured. The results were presented as a total length and a total weight of stems and roots for each batch.

### 2.2 Sample pretreatment procedure

Open acid digestion method was applied. Stems and roots from three replicate batches were mixed. Accurately weighed samples, leaves 1.5 - 2.0 g, roots and seeds - 1 g, were transferred to 150 mL beakers and moistened with 5 mL distilled water and 5 mL of concentrated HNO<sub>3</sub>. The samples were covered with a watch glass and left overnight to prevent foaming and to allow full oxidation of the organic matrix. Thus prepared samples were heated without boiling for 30 min. During heating 4 mL H<sub>2</sub>O<sub>2</sub> in portions of 1 mL each were added. Finally, 2 mL concentrated HNO<sub>3</sub> were added and the digestion continue till clear solution was obtained. After cooling, the solutions were filtered, if necessary, and collected in 50.00 mL measuring flasks, diluted to the volume with distilled water. Each sample was prepared in triplicate. Heavy metal and metalloids concentration were determined by ICP-OES.

### 2.3 ICP-OES determination of heavy metals

An ICP-OES spectrometer Prodigy High Dispersion ICP-OES, Teledyne Leeman Labs, USA equipped with a dual view torch, cyclonic spray chamber, and concentric nebulizer was used. The operating conditions employed for ICP-OES measurements were [7]: coolant gas 18 L min<sup>-1</sup>, auxiliary gas 0.5 L min<sup>-1</sup>, nebulizer gas 34 psi, RF power 1.2 kW, pump rate 1.2 mL min<sup>-1</sup>, sample uptake time 30 sec, integration time 40 sec. High purity Ar 99.999 % was used to sustain plasma and as a carrier gas. Calibration standards were prepared by appropriate dilution of: (1) multi element standard solution ("Ultra scientific", Lot: P00332) containing 24 elements in 5 % HNO<sub>3</sub> at concentration 100 ± 5 mg L<sup>-1</sup> of each element, and (2) arsenic standard solution (As in 5 % HNO<sub>3</sub>) 1000 ± 3 mg L<sup>-1</sup> (VHG Labs, Lot: 112-0017). Each solution was scanned at least three times and a mean analytical signal was calculated. The wavelengths used for direct determination of target metals are presented in Table 1.

### 2.4 Estimation of characteristics of the method

The limits of quantification were calculated as 10 x SD from three measurements of blank solutions and recalculated in mg kg<sup>-1</sup> from 100 mL solution and 1 g sample weight. Extraction efficiency of the metals and arsenic, as well as, matrix effect were evaluated by standard addition method. The plant samples were spiked with analytes before sample digestion. Each analysis was made in triplicate and means were calculated. Spike recovery was calculated. The method precision and trueness were

estimated by analysing certified reference materials: BCR No 62 trace elements in olive leaves and Key comparison CCQM-K89 metals in Herba Ecliptae, provided by the Bulgarian Institute of Metrology. The expanded uncertainty (including germination, sample pretreatment and ICP-OES measurement) was calculated as  $U=k \cdot SD$  ( $k=2$  at 95% confidence interval) from 2 parallel series of germination and bioindicator analysis. Thus obtained expanded uncertainty could be assigned to other measurements without additional experiments.

### 3. Results and discussions

#### 3.1 Analytical characteristics of the method based on ICP-OES and open acid digestion of the sample

Our previous study revealed elevated heavy metal content in the tailing dump material and surrounding soils from the region of closed barite mine, Tarnița, Suceava, Romania [7, 18, 19]. High levels of Fe, Cu, Pb, Zn, as well as some Mn, Cd, Ni and As were found. During the remediation activities the coltsfoot was planted on the contaminated areas; however, the plant scarcely grew up on the second year. It was supposed that the coltsfoot accumulated high levels of heavy metals. Based on the preliminary analysis of coltsfoot from the contaminated area, the analytical wavelengths for the target analytes were chosen. At the found concentrations, spectral interference wasn't assumed and the most sensitive wavelengths were chosen (Table 1). Sensitivity of the method is presented in the Table 1 as a slope of the five points calibration curve in the concentration range corresponding to the actual heavy metal content in the target plants. Limits of quantification were determined by analyzing reagent blank (LOQ 1) and by analyzing sample blank - wheat seeds (LOQ 2) used further in this study as bioindicator. The difference between LOQs was insignificant – up to 0.3 mg kg<sup>-1</sup>.

**Table 1.** Analytical wavelengths and characteristics of the method for determination of heavy metals in plants based on ICP-OES and open acid sample digestion.

Analyte	Wavelength, nm	Sensitivity, L mg <sup>-1</sup>	LOQ 1 <sup>a</sup> , mg kg <sup>-1</sup>	LOQ 2 <sup>b</sup> , mg kg <sup>-1</sup>	Recovery, %
Al	394.210	7.10x10 <sup>-5</sup>	0.7	0.7	105
As	193.759	5.39x10 <sup>-7</sup>	1.1	1.3	97
Cd	226.502	4.83x10 <sup>-6</sup>	0.7	1.0	100
Co	228.615	4.98x10 <sup>-7</sup>	0.6	0.6	100
Cr	205.552	2.89x10 <sup>-7</sup>	0.5	0.6	100
Cu	324.754	1.18x10 <sup>-6</sup>	0.8	0.8	101
Fe	259.940	3.59x10 <sup>-7</sup>	0.5	0.5	101
Mn	257.610	4.44x10 <sup>-6</sup>	0.5	0.5	98
Ni	231.604	3.59x10 <sup>-7</sup>	1.5	1.5	98
Pb	220.353	7.10x10 <sup>-5</sup>	1.0	1.2	99
Zn	213.856	1.18x10 <sup>-6</sup>	0.7	0.7	101

<sup>a</sup>LOQ determined as 10xSD of the signal of reagent blank <sup>b</sup>LOQ determined as 10xSD of the signal of sample blank

The efficiency of sample pretreatment, matrix effect, trueness and uncertainty of the method were estimated by analyzing CRM for plants (Table 2). As CRM for triticum aestivum as bioindicator for contaminated soils was not available, the efficiency of sample pretreatment at the levels found in plants from the studied region and matrix effect were estimated by spiking germinated wheat samples before digestion [20]. Recovery of spikes was between 97 and 105 % (Table 1) and recovery of CRM – between 94 and 104 % (Table 2). The bias for all analytes was within  $\pm 10\%$ , therefore the matrix effects could be considered negligible, and the determinations could be done by calibration using the multipoints external standard method [16]. The obtained recovery of analytes in CRM and in spiked samples (Tables 1 and 2) was below the limits recommended in the Council Directive 98/83/EC [21], indicating acceptable trueness of the sample pretreatment and ICP-OES measurement of target

analytes in plant samples. The uncertainty was calculated as  $U=k \cdot SD$  ( $k=2$  at 95% confidence level), SD was calculated from analysis CRM in triplicates.

**Table 2.** Results from analysis of CRM for plants by open acid digestion and ICP-OES (N=3).

	BCR No 62			CCQM-K68		
	Found $\pm$ U, mg kg <sup>-1</sup>	Certified $\pm$ U, mg kg <sup>-1</sup>	R, %	Found $\pm$ U, mg kg <sup>-1</sup>	Certified $\pm$ U, mg kg <sup>-1</sup>	R, %
Ca	n.a.	n.a.		27.00 $\pm$ .87	26.76 $\pm$ 0.21	100.9
Cd	0.098 $\pm$ 0.032	0.10 $\pm$ 0.02	98.0	3.89 $\pm$ 0.20	4.12 $\pm$ 0.028	94.4
Cu	45.47 $\pm$ 0.03	46.6 $\pm$ 1.8	97.6	n.a.	n.a.	
Mn	55.24 $\pm$ 1.18	57.0 $\pm$ 2.4	96.9	n.a.	n.a.	
Pb	23.63 $\pm$ 1.02	25.0 $\pm$ 1.5	94.5	7.90 $\pm$ 0.22	8.18 $\pm$ 0.021	96.6
Zn	16.56 $\pm$ 1.80	16.0 $\pm$ 0.7	103.5	49.0 $\pm$ 2.7	51.72 $\pm$ 0.26	94.7

### 3.2 Heavy metal accumulation by plants from contaminated area

Accumulation of heavy metals by coltsfoot, a plant used for bioremediation of tailing dump of barite mine in the region of Tarnița, Suceava, Romania, was estimated by the ICP-OES analyses after acid sample digestion. A plant harvested 4-5 kilometres upstream of the tailing dump, on an area considered non-contaminated, was used as a control. Roots and leaves were separated and analysed. The obtained results are presented in Table 3. As can be seen from the results, the main part of heavy metals was accumulated in roots, except zinc. The quantity of zinc in roots and leaves was statistically equal. The quantity of Cu, Fe, Zn, Al, Pb, Ni, and Mn in roots of plants from contaminated and non-contaminated area did not differ at 95% confidence level. In contrast, the results showed elevated content of heavy metals in leaves compared to the control. Pb and Ni were found only in roots of plants grown on the contaminated area. Although, As was found in the soil and tailing dump samples at 100 - 670 mg kg<sup>-1</sup> [18] it was not detected in the coltsfoot samples.

**Table 3.** Heavy metals content in coltsfoot plant: (A) plants sampled nearby tailing dump; (B) plant harvested 4-5 km upstream of the tailing dump (N=2; P=95%).

Sample	Concentration, mg kg <sup>-1</sup>						
	Cu	Fe	Zn	Al	Pb	Ni	Mn
Roots (A)	9.5 $\pm$ 2.1	235 $\pm$ 34	8.2 $\pm$ 1.4	136 $\pm$ 24	2.1 $\pm$ 0.6	1.9 $\pm$ 0.3	10 $\pm$ 1
Roots (B)	5.1 $\pm$ 1.7	301 $\pm$ 48	6.4 $\pm$ 2.0	140 $\pm$ 40	<1.2	<1.5	13 $\pm$ 4
Leaves (A)	3.1 $\pm$ 0.2	97 $\pm$ 23	8.7 $\pm$ 0.4	41 $\pm$ 1	<1.2	<1.5	7.6 $\pm$ 0.2
Leaves (B)	3.6 $\pm$ 0.1	109 $\pm$ 7	7.8 $\pm$ 0.2	35 $\pm$ 2	<1.2	<1.5	6.5 $\pm$ 0.4

### 3.3 Germination test and accumulation of heavy metals in *Triticum aestivum* as bioindicator

To estimate soil and tailing dump material two series of germination tests were made. First, the wheat seeds were planted on untreated material or soil. Second, the seeds were let to germinate after decontamination of the soil by distilled water. Three samples from the studied area were tested. The samples were denoted as follows: soil sample T1 was taken at 30 m distance from the tailing dump; sample T2 was a material from the tailing dump; sample T3 was soil from the river bank near by the tailing dump. Distilled water was used as a control. Three batches of 50 wheat seeds were prepared for each test and the means and confidence interval at 95 % were calculated. The seeds were treated and planted according to the procedure recommended by ISTA [22]. The obtained results are presented on Table 4. The state of the obtained plantlets in samples germinated in decontaminated soil was comparable to the control. However, the length and weight of plantlets germinated on the material from tailing dump were only 30 % of the control. The germination test on soils and tailing material with any treatment was negative – any germinated seeds were found.

**Table 4.** Results from the germination test (N=3; P=95%).

Sample	T1	T2	T3	Control
Germinated seeds, number	43±7	23	41±3	43±3
Dead seeds, number	6.7±7	27	8.7±2.8	6.7±2.7
Weight of germinated seeds, g	2.4±0.3	0.75	2.1±0.2	2.2±0.3
Total length of germinated seeds, cm	402±65	134	390±67	389±54
Weight of the roots, g	2.2±1.1	0.39	1.4±0.7	1.7±0.3

All wheat seeds were analyzed by the proposed method. The results are presented in Table 5 and 6. Non-germinated seeds were used as a control. They were analyzed following the same procedure. As can be seen from Table 5, the dead seeds contained elevated concentrations of heavy metals and arsenic. The results showed that the heavy metals in the soils are highly bioavailable and the contaminated soil wasn't able to support the germination of studied bioindicator.

**Table 5.** Heavy metals accumulation in dead seeds planted on untreated soils (N=3; P=95%).

Sample	Concentration, mg kg <sup>-1</sup>								
	Cu	Fe	Zn	Al	Pb	Ni	As	Mn	Ba
Control	<0.5	45±3	32±1	10.8±0.2	<1	<1.5	<1	41±1	7.1±0.4
T1	85.4±0.1	2018±13	29±1	279±2	10.6±0.5	<1.5	3.8±0.2	24±3	25±7
T2	187±3	6259±179	119±17	219±48	15.1±0.5	10.1±0.7	448±50	29±2	6.4±0.5
T3	127±3	2833±233	44±1	87±5	<1	5.7±0.4	82.±1.5	22±1	7.0±0.3

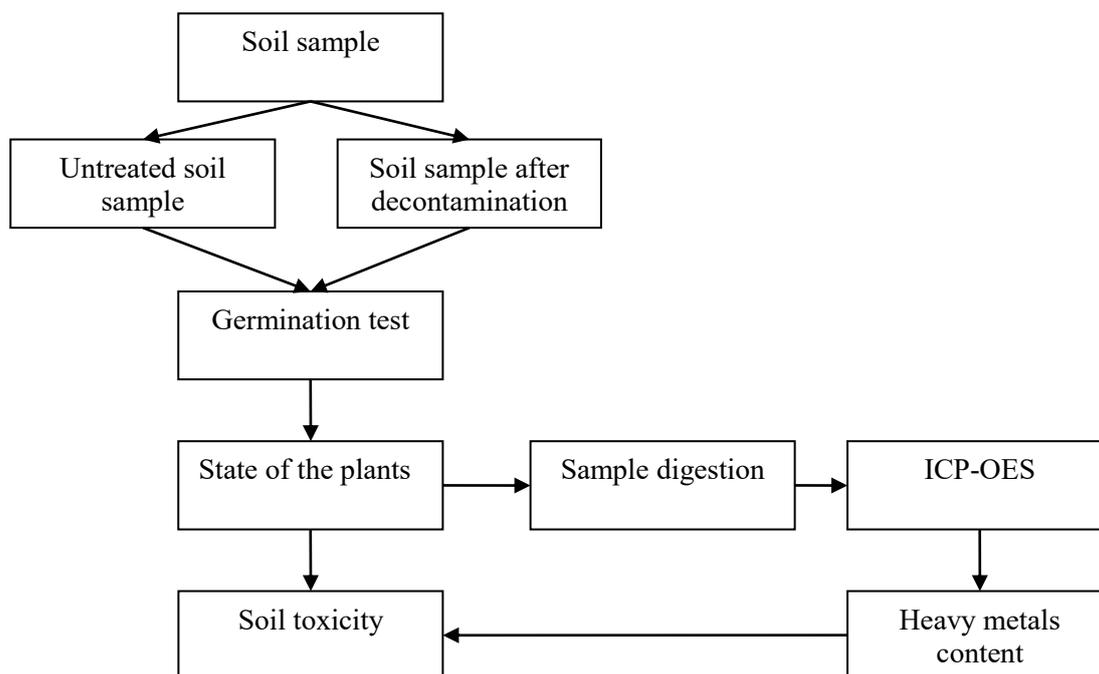
As the results presented in Table 6 showed, the germinated seeds accumulated more heavy metals in its roots than in the leaves. The highest content was observed in plantlets germinated on tailing material washed with distilled water. The results showed that the decontamination of the soils by distilled water decrease heavy metal content and increase the capability of the soil to sustain the germination.

**Table 6.** Heavy metal content in plantlets germinated in decontaminated soil (N=3; P=95%).

Sample	Concentration, mg kg <sup>-1</sup>								
	Cu	Fe	Zn	Al	Pb	Ni	As	Mn	Ba
T1 roots	9.3±1.3	431±118	14±2	129±24	<1	<1.5	<1.1	31±7	98.3±0.8
T1 leaves	5.5±0.8	213±7	10±2	78±5	<1	<1.5	<1.1	12.0±0.4	68±4
T2 roots	200	12270	71	95	737	6.5	49	63	72±7
T2 leaves	8.7	238	10	17	10	1.1	1.4	15	11±2
T3 roots	50±12	6493±1070	54±1	352±83	23±3	2.8±0.2	23±1	33±7	251±46
T3 leaves	7.5±0.2	351±46	9.9±0.7	24±1	1.2±0.3	0.7±0.1	1.6±0.6	10.8±0.1	20.8±0.5
Control roots	7.1±0.9	54.7±0.4	11.6±0.3	25±7	<1	<1.5	<1.1	20±1	9±2
Control leaves	4.2±0.1	22±6	6.6±0.7	2.2±1.3	<1	<1.5	<1.1	10.1±0.2	4.4±0.4

### 3.4 Protocol for estimation of soil toxicity

Based on the obtained results the following protocol for estimation of soil toxicity was proposed (Figure 1). The overall uncertainty of the proposed protocol was determined by germination of two series of three batches of wheat seeds used as bioindicator, sample pretreatment by open acid digestion and ICP-OES determination of the target analytes. The precisions were estimated as RSD under within-laboratory reproducibility conditions.



**Figure 1.** The proposed protocol for estimation of soil toxicity.

**Table 7.** Uncertainty of the developed protocol.

Precision	Cu	Fe	Zn	Al	Pb	Ni	As	Mn	Ba
RSD, % <sup>a</sup>	1.7	2.9	1.5	2.3	3.0	3.2	4.3	0.9	3.0
RSD, % <sup>b</sup>	6.5	8.2	9.2	9.7	6.8	6.4	11	4.7	5.9
U, mg kg <sup>-1</sup>	6.40	179	4.3	22	2.5	1.3	2.5	2.9	2.1

<sup>a</sup>RSD, % - one series of three batches <sup>b</sup>RSD, % - two series three batches each

RSD was calculated from one series of three batches germinated seeds and from two series each containing three batches. The obtained RSD within one series was below 5% for each of the studied analytes, thus indicated good precision of the procedure [23]. The second series of experiments was performed within 3 months using other batches of wheat seeds. Thus, obtained RSD took account on the effect of the wheat seeds used to perform heavy metals and metalloids tests. The obtained RSD was within 10%. The expanded uncertainty of the proposed protocol including germination, sample digestion, ICP-OES measurement, was calculated as  $U=k \cdot SD$  ( $k=2$  at 95 % confidence level) [24]. The SD was calculated from duplicated series. The results are presented in Table 7. Relatively low values of expanded uncertainty showed good precision of the proposed protocol.

#### 4. Conclusions

A protocol for estimation of soil toxicity based on germination test, open acid digestion of plantlets and seeds and ICP-OES determination of heavy metals and metalloids content was proposed. The sample digestion procedure and ICP-OES measurement were validated by analyzing plant certified reference materials. Additionally, standard addition before sample pretreatment was applied for estimation of method recovery at the levels of heavy metals found in plants from contaminated region of closed barite mine in Tarnița, Suceava, Romania. The expanded uncertainty of the protocol, including germination tests, sample digestion and ICP-OES measurements, was estimated. The protocol could be used for estimation of efficiency of decontamination procedures for tailing dump material and surrounding soil. The study is currently in progress in our laboratory.

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