METAL POLLUTION BIOMONITORING IN MINING AREAS USING PERENNIAL RYEGRASS (*LOLIUM PERENNE*)

Levente Levei[1], **Maria Alexandra Hoaghia**[1], **AlexandruOzunu**[2]

[1]*INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 400293 Cluj-Napoca, 67 Donath, Romania*
e-mail: levente.levei@icia.ro
[2]*Babes-Bolyai University, Faculty of Environmental Sciences and Engineering, 400294 Cluj-Napoca, 30 Fântânele, Romania*
e-mail: alexandru.ozunu@ubbcluj.ro

Abstract

The uptake of metals (Pb, Cu, Cd, Zn, Ni, Cr) by perennial ryegrass (*Loliumperenne*), grown on tailings situated in Central and NW Romania, was investigated by calculating the transfer factors (TFs). The mean TFs decreased as follows: $TF_{Pb}>TF_{Cd}>TF_{Zn}>TF_{Ni}>TF_{Cr}>TF_{Cu}$. The metal concentrations in tailings ranged between 19.9-2640 mg/kg Cu, 11.4-5156 mg/kg Pb, 9.10-5328 mg/kg Zn, 0.200-89.6 mg/kg Cd, 3.15-77.6 mg/kg Cr, 1.90-58.9 mg/kg Ni and in ryegrass between 2.25-58.6 mg/kg Cu, 0.129-4815 mg/kg Pb, 5.12-174 mg/kg Zn, 0.010-3.50 mg/kg Cd, 0.410-6.20 mg/kg Cr and 0.440-3.71 mg/kg Ni. The linear relationship between the metal content in ryegrass and tailings indicated a significant correlation for Pb and Zn. Metal concentrations in ryegrass differ in the three areas (Certej, Baia Mare and Aries) and can be used as a biomonitor forPb, Zn pollution.

Introduction

Extraction and use of ores in industrial processes produced significant environmental pollution with metals, which pose a serious threat to human health and ecosystems, due to their toxicity, tendency to bioaccumulate and high persistence [1]. Although some metals (such as Mn, Zn, Cu, Cr, Ni, Fe) act as micronutrients for living organisms, they could induce toxic effects at higher concentrations. Other metals (such as Hg, Cd, As, Pb) are non-essential and are highly toxic even at low concentrations [2].

Different plants have been used to monitor pollution, because they can accumulate toxic compounds without any deleterious effects. Among them, perennial ryegrass (*Loliumperenne*) is suitable as accumulative biomonitor for assessing atmospheric or soil levels of metals [3, 4, 5]. Grass takes up metals via the roots and is also influenced by atmospheric dust impact [4].

The relationship between metal concentrations in tailings and plant is highly specific to the plant species, substrate and metal chemistry, and is generally described by empiric equations [6]. The relationship the between contaminant concentration in plant and the concentration in soil is described using Transfer Factor (TF), which is defined according to Equation 1.

$$TF = \frac{Conc_{lollium}}{Conc_{tailings}} \qquad \text{Eq. 1}$$

where the concentrations are expressed as mg/kg.

The TF values quantify the relative differences in bioavailability of metals to plants and identify the efficiency of a plant species to accumulate a given metal [7].

Experimental

Description of the sampling areas

The environmental impact of nonferrous mining activities is considerable in the Central and Northwestern parts of Romania, where centuries of ore extraction and exploitation generated large amounts of mining waste [8]. The study was conducted in three former mining areas: Baia Mare (NW Romania)and Aries and Certej (CentralWEst Romania), where mining wastes were collected in tailings management facilities [9, 10].

Sample collection and preparation

In autumn 2016, a number of 15 ryegrass samples and the subadjacent tailings collected from the old tailings ponds that are partially revegetated by spontaneous flora. The samples were collected as follows: 5 from Certej, 8 from Baia Mare (BM) and 2 from Aries areas using a hand auger.Samples of tailings were thoroughly mixed, air dried, grounded to pass through 2 mm and then 250 μm sieve. Plant samples were washed in distilled water, dried at 40°C until constant weight and ground to obtain a homogenized powder.

Determination of metals content

The metal contents in tailings were determined after aqua regia(HCl 37.5% and HNO_3 65%) extraction for 16 hours at room temperature and then, 2 hours, at reflux conditions, according to SR ISO 11466:1999 [11]. The extract was analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES) using a SPECTRO FLAME spectrometer (SPECTRO, Kleve, Germany).

An amount of 0.5 g of plant sample was digested in 5 ml $HNO_3$65%and 2 ml $H_2O_2$30%in closed PTFE vessel microwave digestion system, Berghof MWS-3+ (Eningen, Germany). Metals concentrations were measured by inductively coupled plasma mass spectrometer (ICP-MS) ELAN DRC II, Perkin Elmer, USA.

The quantification of ICP-OES and ICP-MS was performed using an external calibration with multi-elemental Merck (Darmstadt, Germany) standard solution. Throughout the experiments, ultrapure water (Millipore from a Direct Q UV 3 Millipore system) was used.

Results and discussion

Descriptive statistics for metal contents in ryegrass in Certej, Baia Mare and Aries areas are shown in Table 1. All concentrations are reported on a dry-weight basis.

Table 1. Descriptive statistics for total metal contents in ryegrass (mg/kg) in Certej, Baia Mare and Aries areas

	Cu	Pb	Zn	Cd	Cr	Ni
Certej area						
min	2.85	0.139	5.12	0.010	0.410	1.00
max	13.7	49.8	49.9	0.840	6.29	3.11
mean	5.65	10.5	25.6	0.374	2.60	2.02
Baia Mare area						
min	2.50	3.34	15.7	0.036	0.645	0.440
max	58.6	4815	174	3.50	5.49	3.71
mean	21.2	1233	84.7	1.56	2.03	1.50
Aries area						
min	2.25	0.129	22.2	0.139	1.71	2.10
max	2.98	0.935	31.4	0.218	2.84	2.29
mean	2.61	0.532	26.8	0.179	2.28	2.20

The obtained concentrations in ryegrass were constantly much higher in Baia Mare area than in Certej and Aries areas (except for 1 sample). In three of the ryegrass samples collected from Baia Mare area, the content of Pb exceeded 500 mg/kg and in 2 samples exceeded 1000 mg/kg. Also, Zn concentration exceeded 100 mg/kg in 3 samples collected in Baia Mare area. The maximum tolerant levels set by the European Commission (Directive 2002/32/EC, 2002: Pb: 10 mg/kg, Cd: 1 mg/kg) [12] for animal feed such as grass and fodder were exceeded for Pb in one sample from Certej area and in 7 samples collected from Baia Mare area and for Cd in 4 ryegrass samples collected from Baia Mare area.
Descriptive statistics for metals contents in tailings in Certej, Baia Mare and Aries areas are shown in Table 2. All concentrations are reported on a dry-weight basis.

Table 2. Descriptive statistics for total metal contents in tailings (mg/kg) in Certej, Baia Mare and Aries area

	Cu	Pb	Zn	Cd	Cr	Ni
Certej area						
min	19.9	11.4	9.10	0.200	3.15	2.39
max	291	170	180	6.09	32.1	38.4
mean	115	70.1	73.6	1.72	16.5	14.8
Baia Mare area						
min	114	501	343	3.40	9.80	1.90
max	2640	5156	5328	89.6	65.1	28.9
mean	1181	1960	1405	18.7	33.5	14.2
Aries area						
min	30.5	16.08	100	4.11	38.8	33.1
max	32.2	32.3	140	5.98	77.6	58.9
mean	31.3	24.1	120	5.05	58.2	46.0

Similar to metals content in plants, the concentration in tailings was much higher in Baia Mare area than in Certej and Aries areas, for all the investigated elements. In three of the samples, the concentrations of Cu exceeded 1000 mg/kg and in two samples, slightly less than this value. Also, Pb contents were higher than 2000 mg/kg in four samples and around 1000 mg/kg in three samples, all in Baia Mare area.
The values of TF calculated for all metals are graphically represented in Figure 1.

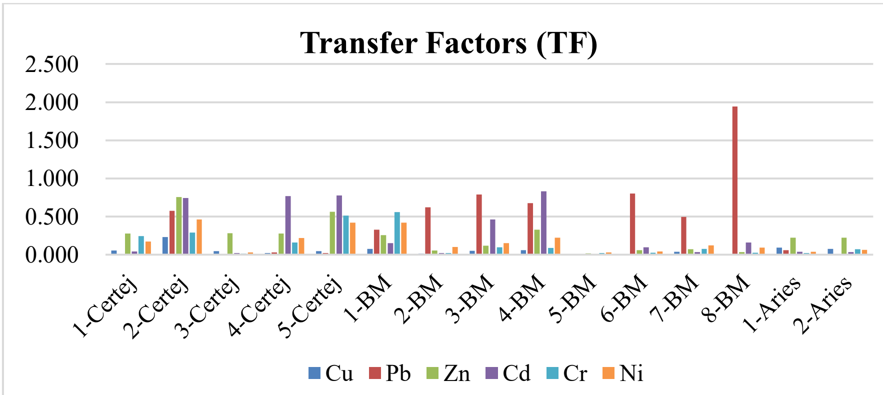

Figure 1. Values of transfer factors for metals in all the sampling points

The values of TF varied largely, over 5 orders of magnitude, from 0.0006 to 1.94, both values for Pb, in Baia Mare area. All the values of TF were lower than 1, except for Pb (1.94) in one sample. The mean values of TF decreased as follows: $TF_{Pb}>TF_{Cd}>TF_{Zn}>TF_{Ni}>TF_{Cr}>TF_{Cu}$.The relationships between metal concentration in ryegrass and tailings were performed for each metal as linear regressions. The obtained equations are showed in Table 3.

Table 3. Relationships between metal concentration in ryegrass and tailings

Metal	Regression equation	Regression coefficient, R^2
Cu	y = 16.79x + 444.69	0.11
Pb	y = 1.21x + 541.46	0.57
Zn	y = 22.64x - 507.20	0.64
Cd	y = 9.91x + 1.48	0.27
Cr	y = -3.35x + 38.68	0.07
Ni	y = 2.87x + 13.58	0.03

For Pb and Zn, the regression coefficients R^2 are higher than 0.5, assuming a significant linear correlation. For the rest of the investigated metals (Cu, Cd, Ni, Cr), non-linear transfer functions could be supposed.

Conclusions

In this study, high levels of Cu, Pb, Zn, Cd, Cr, Ni contents were recorded in tailings and ryegrass, especially in Baia Mare area (NW Romania). The metal content in tailings contributed significantly to metal content in ryegrass. Metal concentrations in ryegrass differ in the three investigated areas (Certej, Baia Mare and Aries), and could be used as biomonitor of metal pollution. Even after decommissioning, tailings deposits represent a continuous pollution source for the surrounding environment.

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