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VARIABILITY ASSESSMENT OF METALS DISTRIBUTIONS DUE TO ANTHROPOGENIC AND GEOGENIC IMPACT IN LEAD-ZINC MINE AND FLOTATION „ZLETOVO" ENVIRONS (MOSS BIOMONITORING)

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A b s t r a c t: Moss species (*Hypnum cupressiforme*, *Scleropodium purum* and *Campthotecium lutescens*) were used as suitable sampling media for biomonitoring the origin of heavy metal pollution in the lead–zinc mine and flotation environ near the town of Probištip. The 21 metals contents were determined by atomic emission spectrometry with inductively coupled plasma (ICP–AES). Data processing was applied with combinations of multivariate statistical methods: factor analysis, principal component analysis and cluster analysis. The main anthropogenic markers in the investigated area were Pb and Zn (maximal values of 200 and 186 mg kg–1, respectively). The factor analysis singled out (in the increasing scale) the following associations: F1/D1: Fe < Mo < Pb < Na < Cd < Mg < Zn < Ag < Cu and F2/D2: Mn < Ni < K < P < Ba < Sr < Ca < As < Cr < Al < V < Li. The anthropogenic elements contents vary independent from the moss species, but depending on the distancing from the pollution source, there are positive correlation. Long distance distribution from the emission source doesn't occur.

Key words: metals distribution; moss species; multivariate analysis; ICP–AES; Pb–Zn mine.

INTRODUCTION

The atmosphere is simple as well as complex. Therefore it's simple so long as it remains clean, but it's get complex when industrial activities (emissions) get grow. To achieve this complexness, numerous of monitoring programs where continuously conducted for almost five decades; to comprehend this complex nature of the atmospheric pollution with toxic elements (Athar and Vohora, 1995). Bio-indication of the heavy metals contents distributed thought the air showed very realistic measurements for the metals areal deposition in environ (Markert et al., 2003, Harmens et al., 2010; 2013). Small scaling monitoring present an challenge, considering the interference of many factors, like climatic condition with emphasis on winding, geographical location etc. (Fernández et al., 2007; Balabanova et al., 2010; 2013). Mosses have frequently been used to monitor time integrated bulk deposition of metals as a combination of wet, cloud, and dry deposition, thus eliminating some of the complications of precipitation analysis due to the heterogeneity of precipitation (Ceburnis et al., 1999; Ceburnis and Valiulis, 1999a; Aničić et al., 2009). Additional advantages of using mosses as heavy metal biomonitors include their stationary nature, wide spread geographic distribution, and low genetic variability between populations (Zechmeister et al., 2003; Dolegowska et al., 2013; Harmens et al., 2013). Heavy metal concentrations in mosses provide a complementary, time–integrated measure of the spatial patterns and temporal trends of heavy metal deposition from the atmosphere to terrestrial systems, at least for cadmium and lead (Aboal et al., 2010; Harmens et al., 2010; 2013). However, moss as suitable sampling media can reach the researcher a variety of results, but main problem occurs in the data processing and interpretation of semiquantitative results.

The data processing plays an important role in interpretation of obtained elements contents for areal distribution in environ. In the process of the biomonitoring enormous data can be collected, but the critical moment is the data processing and appropriate data interpretation. Therefore, there are different ways of interpreting the data obtained in this kind of monitoring programs. Variables differ in "how well" they can be measured; how much measurable information their measurement scale can provide. There is obviously some measurement error involved in every measurement, which determines the "amount of information" that can be obtained. Another factor that determines the amount of information that can be provided by a variable is its "type of measurement scale".

Generally speaking, the ultimate goal of every scientific analysis is finding relations between variables. The philosophy of science teaches that there is no other way of representing "meaning" except in terms of relations between some quantities or qualities; either way involves relations between variables. Correlation study involves measuring such relations in the most straightforward manner. Multivariate analysis is commonly used for statistically data processing of values obtained for the metals contents. Factor analysis (FA), principal component analysis (PCA) and clustering use different settings for processing and interpretation of the results, but the ultimate effect of data set are very similar (Anderson, 2001). The methodology of the above mentioned statistic analysis is considering as a data reduction methods, for reducing the number of variables. The question then is, how many *synthetic variables* (association of elements) should be extracted? The decision of when to stop extracting factors basically depends on when there is only very little "random" variability left. Reducing the numerous variable, selection of the most extensive variable, and interpretation of the synthetic variables (simplified as "grouping"), must be corresponding to the elements natural distribution and geology of the area. Commonly, FA, PCA and clustering are used in environmental monitoring where grouping of variables are expected as responsiveness of the two types of environmental impacts: anthropogenic, from one hand, and geogenic distribution of metals in potentially polluted area.

The conducted monitoring present an overview of the implementation and expression measurement of moss species stability in small area. Moss monitoring program was conducted in lead–zinc mine and flotation as potentially polluted environ with toxic heavy metals (Cd, Cu, Mn, Pb, Zn). The lead and zinc mine and flotation near the town of Probištip, Republic of Macedonia, potentially polluted area, were selected as study area where natural and anthropogenic distribution of certain heavy metals was expected. A total of 21 elements (Ag, Al, As, Ba, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V and Zn) were analyzed, how can be create a medium matrix for multivariate data processing.

MATERIALS AND METHODS

Investigating area

A total of ~400 km^2 area was monitored, limited with coordinates N: 41°51' – 42°08' and E: 22°01' – 22°25', located in the eastern part of the Republic of Macedonia (Fig. 1). Lead–zinc mine and flotation environ was monitored as potentially polluted area with anthropogenic introduced higher contents of certain heavy metals. In terms of climate field is located in the Southern-North temperate zone, including areas that are experiencing the effects of a Mediterranean climate (Kočani Valley and terrain) and Osogovo Mountains, where reigns quite mountainous climate. This geographical position conditioned its climate is characterized by elements of moderate continental (Lazarevski, 1993). The altitude varies between 300 and 1500 m. The average annual rainfall is amounted to 600–650 mm with large variations from year to year. Most frequent winds in the region are those from the west with frequency of 199 ‰ and 2.7 m s^{-1} speed, and winds from the east with frequency of 124 ‰ and 2.0 m s^{-1} speed. Climatic condition in the region allow air-distribution of fine dust particles generated as a result of mine activities and exposure flotation tailings at open.

The Zletovo Pb-Zn deposit is situated along the active continental margin and is intimately associated with the Tertiary volcanism and hydrothermal activity of the area. The Zletovo mine is located 5 km NW from the Zletovo village and about 7 km from the city of Probištip (Fig. 1). Continuous exploitation of the mine started after the Second World War and it has an annual capacity of 300,000 tons (9% Pb and 2% Zn) and significant concentrations of Ag, Bi, Cd, and Cu. The mine is active to date with production of Pb-Zn concentrate. Mineral association comprises galena (principal ore mineral) and sphalerite, with subordinate pyrite, lesser amounts of siderite, chalcopyrite, and occasional pyrhotine, marcasite, and magnetite. Ore is concentrated by flotation at Probištip town and tailings stored in two impoundments situated in adjacent valleys (Serafimovski et al., 2006).

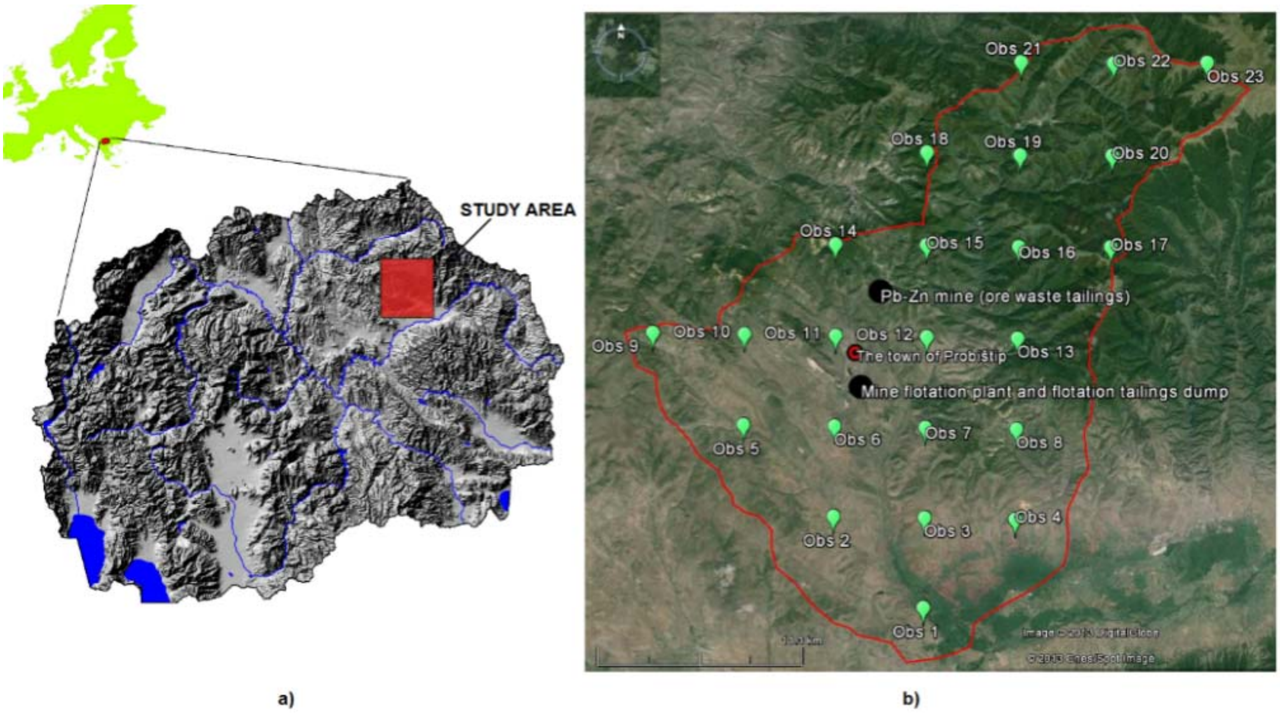


Fig. 1. The location of the study area (a) and sampling network within Probištip region (b)

Sampling and samples treatment

Three local characteristic moss species were used as biomonitors (*Hypnum cupressiforme, Scleropodium purum* and *Campthotecium lutescens*) reproducing dense sampling network of 5×5 km. The moss sampling protocol was performed according to set standard rules for collection of such samples (Tuba, et al. 2007), and it was done in this order: one sampling spot is formed by collecting five sub-spots in the area of 50×50 m^2. Every spot of sampling network must be in a distance of minimum 300 m from main roads, 100 m from local roads, and 200 m from villages. Moss samples were collected using polyethylene gloves, to prevent any further samples contamination. The collected material was stored in paper bags. After it was cleaned from other plant species and soil individual plant samples and air dried for several days. Dry samples were again placed in paper bags until analyses were performed.

For digestion of moss samples, the microwave digestion system (CEM, model Mars) was applied. Precisely (with accuracy of 0.0001) measured 0.5 g of moss samples, than 5 ml concentrated nitric acid HNO_3 and 2 ml hydrogen peroxide (H_2O_2) (30%, m/V) were added. The teflon vessels were carefully closed and the microwave digestion method was applied. Digestion method was performed in to two steps for total dissolving of moss tissue at 180 °C and applied pressure of 600 psi, as previously published (Balabanova, et al. 2010). After the digestion method was finished, digests were quantitatively transferred into 25 ml volumetric flaks. Thus way prepared digests from moss tissue were analyzed for the total elements contents.

Instrumentation

The analyses of total 21 elements contents in digest samples was performed by atomic emission spectrometer with inductively coupled plasma, ICP-AES (Varian, 715ES) applying an ultrasonic nebulizer CETAC (ICP/U–5000AT+) for better sensitivity (Balabanova et al., 2010). 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%. The same methods were applied for the determination of the analyzed elements in certificated reference materials M2 and M3 (moss samples) and the difference between measured and certified values was satisfied ranging within 15%. The sensitivity in regard to the lower limit of detection was done: 0.0015 mg kg^{-1} for Mn; 0.006 mg kg^{-1} for Fe; 0.003 mg kg^{-1} for Zn; 0.005 mg kg^{-1} for Cd; 0.0125 mg kg^{-1} for Al, Cu; 0.02 mg kg^{-1} for Ag, Mo; 0.025 mg kg^{-1} for Ba, Ca, Mg, Sr; 0.05 mg kg^{-1} for Cr, Li, V; 0.25 mg kg^{-1} for Ni; 0.5 mg kg^{-1} for As, P, Pb; 2.5 mg kg^{-1} for Na; 5 mg kg^{-1} for K.

Data processing

The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics. Data distribution was examined with the application of normality tests and visual comparative analysis. Regardless of their type, two or more variables are related if in a sample of observations, the values of those variables are distributed in a consistent manner. The application of bivariate statistic showed how chemical elements correlate between their content in different sampling media. For that issue the linear coefficient of correlation was used (examine the $p < 0.05$). The use of quantitative methods to exclude outliers, For example, they exclude observations that are outside the range of ±2 standard deviations (or even ±1.5 SDs) around the group or design cell mean. In some areas of research, such "cleaning" of the data is absolutely necessary. In this study case outliers are considered as higher contents of some elements and anthropogenic introducing in environment.

Multivariate statistic method (PCA, 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 (Reimann et al., 2002; Filzmoser et al., 2005; Žibret and Šajn, 2010). The variances extracted by the factors are called the eigenvalues. At the "heart" of factor analysis is the eigenvalue problem that is solved in this program via the Householder method, as presented by Golub and Van Loan (2013). Eigenvalues are calculated via least squares procedures. The sum of the eigenvalues is equal to the sum of the diagonal elements of the matrix (correlations or covariances) that is analyzed acording to Equation (1):

$$\sum l_j = \text{trace} \,/\, S \,/ = \sum S_{ii} \tag{1}$$

where l_j is the j'th eigenvalue; /S/ is the variance/covariance matrix or correlation matrix; S_{ii} are the diagonal elements of the variance/covariance matrix or the correlation matrix.

As a measure of similarity between variables, the product-moment correlation coefficient (r) was applied. There are various rotational strategies that have been proposed (Žibret and Šajn, 2010). The goal of all of these strategies is to obtain a clear pattern of loadings, 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, and we want to obtain a pattern of loadings on each factor that is as diverse as possible, lending itself to easier interpretation. Varimax rotation is the most common of the rotations that are available. This first involves scaling the loadings. We will scale the loadings by dividing them by the corresponding communality as shown below:

$$\tilde{l}_{ij} = \hat{l}^*_{ij} \,/\, \hat{h}_i \tag{2}$$

Here the loading of the I^{th} variable on the j^{th} factor after rotation, where $\hat{h}_i$is the communality for variable i. What we want to do is to find the rotation which maximizes this quantity.The varimax procedure, as defined below, selects the rotation to find this maximum quantity:

$$V = \frac{1}{p}\sum_{j-1}^{m}\left\{\sum_{i-1}^{p}\left(\tilde{l}^*_{ij}\right)^4 - \frac{1}{p}\left(\sum_{i-1}^{p}\left(\tilde{l}^*_{ij}\right)^2\right)^2\right\} \tag{3}$$

The success of the analysis can be judged by how well it helps you to make the data interpretation. If this does not help you then the analysis is a failure. If it gives some insight as to the pattern of variability in the data, then a successful analysis have been made.

The cluster analysis module was used for computing various types of distance measures, or the user can compute a matrix of distances. The purpose of this algorithm is to join together variables (elements, in this case) into successively larger clusters, using some measure of similarity or distance. The first choice that must be made is how similarity (or alternatively, distance) between gene expression data is to be defined. There are many ways to compute how similar two series of numbers are; the cluster provides eight options. These distances can be based on a single dimension or multiple dimensions. The most straightforward way of computing distances between objects in a multidimensional space is to compute Euclidean distances. This is probably the most commonly chosen type of distance. It simply is the geometric distance in the multidimensional space. It is computed as:

$$\text{Euclidean distance}(x,y) = \left\{\sum{}_i (x_i - y_i)^2\right\}^{1/2} \quad (4)$$

Commonly used similarity metrics are based on Pearson correlation. The Pearson correlation coefficient between any two series of numbers $x = \{x_1, x_2, ..., x_n\}$ and $y = \{y_1, y_2, ..., y_n\}$ is defined as given in Equation (3). *Pearson correlation coefficient* is used to cluster together variable or samples with similar behavior. Pearson correlation measures the similarity in shape between two profiles.

$$\text{Pearson correlation } (x,y) = 1 - r \quad (5)$$

where: $r = Z(x) * Z(y) / n$.

The dendrograms were performed using the linkage distance reported as $D_{\text{link}}/D_{\max}$; quotient between the linkage distances for a particular case divided by the maximal linkage distance as given by Dolegowska (2013).

RESULTS AND DISCUSSION

All values for the element contents were statistically processed using descriptive statistics as presented in Table 1. Using histograms plots, for the elements Al, Cr, Fe, Mn, Ni, and V normal distributions were assumed. For the rest of the elements, the normality was assumed on the bases of the logarithms of their contents. Higher contents of Pb and Zn were obtained with the average values of 34 and 46 mg kg^{-1}, respectively. The average values, when anthropogenic enrichments are introduced in environ, are not the best choice for comparative analysis. Thus way, the better choice is using the median values as less influenced statistical parameter. The median values for the main anthropogenic elements were 15.4 and 33.8 mg kg^{-1}, respectively. Compared to corresponding values for the whole territory of the R. Macedonia enrichments values were found, using the data obtained from Barandovski et al. (2013). For the Pb contents the large scale/small scale enrichments were calculated as 3.35. For the Zn content the enrichment value was 1.7 (20 mg kg^{-1} for whole territory of the R. Macedonia). Almost whole of the Probištip region is characterized with Pb deposition in moss samples ranging from 8 to 25 mg kg^{-1}. The contents bigger than 25 mg kg^{-1} are deposited in the western part of the region as presented at Figure 2. Zinc contents bigger than 25 mg kg^{-1} are deposited more disperse than the Pb distribution, in southern direction from the town of Probištip, and in smaller areas around the "Zletovo" mine, due to the Pb-Zn mineralization in this region.

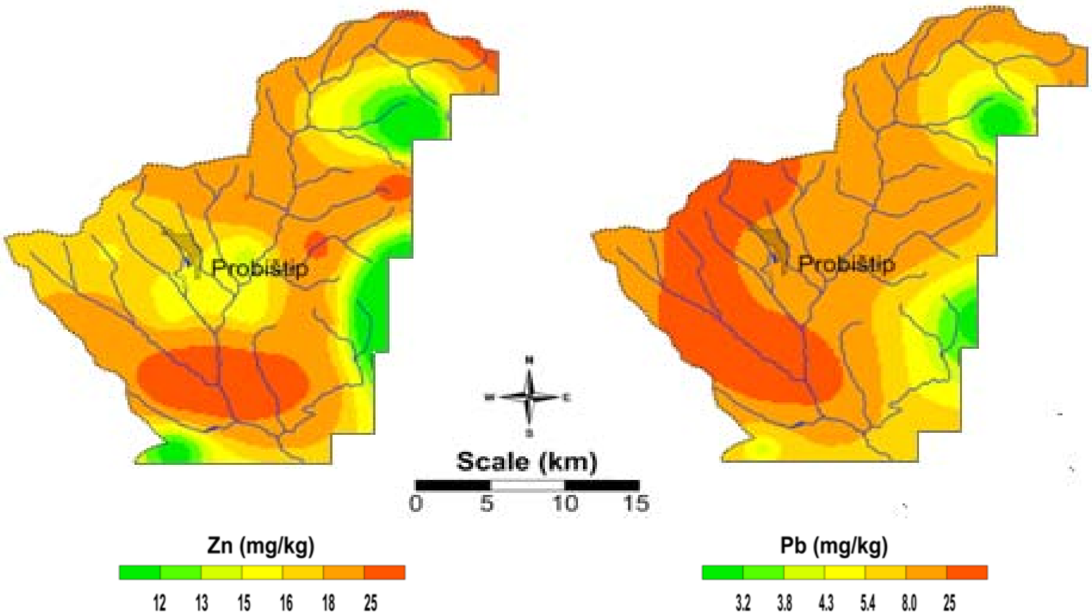


Fig. 2. Areal distributions of Zn and Pb in the investigated area

T a b l e 1

Matrix for basic descriptive statistics parameters for the elements contents in moss samples (values for elements contents are given in mg kg^{-1})

	Dis	X_a	X_g	Md	min	max	P_{10}	P_{90}	s	*CV*	*A*	*E*
Ag	log	0.12	0.09	0.09	0.03	0.4	0.04	0.29	0.1	85.2	1.67	2.31
Al	N	3925	3578	3969	1530	7169	1973	6158	1686	42.9	0.43	–1.01
As	log	2.35	1.67	1.67	0.51	8.35	0.51	4.38	2.11	89.7	1.71	2.74
Ba	log	68.4	61.2	58.8	21.3	226	36.8	94.7	39.8	58.2	2.98	11.7
Ca	log	10871	10338	10585	3708	17838	7194	14979	3272	30.1	0.02	0.04
Cd	log	0.31	0.23	0.21	0.08	1.73	0.13	0.66	0.35	114	3.45	13.1
Cr	N	3.47	2.99	3.44	0.51	7.43	1.34	6.12	1.72	49.8	0.49	–0.03
Cu	log	9.28	8.34	6.82	4.11	21.4	5.52	17.6	4.88	52.5	1.46	1.22
Fe	N	3795	3405	3624	1345	8269	1677	5853	1777	46.8	0.76	0.36
K	log	3850	3666	3490	2231	7178	2837	5957	1336	34.7	1.42	1.5
Li	log	2.01	1.78	1.99	0.604	3.62	0.99	3.19	0.91	45.6	0.17	–1.18
Mg	log	2127	1938	1602	1130	4367	1160	3768	1010	47.5	1.15	0.04
Mn	N	180	167	169	55.5	376	102	280	72.1	39.9	1.03	1.47
Mo	log	0.23	0.17	0.18	0.07	0.6	0.07	0.56	0.18	81.1	1.09	–0.06
Na	log	45.7	42.5	44.4	22.8	78.2	26.6	71.6	17.6	38.5	0.5	–1.01
Ni	N	4.07	3.63	3.75	0.94	11.1	2.21	6.61	2.08	51.1	1.73	4.84
P	log	1499	1392	1511	597	2930	691	1902	563	37.5	0.49	0.82
Pb	log	33.8	18.9	15.4	4.01	200	5.94	54.85	50.6	149	2.85	7.51
Sr	log	44.3	39.3	36.5	18.6	123	20.3	71.2	24.1	54.2	1.73	4.15
V	N	8.20	7.36	8.11	2.16	15.8	4.08	12.3	3.57	43.6	0.22	–0.59
Zn	log	46.5	37.8	33.8	12.8	186	22.5	98.6	39.1	84.2	2.63	7.41

Dis.– distribution (log-log normal; *N* – normal); X_a – arithmetical mean; X_g – geometrical mean; Md – median; min – minimum; max – maximum; P_{10} – 10 percentile; P_{90} – 90 percentile; *s* – standard deviation; *CV* – coefficient of variation; *A* – skewness; *E* – kurtosis

The contents of Cd, Cu and Mn were also assumed as anthropogenic influenced from the emission source, in order of their range values: 0.08–1.73 mg kg^{-1}; 4.11–21.4 mg kg^{-1} and 55.5–376 mg kg^{-1}, respectively. The manganese distribution was assumed as normal based on the histograms visualization of data set and comparison with the geology maps of distribution. However, the mine and flotation works influence as anthropogenic enrichment, give another point of view. Median values were compared with the corresponding values from whole territory of the R. Macedonia and there were no significant differences for the Cd and Mn. Only for Cu contents enrichments were found, calculated ~2 times (Barandovski et al., 2013).

Values for the contents of plant-biogenic elements (Ca, K, Na, P, Mg) undergoes with the contents of macro- and micro-element nutrients in moss tissue. Their statistical data distributions were assumed as lognormal, consequent as natural phenomena. Calcium contents vary in the range of 0.3%–1.8%, for K: 0.2%–0.7%; for P: 0.05%–0.3% and for Mg: 0.1%–0.37%. The less enriched contents occurred for Na (23–78 mg kg^{-1}) and for Sr (17–123 mg kg^{-1}). There is no significant trend for variations in moss tissue biogenic elements compared to moss study issue for whole territory of the R. Macedonia (Barandovski et al., 2013).

The contents for the lithogenic elements (Al, Cr, Fe, Mo, Ni) relays on the geology of the region. Aluminum contents in the moss species vary in the range 0.15%–0.7% with a median value of 0.4%, and characteristic normal data distribution. As it been previously presented from Barandovski et al. (2013), the trend for Al distribution for the whole territory of R. Macedonia vary in the range of 0.05% to 0.87%, and strongly relays on the lithology of the area (dominant magmatic and volcanic rocks, enriched with quaternary sediments). Iron contents in moss tissue were accumulated in range of 0.13%–0.83%. The Fe distribution follows the Al distribution, probably on the same lithological impact. For the rest of the lithological distributed elements Cr, Mo and Ni median values were in accordance with their normal distribution for whole territory of the R. Macedonia (3.44, 0.18 and 3.75 mg kg^{-1}, respectively). It is relatively difficult to distinguish and determine the type of deposition and distribution based only on descriptive statistics on the content of elements in the investigated area.

In order to determine correlation between elements how can be determine relationships in elements contents, matrix of correlation coefficients was processed (Table 2). As it can be seen constructive conclusion can't be reproduced only within the matrix. Bold values indicate significant correlation between elements, different from 0 with a significance level alpha = 0.05. Reproduced correlation matrix includes 21 elements contents, for which the interpretation of the variable associations is quite complex. As lithological elements significant correlations are produced for: Cr–Ni; Fe–Al; Cu–Ag; Al–V; Li–Cr; Ag–Zn. For the matrix elements for plant tissue, the relations of elements were considered as significant: P–Ca; Mg–Na; Ba–Sr; Mg–Zn. Potentially hazardously relation of elements that undergoes with the pollution emissions are: Pb–Zn; Cu–Zn; Cu–Pb; Cu–Cd; Cd–Zn; Mn–Zn. As it can be seen, distribution of 21 elements is very hard to interpret only within bivariate correlations. For that issue multivariate analysis of factor components and principle components was applied.

Table 2

Reproduced correlation matrix

	Ag	Al	As	Ba	Ca	Cd	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	P	Pb	Sr	V	Zn
Ag	**1**																				
Al	–0.06	**1**																			
As	0.11	**0.56**	**1**																		
Ba	0.08	**0.56**	**0.42**	**1**																	
Ca	0.02	0.27	0.06	0.11	**1**																
Cd	**0.83**	–0.01	0.18	0.14	0.10	**1**															
Cr	–0.09	0.40	**0.46**	0.13	**0.41**	–0.02	**1**														
Cu	**0.90**	0.01	0.20	0.25	–0.04	**0.75**	–0.08	**1**													
Fe	**0.47**	**0.68**	**0.58**	0.24	0.12	0.40	0.37	**0.52**	**1**												
K	0.34	0.32	**0.70**	0.26	0.16	0.21	**0.41**	**0.48**	**0.59**	**1**											
Li	–0.07	**0.81**	**0.44**	0.19	**0.61**	–0.02	**0.59**	–0.10	**0.64**	0.35	**1**										
Mg	**0.77**	0.24	0.37	0.05	–0.03	**0.57**	0.01	**0.81**	**0.77**	**0.61**	0.19	**1**									
Mn	**0.41**	0.24	**0.47**	0.26	0.29	**0.58**	0.22	0.36	0.35	**0.41**	0.29	0.29	**1**								
Mo	**0.58**	–0.05	0.12	0.26	0.23	**0.64**	–0.19	**0.66**	0.18	0.27	–0.08	**0.45**	0.15	**1**							
Na	**0.63**	0.33	0.31	0.27	–0.16	**0.52**	–0.19	**0.73**	**0.65**	0.35	0.06	**0.82**	0.12	**0.54**	**1**						
Ni	–0.30	0.22	–0.06	0.04	0.35	–0.08	**0.63**	–0.29	–0.01	–0.03	0.39	–0.25	–0.15	–0.14	–0.30	**1**					
P	0.06	0.19	0.23	0.28	**0.78**	0.12	0.25	0.07	0.01	0.36	0.32	0.01	0.32	0.41	–0.10	0.07	**1**				
Pb	**0.67**	0.08	–0.09	0.28	0.27	**0.50**	–0.04	**0.71**	**0.41**	0.36	0.11	**0.57**	0.06	**0.65**	**0.55**	–0.02	0.26	**1**			
Sr	–0.25	**0.47**	0.23	**0.78**	0.29	–0.05	0.22	–0.14	–0.10	0.04	0.21	–0.32	0.13	0.11	–0.15	0.35	**0.47**	0.02	**1**		
V	0.15	**0.86**	**0.43**	**0.46**	**0.54**	0.19	**0.47**	0.17	**0.70**	0.33	**0.85**	0.29	0.34	0.17	0.34	0.24	0.39	0.35	0.35	**1**	
Zn	**0.82**	–0.06	0.11	0.16	0.14	**0.96**	0.01	**0.76**	0.36	0.26	–0.04	**0.53**	**0.53**	**0.66**	**0.50**	–0.01	0.15	**0.62**	–0.04	0.19	**1**

Values in red and bold are different from 0 with a significance level alpha = 0.05

The data distribution was reduced with the application of factor analysis. The extraction of the factors was made by analyzing the *eigenvalues* of the data matrix set (elements contents). Primarily, eighth factors were extracted where total variance and communalities were expressed in percent (Table 3). As it can be seen, Factor 1 accounts for 33% of the variance, while Factor 2 for 20%. Using *Kaiser* criterion factors with eigenvalues greater than 1 were retained (factors F1, F2, F3 and F4) retain. However, the value for the total variance for F3 and F4 (<10), refer to rejecting these factors. Thus way, as Cattell (1966) suggests to find the place where the smooth decrease of eigenvalues appears to level off to the right of the scree plot (as graphical method), as it can be seen in Figure 3.

T a b l e 3

Eigenvalues for synthetic factors

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	**6.98**	**4.28**	**1.74**	**1.06**	0.87	0.56	0.41	0.06
Variability (%)	33.2	20.4	8.28	5.05	4.15	2.69	1.93	0.31
Cumulative (%)	33.2	53.6	61.9	66.9	71.1	73.8	75.7	76.0

Red and bold values indicate retaining the factors; eigenvalues > 1

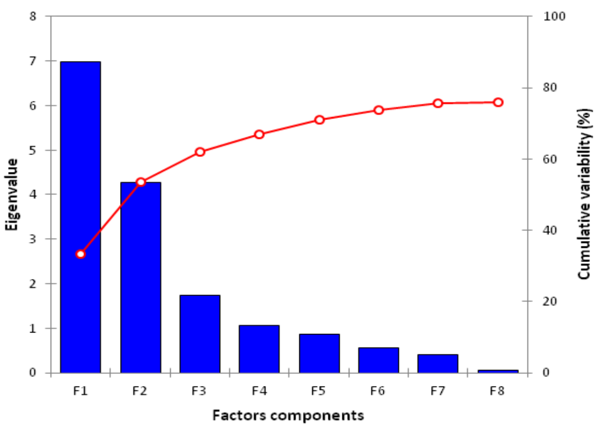


Fig. 3. Scree plot for eigenvalues

Therefore, the two factors (F1 and F2) were expressed as statistically significant elements associations. The first factor associates the following elements: Ag, Ba, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Pb, Zn. The second factor associates the elements: Al, Cr, Li, and V, as presented in Table 4. Factor 1 represents the anthropogenic factor due to include anthropogenic introduced elements as: Ag, Cd, Cu, Fe, Mn, Pb, and Zn in the lead–zinc mine and flotation environ. On the other hand, the elements as Ba, K, Mg, and Mo incorporate in this factor because of the enrichments of these elements as biogenic elements in moss tissue. Factor 2 present a typical geogenic associations of elements due to the geology of the region, their distribution is not correlated to any anthropogenic influence in the study area (Serafimovski et al., 2006).

T a b l e 4

Matrix of dominant Factor patterns

Element	F1	F2	Initial comm.	Final comm.	Specific var.
Ag	**0.79**	0.52	0.97	0.89	0.11
Al	**0.45**	**–0.72**	0.99	0.73	0.27
As	**0.49**	–0.40	0.98	0.40	0.60
Ba	**0.39**	–0.33	0.94	0.26	0.74
Ca	**0.27**	–0.48	0.97	0.30	0.70
Cd	**0.73**	0.37	0.99	0.68	0.32
Cr	0.21	**–0.63**	0.93	0.45	0.55
Cu	**0.84**	0.49	0.98	0.94	0.06
Fe	**0.77**	–0.23	0.98	0.65	0.35
K	**0.62**	–0.20	0.99	0.42	0.58
Li	0.40	**–0.79**	0.99	0.79	0.21
Mg	**0.81**	0.27	0.99	0.73	0.27
Mn	**0.52**	–0.12	0.95	0.28	0.72
Mo	**0.61**	0.30	0.89	0.46	0.54
Na	**0.72**	0.29	0.99	0.59	0.41
Ni	–0.08	–0.47	0.96	0.23	0.77
P	0.30	–0.36	0.96	0.22	0.78
Pb	**0.67**	0.22	0.97	0.49	0.51
Sr	0.07	**–0.51**	0.95	0.27	0.73
V	0.62	**–0.66**	0.99	0.82	0.18
Zn	**0.73**	0.37	0.99	0.67	0.33

Values in red and bold correspond for each variable to the factor for which the squared cosine is the largest; comm.– communality; var.– variance

An initial correlation of separate elements with the factors patterns was made (Table 5). Due to correlation patterns for the As, Ba, Ca, Ni, P and Sr; the elements should be excluded from further analysis because these elements does not indicate any correlation with F1 and F2. As before, we want to find a rotation that maximizes the variance on the new axes; put another way, to obtain a pattern of loadings on each factor that is as diverse as possible, lending itself to easier interpretation. The values of rotated factor loadings are given in Table 5. The *varimax rotation method* was applied and no significance in correlations of these elements was found in their factor patterns (Table 5). The factor patterns for Ba, Ca, P and Sr (as biogenic elements) doesn't rich more than 0.60 values, with and without *varimax* rotation. The same effect was obtained and for the lithogenic elements As and Ni.

Table 5

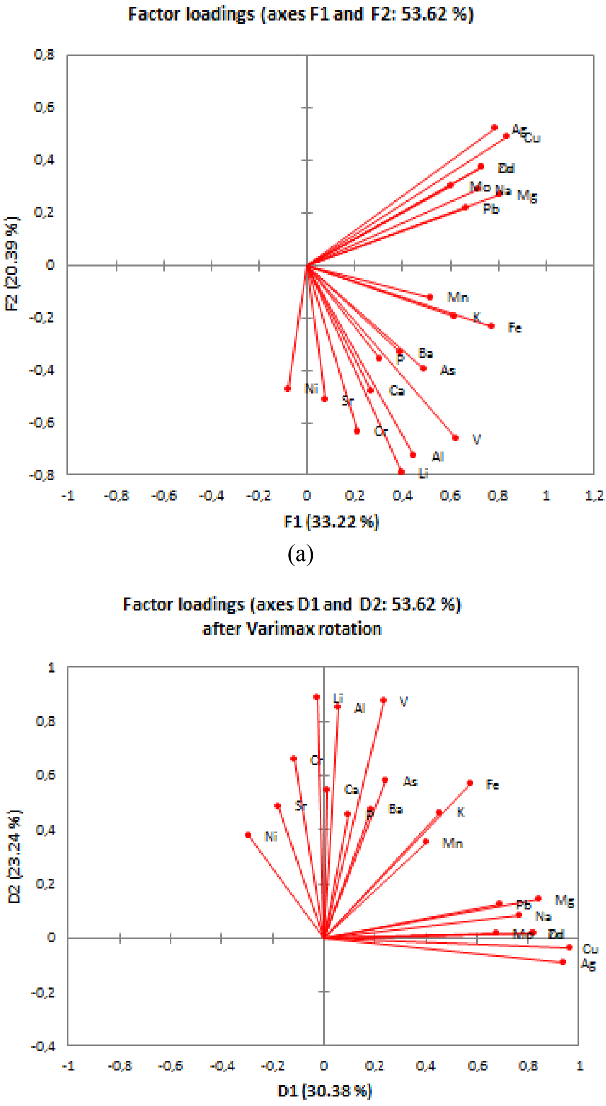
Correlations between variables and factor loadings (F > 0.60)

	Before varimax rotation		After varimax rotation	
Element	F1	F2	D1	D2
Ag	***0.78***	0.49	***0.94***	–0.09
Al	0.45	***–0.68***	0.05	***0.85***
As	**0.49**	**–0.37**	**0.25**	**0.58**
Ba	**0.39**	**–0.31**	**0.19**	**0.48**
Ca	**0.27**	**–0.45**	**0.01**	**0.55**
Cd	***0.73***	0.35	***0.82***	0.02
Cr	0.21	***–0.60***	–0.11	***0.66***
Cu	***0.83***	0.46	***0.97***	–0.03
Fe	***0.77***	–0.22	***0.57***	0.57
K	***0.62***	–0.18	0.46	0.46
Li	0.40	***–0.75***	–0.02	***0.89***
Mg	***0.81***	0.26	***0.84***	0.14
Mn	0.52	–0.12	0.40	0.35
Mo	***0.60***	0.29	***0.68***	0.02
Na	***0.71***	0.27	***0.77***	0.08
Ni	**–0.08**	**–0.45**	**–0.29**	**0.38**
P	**0.30**	**–0.34**	**0.10**	**0.46**
Pb	***0.66***	0.20	***0.69***	0.12
Sr	**0.07**	**–0.48**	**–0.18**	**0.49**
V	***0.62***	***–0.62***	0.24	***0.88***
Zn	***0.72***	0.35	***0.82***	0.02

Red and bold values indicate rejecting from factor associations because of their low correlation within the factors. Value in italic correspond for each variable to the factor for which the squared cosine is the largest

Graphical schematizing of the factor loadings before and after varimax rotation for better visibility was done (Fig. 4). Rejecting the selected variables present a significant step in the data processing. The factor loadings >0.30 were included, thus way rejection of the variables was reduced including the 21 elements distribution.

After the varimax rotation, stabilization in data distribution was showed in the increasing scale: F1/D1: Fe < Mo < Pb < Na < Cd < Mg < Zn < Ag < Cu and F2/D2: Mn < Ni < K < P < Ba < Sr < Ca < As < Cr < Al < V < Li, according the calculated factor loadings with varimax rotation, as presented in Table 5 and Figure 4.



(a)

(b)

Fig. 4. Effects of varimax rotation on elements contents *vs.* F1/F2 (a) and D1/ D2 after the varimax rotation (b)

Principal components and classification analysis as data reduction method for a description of supplementary variables and cases was applied on the same data set (elements contents) and moss species as cases. In principal components analysis, after the first factor has been extracted, it should be defined another line that maximizes the remaining variability. In this manner, consecutive principle components are extracted as PC1 and PC2 (using scree plot as extraction method). Because each consecutive factor is defined to maximize the variability that is not captured by the preceding factor, consecutive factors are independent of each other. Put another way, consecutive factors are uncorrelated or orthogonal to each other (Table 6 and Figure 5). The first principal component PC1 associates the elements: Cu > Ag > Mg > Cd ~ Zn > Na > Pb ~ Mo, sequenced according the expressions of component patterns. The second principle component PC2 associates the elements: Li > V > Al > Cr > Ca ~ As. The elements Ba, Fe, K, Mn, Ni, P and Sr (marked in red) in Table 6), does not belong to any group, because of very low component pattern for the PC1 and PC2. Almost identical results were obtained using principle factors and principle components, with variation for the elements Al, Fe, and Mn.

Table 6

Correlations between elements contents and principle components after varimax rotation (PC > 0.60)

Element	PC1	PC2
Ag	***0.93***	–0.09
Al	0.06	***0.85***
As	0.26	***0.62***
Ba	**0.20**	**0.53**
Ca	0.01	***0.62***
Cd	***0.84***	0.02
Cr	–0.13	***0.71***
Cu	***0.95***	–0.03
Fe	**0.59**	**0.56**
K	**0.48**	**0.49**
Li	–0.02	***0.88***
Mg	***0.86***	0.13
Mn	**0.43**	**0.39**
Mo	***0.72***	0.02
Na	***0.80***	0.06
Ni	**–0.33**	**0.44**
P	**0.10**	**0.53**
Pb	***0.72***	0.12
Sr	**–0.20**	**0.56**
V	0.24	***0.86***
Zn	***0.84***	0.02

PC – Principal component pattern
Values marked in red and bold does not belong to any group; values in italic represent principal component pattern > 0.60

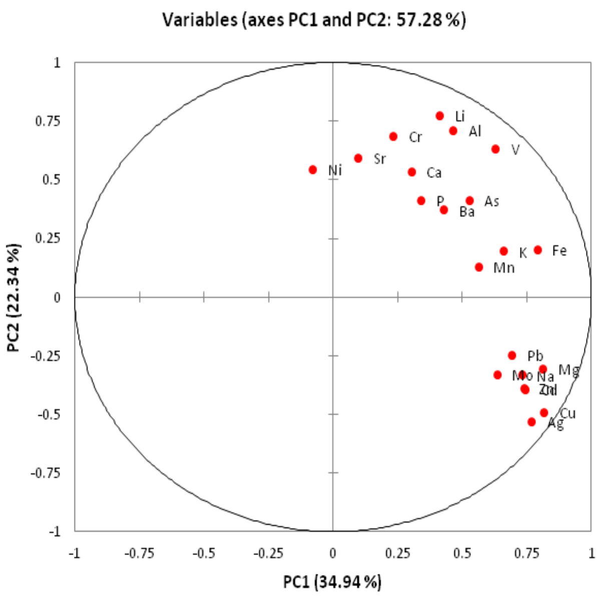


Fig. 5. Principal components for elements contents

The advantage of the principle component analysis is to reveal the two dimensional observation for the variables on one hand and cases at the other hand. Thus way can be visualized the correlations between factor components extracted from the elements contents (as variable) and the expressiveness of two moss species (as cases), as presented in Table 7 and Figure 6. Locations of the mentioned observations were very close to the emission source. Thus way can be concluded that the higher contents of the elements are correlated with the distance of the location of the sampled moss species to the emission source.

Unlike many other statistical procedures, cluster analysis methods are mostly used when we do not have any *a priori* hypotheses, but are still in the exploratory phase of our research. In a sense, cluster analysis finds the "most significant solution possible." Therefore, statistical significance testing in the traditional sense of this term is really not appropriate here, but the linkage of the reproduced clusters have meanings when is used the appropriate method. Ward's method has been shown as more appropriate compared with *complete linkage* and *unweight pair–group average*; this method is regarded as very efficient, however, it tends to create clusters of small size. For distance measuring of clusters were used Euclidean distance measuring and distance measuring on the Pearson correlation. Therefore, the second choice had revealed better expression in the data similarity (Figure 7). Elements contents were cluster as two clusters C1 (Ag–Cu–Cd–Zn–Mo–Pb–Fe–Mg–Na) and C2 (Al–V–Li–Cr–Ni–Ba–Sr–Ca–P–As–K–Mn).

Table 7

Factor scores presented in dependences of observations (moss species)

Observation	Moss species	PC1	PC2	Cluster class
Obs 1	*Hypnum cupressiforme*	**2.716**	–0.546	1
Obs 2	*Hypnum cupressiforme*	**2.299**	0.592	1
Obs 3	*Hypnum cupressiforme*	**1.561**	0.226	2
Obs 4	*Scleropodium purum*	**1.012**	0.558	3
Obs 5	*Campthotecium lutescens*	0.819	**–1.635**	3
Obs 6	*Campthotecium lutescens*	–0.322	**–0.978**	2
Obs 7	*Scleropodium purum*	–0.131	**0.216**	1
Obs 8	*Scleropodium purum*	–0.532	**2.518**	2
Obs 9	*Campthotecium lutescens*	0.069	**–0.812**	2
Obs 10	*Campthotecium lutescens*	–0.292	**0.930**	3
Obs 11	*Campthotecium lutescens*	–0.980	**0.993**	3
Obs 12	*Hypnum cupressiforme*	–0.379	**–1.005**	3
Obs 13	*Hypnum cupressiforme*	–0.514	**–0.843**	3
Obs 14	*Campthotecium lutescens*	**–1.003**	–0.465	3
Obs 15	*Hypnum cupressiforme*	**–0.149**	0.111	3
Obs 16	*Hypnum cupressiforme*	**–0.457**	0.328	1
Obs 17	*Hypnum cupressiforme*	**–0.898**	–0.775	3
Obs 18	*Hypnum cupressiforme*	–0.541	**2.491**	3
Obs 19	*Campthotecium lutescens*	0.129	**–0.278**	1
Obs 20	*Campthotecium lutescens*	–1.050	**–1.120**	3
Obs 21	*Scleropodium purum*	**–0.827**	0.035	3
Obs 22	*Campthotecium lutescens*	–0.015	**–0.246**	3
Obs 23	*Scleropodium purum*	**–0.514**	–0.296	1

Bold values correspond for each observation to the factor for which the squared cosine is the largest;
Red values – differences in patterns scores after varimax rotation

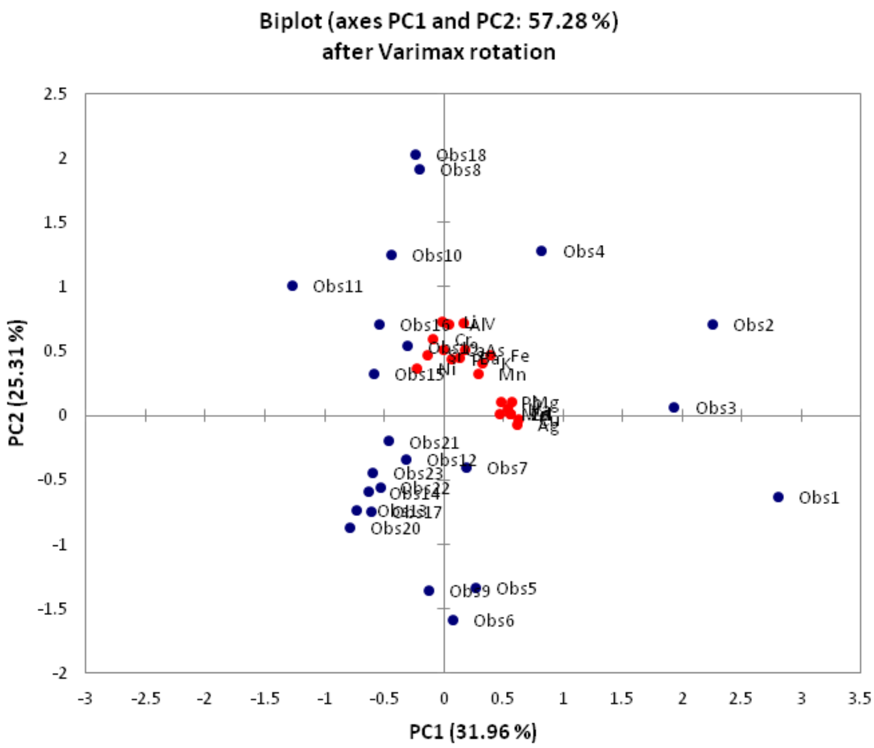


Fig. 6. Biplot for moss species *vs.* elements contents

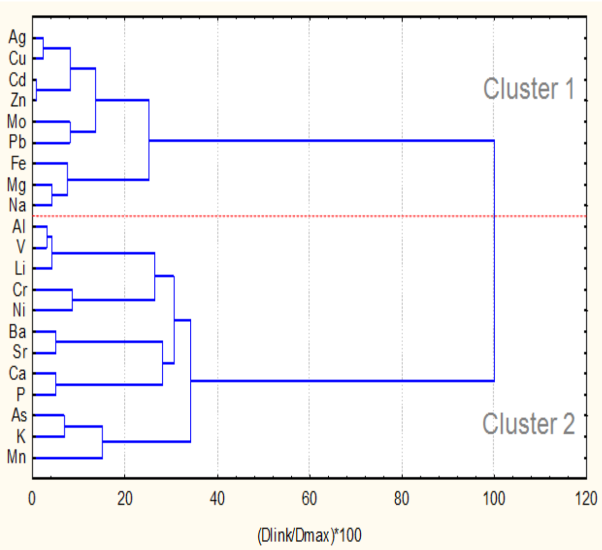


Fig. 7. Clusters for elements associations (Tree diagram, Vard's method, 1-Pearson r)

Almost identical results were obtained as previously made FA and PCA analysis with the exception of As, Mn and Ba.

The same method was used for the observations (moss samples locations and species), how to determine the similarity, without previously set hypothesis. There were no significant correlations in the moss species. However, it can be concluded that the grouping is performed in order of the contents of elements in moss tissue (Fig. 8). Thus way regression analysis can determine the correlation of the higher contents in moss species *vs.* distance from the pollution source or areal distribution as suggested from Fraley and Raftery (1998), (Fig. 1, sampling locations). There are no significant dependences between the moss species, determination differences in accumulation abilities of moss species: *Hypnum cupressiforme* vs. *Scleropodium purum* vs. *Campthotecium lutescens.* Similarity measurements defined three clusters groups from cases (sampling location or moss species), specified as: Cluster 1: observations (9–3–6–8); Cluster 2 observations (19–1–2–7–16) and Cluster 3: observations [(22–20–10–17–18) (4–5–11–12–13)]. The anthropogenic elements contents vary independent from the moss species, but depending on the distancing from the pollution source, there are positive correlation. Long distance distribution from the emission source doesn't occur, but the impact of the flotation tailings is obvious, because of the location of the flotation tailings dump, very close to the town of Probištip.

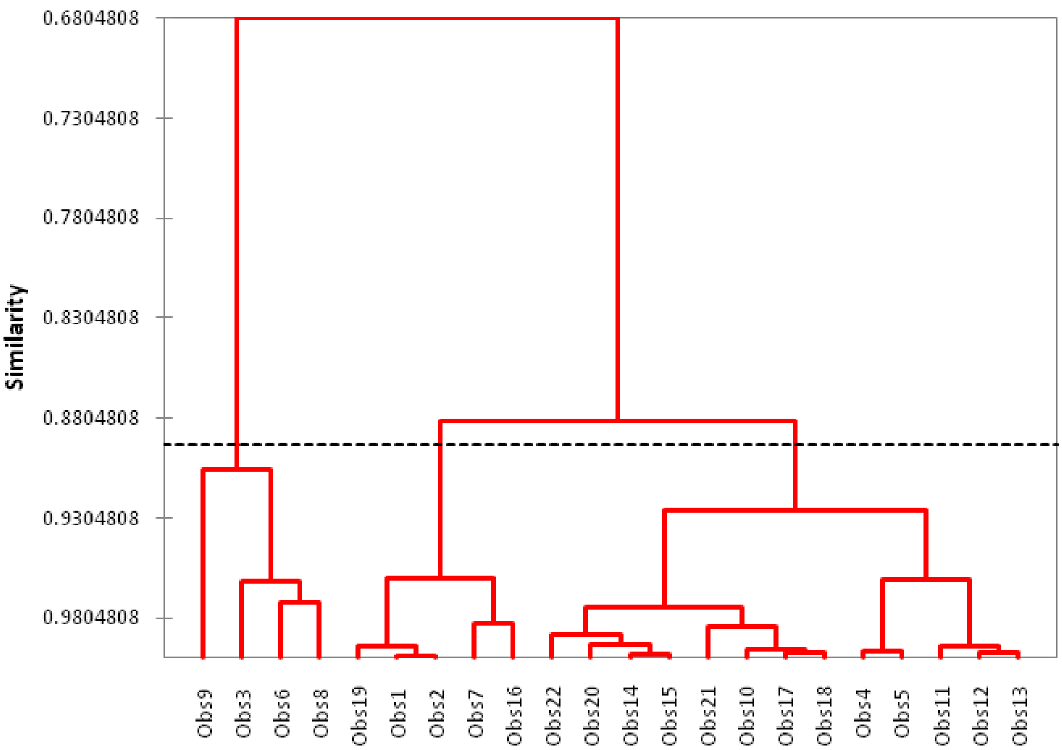


Fig. 8. Cluster analyses for observations (moss species locations)

CONCLUSION

From the obtained and processed results it can be concluded that moss species is readily available and it can be efficiently analyzed for certain heavy metals. The collected moss species shows landscape and canopy-dependent accumulations that generally correspond to expected concentrations. The results also suggest that smaller scale, local deposition patterns are variable and could be completely studied using moss sampling media. The smaller scale monitoring, due to emissions in lead-zinc mine and flotation tailings undergoes primarily with the winding in the investigated area. Moss species variability in elements accumulation wasn't found. For the application of the multivariate techniques (FA, PCA and CA), further suggestion can be specified as: dominant using of FA (with varimax rotation for data normalization) for reducing the numerous variables (chemical elements). Principal components are more useful in determination of the correlations of the elements contents with lithological bases (Mn, Ni, K, P, Ba, Sr, Ca, As, Cr, Al, V, Li). Significant variability between the PCA and FA were obtained for the Al, Mn and Fe, despite their normally data distribution. For better extraction of the synthetic factors/components, clustering is suggested for the dissimilarity/similarity measurements.

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Р е з и м е

ВАРИЈАБИЛНОСТ ВО ДИСТРИБУЦИЈАТА НА МЕТАЛИТЕ КАКО РЕЗУЛТАТ НА АНТРОПОГЕНОТО И ГЕОГЕНОТО ВЛИЈАНИЕ ВО ОКОЛИНАТА НА РУДНИКОТ И ФЛОТАЦИЈАТА ЗА ОЛОВО И ЦИНК „ЗЛЕТОВО“ (БИОМОНИТОРИНГ СО ПРИМЕРОЦИ МОВ)

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Клучни зборови: дистрибуција на метали; видови мов; мултиваријантни аналитички методи; ICP-AES; Pb-Zn-рудник.

Различни видови мов (*Hypnum cupressiforme*, *Scleropodium purum* and *Campthotecium lutescens*) се користени како погоден материјал за биомониторинг на загадувањето со тешки метали во околината на рудникот и флотацијата за олово и цинк, во близината на градот Пробиштип. Содржината на 21 метал беше одредувана со примена на атомска емисиона спектрометрија со индуктивно спрегната плазма (ICP-AES). Обработката на податоците беше направена со примена на мултиваријантни аналитички методи: факторна анализа, компонентна анализа и кластерна анализа. Главни антропогени маркери во испитуваното подрачје претставуваат Pb и Zn со максимални вредности за содржината од 200 и 186 mg kg^{-1}, соодветно. Факторната анализа ги издвојува следниве асоцијации (според зголемувањето на факторите на оптоварување): F1/D1: Fe < Mo < Pb < Na < Cd < Mg < Zn < Ag < Cu; and F2/D2: Mn < Ni < K < P < Ba < Sr < Ca < As < Cr < Al < V < Li. Содржината на антропогено внесените елементи се променува независно од видовите на мов, но зависи од растојанието од изворот на загадувањето, за што се добиени позитивни корелации. Не е утврдена дистрибуција на овие елементи на поголеми растојанија од рудникот и флотацијата.