

Applicability of Atomic Emission and Atomic Absorption Spectrometry for Variability Assessment of Trace and Macro-Elements Content in Moss Species from Pb-Zn Mine Environment

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Abstract

The aim of this study was to assess the applicability of the Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) Electro Thermal Atomic Absorption Spectrometry (ETAAS), and Cold Vapour Atomic Absorption Spectrometry (CVAAS) for the analysis of total content for 23 elements. Deposition and distribution of metals in the air was determined by biomonitoring a widely prevalent moss species from a lead and zinc polluted area of the "Toranica" mine, Republic of Macedonia. Moss species *Hypnum cupressiforme*, *Homalothecium lutescens*, *Camphothecium lutescens* and *Brachythecium glareosum* were used as very specific and suitable sampling biomonitors. Moss samples were digested by the application of a closed wet digestion using a microwave digestion system. The applied instrumental techniques were useful in order to determine a wider range of content for the analyzed elements; macro contents of Ca, Mg, K and P to trace contents of As, Cd, Co, Ga, and Hg. From data processing the values for Pb and Zn were used as anthropogenic markers. Higher contents of As, Cd and Cu in moss samples from this region were also determined, confirming the impact of mining activities on anthropogenic air pollution in this area. Multivariate factoring identifies four chemical associations: F1 (As-Cd-Ca-Cu-Fe-Mn-Pb-Zn), F2 (Co-Cr-Li-V), F3 (Hg-P) and F4 (K).

Keywords: Air pollution; Moss biomonitoring; Heavy metals; ICP-AES; ETAAS; CVAAS

Introduction

Environmental pollution has been known as one of the most important problems in modern societies. Heavy metals are a major source of environmental pollution, and determining their environmental concentrations is an important part of understanding biogeochemical processes and gauging ecosystem's health [1]. Human activities, such as mining and tailings discharge processes are rapidly increasing environmental pollution [2,3]. Emissions into the air constitute the greatest source of the toxic metals pollution. Even the metals emitted naturally in wind-blowing dust are often of anthropogenic sources. Therefore, it is necessary to maintain a close watch on heavy metal depositions, even when local or regional areas are monitored. Specific danger arises from waste processing ore and flotation tailings in mine plants surroundings. Metals content quantifications present an ecological for the determination not only of the presence of metals but also their distribution and extent of toxicology for the environment and human health [4].

The use of mosses as biomonitors in smaller scale is a convenient way of determining levels of atmospheric deposition [5-8]. This is possible because mosses absorb/adsorb nutrients, and also contaminants, directly from the atmosphere. Compared to higher plants, mosses have several advantages which make them more suitable for this kind of study [9]. Mosses lack a root system and a well-developed cuticle, thus the substrate has little influence on the levels of contaminants in their tissues and they readily take up atmospheric contaminants. Moreover, the cationic exchange capacity in mosses is high and they possess a high surface area to volume ratio, factors which both favor the accumulation of large amounts of pollutants [10]. Bryophytes are especially suitable organisms for purposes of monitoring investigations, because procedures of sampling and chemical analyses are relatively simple and of low-cost. Bryophytes include evergreen and perennial plants, making it possible to collect them year round. Most of their

species are widespread, and, thus, heavy metal concentrations of distant areas can also be compared [11].

Determination of enriched contents for certain toxic elements (As, Cd, Cr, Cu, Hg, Ni and Pb) occurring in moss species as an accumulator medium, present a challenge for analytical determination. These elements occur from trace contents to micro and macro contents in polluted areas. Thus it is necessary to find the most appropriate techniques for their quantification. Widely used instrumental techniques for multielement analyses like Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) are used by numerous investigations [12-14]. However, the elemental analyses like Electro Thermal Atomic Absorption Spectrometry (ETAAS) for ultratrace elements (As, Co) and Cold Vapour Atomic Absorption Spectrometry (CVAAS) for mercury determination are still the most efficient instrumental analytical techniques [5].

The present study is the first attempt to characterize the atmospheric deposition of 23 elements in "Toranica" lead-zinc mine vicinity by means of mosses. Therefore, the aims of this study were: 1) quantification of metals in the air in terms of determination of elements contents in mosses as well as by calculated deposition rates; 2) patterns

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Parameter	As	Cd	Co
Wavelength, nm	193.7	288.8	242.5
Spectral width slit, nm	0.2	0.5	0.2
Calibration mode	Peak height		
Lamp current, mA	10.0	4.0	7.0
Step 1 (drying)			
Temperature, °C	120	120	120
Ramp time, s	55	55	55
Hold time, s	-	-	-
Step 2 (pyrolysis)			
Temperature, °C	1400	250	400
Ramp time, s	10	5	5
Hold time, s	35	15	22
Step 3 (atomizing)			
Temperature, °C	2600	1800	2300
Ramp time, s	1	1	1
Hold time, s	2	2	2
Step 4 (cleaning)			
Temperature, °C	2600	1800	2650
Time, s	2	2	5

Table 2: Operating condition and wavelengths for As, Co and Cd for ETAAS.

Elements	Detection Limit (in mg kg ⁻¹)
ETAAS	
Cd, Co	0.1
As	0.2
ICP-AES	
Mn	0.0015
Zn	0.003
Fe	0.006
Al, Cu	0.0125
Ga, Ba, Ca, Mg, Sr	0.025
B, Cr, Li, V	0.05
Ni	0.25
P, Pb	0.5
Na	2.5
K	5
CVAAS	
Hg	0.01

Table 3: Lower detection limits of determinations for ETAAS, CVAAS and ICP-AES for analyzed elements.

the absorption cell. The mercury absorption line is has the wavelength of 253.7 nm. The magnitude of the signal shows the amount of mercury present in the samples.

The QC/QA of the applied techniques was performed by standard addition method, and it was found that the recovery for the investigated elements ranges for ICP-AES 98.5-101.2%, for ETAAS and CVAAS 96.9-103.2%. The same methods were applied for the determination of the elements in moss certificated reference materials M2 and M3 [20]. The sensitivity in regard to the lower limit of detection was done as given in table 3.

Data processing for elements contents

The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics. The application of bivariate statistics was used for data check of elements contents correlations. For that issue the linear coefficient of correlation was used. Two-dimensional scatterplots were used to visualize relations between two data sets. Individual data points were represented by point markers in two-dimensional space, where axes represent the variables.

Multivariate statistical method (cluster and R-mode factor analyses) was used to reveal the associations of the chemical elements. The factor analysis was performed on variables standardized to zero mean and unit standard deviation [21,22]. As a measure of similarity between variables, the product-moment correlation coefficient (r) was applied. There are various rotational strategies that have been proposed [22]. The goal of all of these strategies is to obtain a clear pattern of factor scores, that is, factors that are somehow clearly marked by high loadings for some variables and low loadings for others. The elements with low communalities were excluded because of their lack of significant associations. In this study, the varimax method was used for orthogonal rotation. As before, we want to find a rotation that maximizes the variance on the new axes; put another way, we want to obtain a pattern of loadings on each factor that is as diverse as possible, lending itself to easier interpretation. Statistical softer *Statistica 8.0.* was used for data processing the obtained values for elements contents.

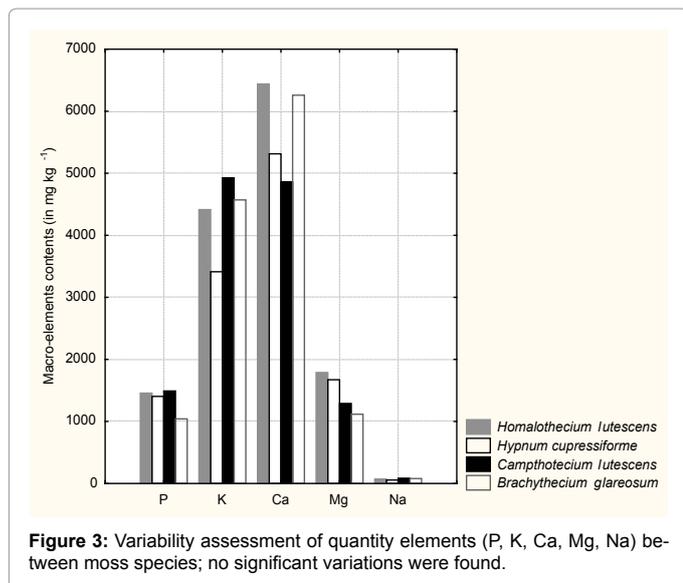
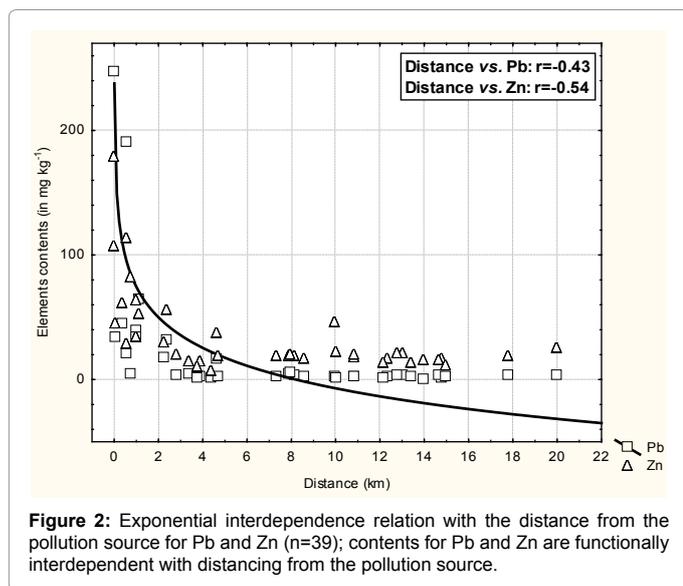
Results and Discussion

The descriptive statistics of analyzed elements is presented in table 4. On the basis of the normality tests and compared with histograms of distribution for the content of all analyzed elements in moss samples, the normality was assumed for the values of Al, Ca, Cr, Hg, K, Na, and Sr. High contents of Pb and Zn were assumed (average values 60 and 75 mg kg⁻¹, respectively) in the area very close to the pollution source. However, distribution of dust with high Pb-Zn contents was expected in the investigated area. The maximum values (420 mg kg⁻¹ for Pb and 180 mg kg⁻¹ for Zn) for the contents in moss occur in very close vicinity

Element	x_g	x_g	Md	Min	Max	P ₁₀	P ₉₀	S	CV	A	S
Al	1038	907	989	203	2818	343	1892	535	51.5	1.30	2.76
As	0.80	0.30	0.25	0.03	10.5	0.09	1.62	1.86	233	4.31	20.4
B	5.17	3.71	3.62	1.13	26.7	1.52	9.85	5.25	102	2.98	11.2
Ba	33.9	28.0	26.3	5.76	98.2	8.54	70.1	21.5	63.4	1.30	1.28
Ca	5929	5659	5915	2360	10587	4136	8098	1795	30.3	0.57	0.79
Cd	0.31	0.19	0.15	0.06	1.62	0.06	0.96	0.37	120	2.17	4.19
Co	0.30	0.17	0.15	0.02	1.36	0.04	0.80	0.35	117	1.98	3.34
Cr	1.73	1.62	1.62	0.48	4.00	0.97	2.64	0.66	37.8	1.10	2.74
Cu	6.20	5.73	5.56	2.23	16.3	3.62	10.1	2.69	43.3	1.60	3.88
Fe	1408	1046	1158	164	7113	331	2790	1270	90.2	2.78	10.3
Ga	1.61	1.37	1.51	0.21	2.88	0.99	2.69	0.80	49.4	0.18	-0.50
Hg	0.02	0.02	0.02	0.01	0.04	0.01	0.03	0.01	46.0	-0.03	1.13
K	4065	3927	3896	2184	8300	2640	5412	1131	27.8	1.35	3.92
Li	0.69	0.57	0.56	0.13	2.52	0.23	1.36	0.50	72.8	2.46	6.64
Mg	1692	1607	1649	846	2489	975	2376	525	31.0	-0.05	-1.40
Mn	209	159	156	30.4	815	48.1	430	160	76.1	1.68	4.13
Na	65.3	60.7	59.5	22.3	133	32.3	106	25.4	38.9	0.79	0.51
Ni	2.88	2.51	2.84	0.45	9.43	1.05	4.75	1.56	54.0	1.93	7.27
P	1419	1382	1350	1028	2172	1041	1713	347	24.5	0.79	0.32
Pb	31.6	6.25	3.21	0.43	421	1.44	64.1	80.8	255	3.81	15.4
Sr	18.6	17.1	18.1	7.5	49.0	8.6	26.6	7.92	42.7	1.66	4.96
V	2.18	1.68	2.01	0.21	7.06	0.69	4.82	1.53	70.2	1.33	1.83
Zn	34.7	25.6	19.4	6.7	179	12.7	81.3	34.5	99.4	2.60	7.75

Table 4: Descriptive statistics for the analyzed elements in collected moss species (n=39)

Values given in mg kg⁻¹; X_g : mean; X_g : Geometric Mean; Md: Median; Min: Minimum; Max: Maximum; P₁₀: 10 percentile; P₉₀: 90 percentile; S: Standard Deviation; CV: Coefficient of Variation; A: Skewness; E: Kurtosis; S: Skewness; bolded values indicated higher anthropogenic introducing in the air of these elements



of the pollution source. The median value for lead contents (3.2 mg kg^{-1}) compared to the same value for the whole territory of the Republic of Macedonia (4.6 mg kg^{-1}) shows no enrichments at all [14]. If close mine locations are isolated from the rest of the study area an enrichment of 10 times was obtained for 12 samples (33.4 mg kg^{-1}). Fine dust from flotation tailings is distributed due to winds caring out, and deposited on moss. Almost 0.05% of these heavy metals are accumulated in the moss plant tissue, confirming how good adsorbers/absorbers of heavy metals are. Significant skewed distributions were main problem in data processing. The rest of the potentially hazardous elements distributes in ranges for As ($0.03\text{-}10.5 \text{ mg kg}^{-1}$), Cd ($0.06\text{-}1.62 \text{ mg kg}^{-1}$), Hg ($0.01\text{-}0.04 \text{ mg kg}^{-1}$), Ni ($0.45\text{-}9.43 \text{ mg kg}^{-1}$). The lead-zinc deposits usually are characterized with Mn contents as well. Therefore, the Mn contents were ranged in $30.4\text{-}815 \text{ mg kg}^{-1}$, wherefrom it can be considered geo-enrichments in the study area.

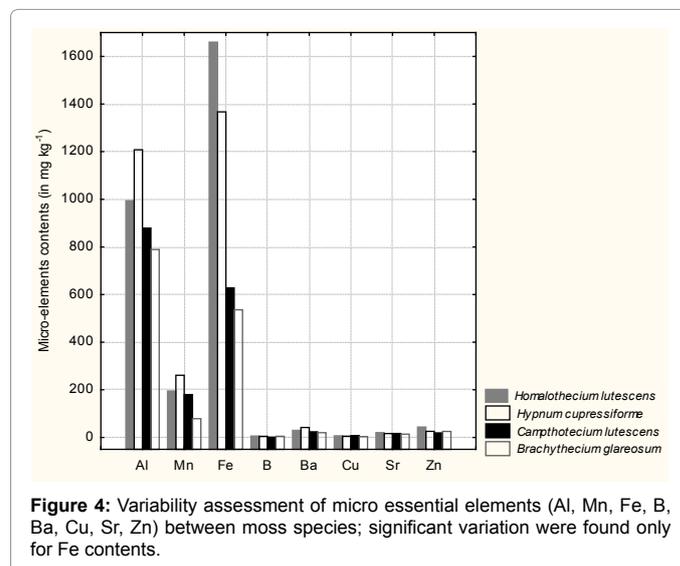
Lead and zinc were distinguished as main anthropogenic introduced elements in the mine environment. The logarithmic values were used in the interpretation of the Pb and Zn distribution. Aerial distribution of

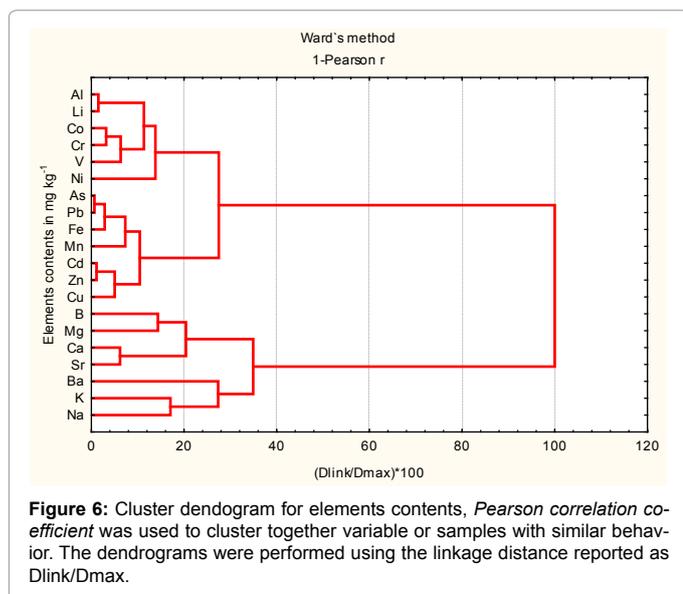
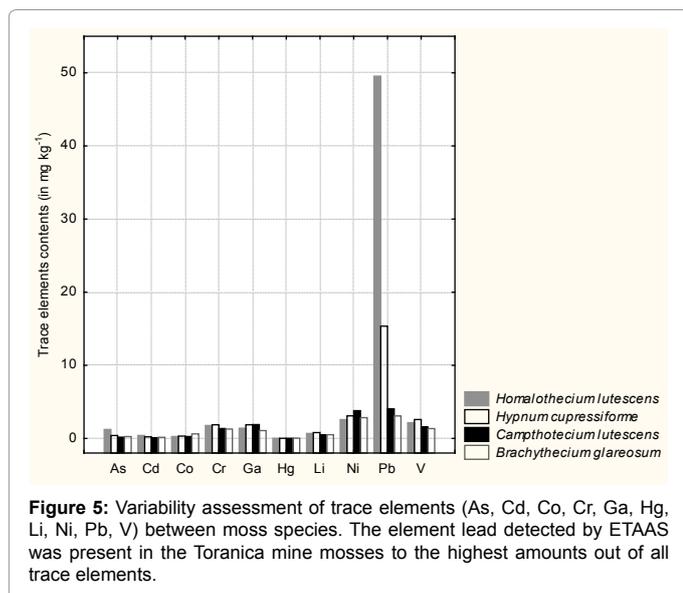
dust particles is certainly in relation to the distance at which they can reach. Therefore, a functional dependence of the content versus distance from the emission source was established. Functional interdependence relation of elements contents across the distance from the pollution source was determined. Significant higher negative correlation ($r = -0.43$ and -0.54 , respectively for Pb and Zn) was derived from the relation given in figure 2, indicating that distancing from emission source, results in decrease of bio-accumulation of these heavy metals in moss (Table 4).

Interspecies comparison of bio-indicating ability of the collected four moss species was performed in order to determine whether there is a statistically significant difference in the accumulation of certain elements. The macro elements content does not show any significant variations in moss tissues as given in figure 3. Phosphorus is accumulated in the ranges $\sim 0.1\%$ as same as Mg. Calcium contents ranges from $0.4\text{-}0.6\%$ in all four moss species. Sodium is the mainly accumulated macro element in ranges of $55\text{-}85 \text{ mg kg}^{-1}$.

Micro elements (Al, Mn, Fe, B, Ba, Cu, Sr, and Zn) similar to macro elements contents do not significantly vary between the moss species based on their average values (Figure 4). However, if we consider that the higher content of certain micro elements are introduced in the mine environment, contents enrichment occurred. Such is the case with Mn and Zn contents where the maximum values of 815 and 179 mg kg^{-1} , respectively, are obtained from *Homalothecium lutescens* moss tissue sample. *Hypnum cupressiforme* showed higher bioaccumulation ability for Al and Ba as micro elements and Cr, Ga and Li as trace elements. Certain predominance of *Homalothecium lutescens* occurs in relation to the accumulation of potentially hazardous elements As, Cd, Cr and Pb, but it should be considered that this is the dominant collected species in the study area (Figure 5). *Campthotecium lutescens* and *Brachythecium glareosum* are less dominant species but no deviation in average values for all analyzed elements were found. Considering this, no specific trend in the elements distribution pattern in mosses species were found (Figures 3-5). This evidence suggests that environmental factors influence element distribution between moss species.

By the application of multivariate statistics, three geogenic and one anthropogenic association were established on the basis of: a) visual inspection of similarities of spatial distribution of element patterns in moss samples; b) comparison of basic statistical parameters; c) the





correlation coefficient matrices; d) the results of multivariate (cluster and Factor Analysis, FA) analyses (Figure 6 and Table 5). *Pearson correlation coefficient* was used to cluster together variable or samples with similar characteristics. The distribution of the normal variable in FA was reduced, excluding Al, Na, Sr, Mg, Ga, Ba and B, because of their very low factor loadings.

Four factors were identified, when applying FA that includes 87% of variability of the treated elements. Factor 1 (F1) is the strongest factor representing 40% of the total variability. This factor principally associates As, Cd, Ca, Cu, Fe, Mn, Pb, and Zn, which for the most of the elements are presumed to be of anthropogenic origin. Only the Ca distribution does not rely on anthropogenic introduction, but higher content as macro-element and mechanisms of adsorption as Ca^{2+} ion metal, consequted with stronger correlation with the others elements from F1. Factor 2 (F2) is the second strongest factor, with 23% of total variability. With this factor were associated elements Co, Cr, Li and V. The group also links elements that are probably naturally and anthropogenically distributed. Second geochemical association

represents chemical elements that are probably naturally distributed. Factor 3 (F3) is the third strongest factor, with almost 12% of total variability. Significantly higher correlation of Hg-P was estimated for F3, due to obtained factor loadings (0.90 and -0.92, respectively). Therefore, the Hg accumulation in moss plant species probably is consequently dependent with phosphorus accumulation. The weakest expressive Factor 4 - F4, includes only K, represents 8% of the total variability. However, clustering extends the elements distribution linkage to K with Na and Ba (Figure 3). The same effect was achieved for the F3 where Ni was linked with the geochemical association Al-Li-Co-Cr-V-Ni (Figure 6 and Table 5).

Clustering the 23 elements distributions to four *synthetic variables* (elements associations) was more reproductive vs. FA. The geochemical association Al-Li-Co-Cr-V confirms the dust dispersion in the investigated area, due to that element are mainly characteristic to soil particles. Moss species placed the particles with the physical adsorption or adsorption from moss surface. The geochemical associations of B-Mg-Ca-Sr and Ba-K-Na associate elements primarily affected by natural factors such as lithological background with no characteristic anthropogenic influence. Implementing clustering the elements Hg, Ga, Ni and P were excluded, consequently because of no similarity linkage.

Anthropogenic impact occurs at geochemical association distribution of As-Pb-Fe-Mn-Cd-Zn-Cu. Typical elements which are consequences of air transport from flotation tailings and are not influenced by lithological background. High values are found in the close mine environ along the Kriva Reka Canyon. This factor is connected with pollution by the lead and zinc flotation tailing dam dust dispersion. Association of Fe in this factor indicates anthropogenic influence, although all southeastern countries Serbia, Bulgaria, Turkey and Macedonia reported high median values for Fe indicating a natural source of this element [14]. Normal distribution of Mn in moss species ranges 30–200 $mg\ kg^{-1}$, but in the present case, the maximal value was 815 $mg\ kg^{-1}$, indicating a significant Mn anthropogenic introduction in the investigated area. Considering this, Mn occurring in higher contents is expected phenomena, due to the lead-zinc ore deposits containing significant amounts of Mn.

Areal distribution maps were constructed in order to be determine

Elements	F1	F2	F3	F4	Comm.
As	0.92	0.16	0.02	0.05	0.98
Ca	0.76	0.01	0.34	-0.03	0.85
Cd	0.91	0.09	0.16	-0.02	0.96
Cu	0.85	0.06	-0.27	-0.15	0.84
Fe	0.88	0.39	0.00	0.21	0.98
Mn	0.77	0.21	-0.13	0.26	0.87
Pb	0.94	0.15	0.02	0.03	0.98
Zn	0.97	0.09	0.07	-0.01	0.99
Co	-0.23	0.80	0.21	-0.40	0.89
Cr	0.51	0.70	-0.02	0.30	0.91
Li	0.16	0.89	0.08	0.11	0.89
V	0.28	0.82	0.03	0.20	0.89
Hg	0.10	0.06	0.90	0.02	0.78
P	0.01	-0.08	-0.92	-0.10	0.77
K	-0.05	-0.27	-0.18	-0.83	0.71
Var.	0.40	0.23	0.12	0.08	0.87

Table 5: Factor loadings for elements contents in moss samples. F1, F2, F3, F4 - Factor loadings values; Comm: Communality; Var: Specific variance.

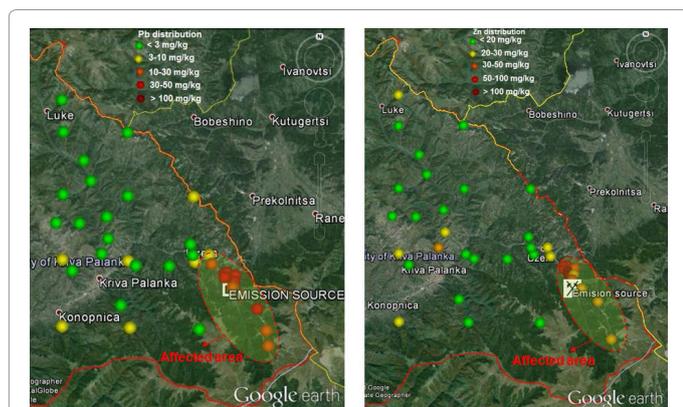


Figure 7: A real total contents distribution of main anthropogenic markers of Pb (left) and Zn (right) in “Toranica” lead-zinc mine environ. Distribution range for Pb is 0.4-420 mg kg⁻¹ and for Zn is 6.7-180 mg kg⁻¹, set in five sub contents.

the very close areas that are affected with hazardous contents of lead and zinc (contents >100 mg kg⁻¹), as presented in figure 7. The topography of the region is characterized as mountainous with reduced dust air distribution. The effect of contamination caused by flotation tailings open dam is occurring along in north direction from the flotation dam. Long distance distribution from the pollution source does not occur.

Conclusions

The applicability of ICP-AES, ETAAS and CVAAS for determinations of twenty three elements contents in moss species is presented. The ICP-AES technique was applied for determining the total contents of Al, B, Ba Ca, Cr, Cu, Ga, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Sr, V, Zn; the ETAAS for determination of As, Cd, Co; and CVAAS for determining the total content of mercury in moss samples. It was established that the applied instrumental techniques are useful in order to determine a wider range of concentrations for the analyzed elements. Metals biomonitoring with naturally growing mosses technique was used in dependence of four moss species (*Homalothecium lutescens*, *Hypnum cupressiforme*, *Brachythecium glareosum* and *Camptothecium lutescens*) in the Pb-Zn mining area of “Toranica” mine, Republic of Macedonia. Results show that Pb and Zn, occurring as main markers for anthropogenic impacts of the pollution source associated with As, Cd, Cu, and Mn, strongly correlated in moss samples. Highest bioaccumulation values are found immediately near the flotation tailing dam, decreasing rapidly with distance. Dispersion of fine dust from mining operations presents a serious environmental problem. Deposition patterns resulting from metal biomonitoring may help regional authorities to locate precipitation collectors for direct chemical analysis, in order to conduct further ecological and/or epidemiological surveys.

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