

## Characterisation of Heavy Metals in Lichen Species *Hypogymnia physodes* and *Evernia prunastri* due to Biomonitoring of Air Pollution in the Vicinity of Copper Mine

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**ABSTRACT:** Lichen species (*Hypogymnia physodes* and *Evernia prunastri*) were used for biomonitoring the possible air pollution with heavy metals in the vicinity of copper mine in the eastern part of the Republic of Macedonia. The contents of 18 elements were analysed by ICP-AES and ETAAS techniques. For data processing basic statistical methods and multivariate exploratory (factor and cluster analysis) techniques were applied. Three geogenic and one anthropogenic group of elements were singled out from factor analysis. Maps of spatial distribution of elements from the anthropogenic group showed an increased content of the anthropogenic elements only in close vicinity to the mine. A maximum value obtained for Cu content was 130 mg/kg and for Pb content was 120 mg/kg. Lichen talus of the collected species showed high retention power for accumulation of the anthropogenic group of elements compared to moss species sampled from the same study area ( $r=0.86$  and  $r=0.68$ ). Here we show that lichens can be used as biomonitors for atmospheric distribution of anthropogenic-introduced elements.

**Key words:** Atmospheric pollution, Heavy metals, Biomonitoring, Lichens, Copper mine

### INTRODUCTION

Atmospheric pollution with heavy metals is a global problem. A significant factor for this problem is mining work, because of the direct exposition of metals to the atmosphere. Most of the studies based on atmospheric pollution explore metals adsorption by atmospheric particles deposits on soil and terrestrial environment (Järup, 2003; Alloway and Ayres, 2004; Ansari *et al.*, 2011). Atmospheric emissions attributed to the extraction stage of mining come mainly from the wind action on disturbed land and stockpiles of ore and waste material (Salomons, 1995). Another serious consequence in atmospheric terms is acid deposition, which removes other pollutants (Cortes-Maramba *et al.*, 2006). The ore and ore tailings are continually exposed to open air, allowing spreading of the fine particles in the atmosphere. On the other hand, after the cessation of mining activity in sulfide-ore mines, the drainage of waste rock dumps carries harmful dissolved and

particulate products to the environment (Kargar *et al.*, 2012; Nasrabadi *et al.*, 2009; Rafati *et al.*, 2011; Bhakta and Muneke, 2011). Such drainage waters, that have a low pH, often have high dissolved metal concentrations as well as sulfide ore particles (Thothon, 1996; Dudka, 1997; Rafiei *et al.*, 2010). Bio removal of different heavy metals have been considered by lots of researchers for a variety of biosorbents (Gomes *et al.*, 2011; Dekhil *et al.*, 2011; Ekmekyapar *et al.*, 2012; Mane and Bhosle, 2012). Lichen biomonitoring has proved to be a very useful technique for determining the atmospheric deposition of heavy metals in potentially polluted areas (Jeran *et al.*, 2002; Loppi *et al.*, 2004; Adamo *et al.*, 2007). Based on measurements of selected physiological parameters (e.g. composition of assimilation pigments, fluorescence of chlorophyll a and composition of amino acids, including content of soluble proteins), it has been demonstrated that lichens can be physiologically well adjusted to metal-rich substrata

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(Bačkor *et al.*, 2009). Based on physical, chemical and biological properties, lichens are used as monitors of metal deposition from the atmosphere, since they can accumulate trace elements to levels far greater than their expected physiological needs (Pirintzos *et al.*, 2006; Bačkor and Loppi, 2009). Lichen are suitable monitors due to their occurrence in almost all terrestrial ecosystems and their ability to tolerate long periods of extreme environmental conditions (Garty, 2001). Heavy metal tolerance in lichens has been mainly attributed to cell wall immobilisation and/or organic acid-chelating. Soluble metals, if are located extracellularly, assumed to be less important in causing immediate metabolic effects vs. metals located intracellularly. As reported by various authors, heavy metals mostly are accumulated on the surface layers of the lichen cell walls because of the negatively charged anionic sites present in these structures; and only traces of supplied metals are observed intracellular (Branquinho *et al.*, 1999; Artiola *et al.*, 2004; Purvis *et al.*, 2006; Nieboer *et al.*, 2007). Possible locations and uptake mechanisms of metals in the lichen thallus are: (1) Metal rich particulate entrapment on the lichen surface and in intercellular spaces of fungal filaments; (2) Intracellular complexation to metallothioneins; (3) Extracellular complexation to the functional groups of fungal macromolecules from the cell walls; (4) Extracellular complexation to organic acids, such as oxalate (Salemaa *et al.*, 2004). Phytochelatins, rather than metallothioneins are involved in intracellular complexation of heavy metals in lichens, specifically in the cells of algal partner forming lichen thalli (Bačkor *et al.*, 2007). In addition, lichen elemental concentration can be converted into atmospheric deposition values providing metal uptake efficiency (Sloof, 1995; Rusu *et al.*, 2006; Market, 2007; Williamson *et al.*, 2007; Dzubaj *et al.*, 2008). Therefore, because of all the above-mentioned reasons, specific lichen species are being used as a part of monitoring programs for air pollutants worldwide.

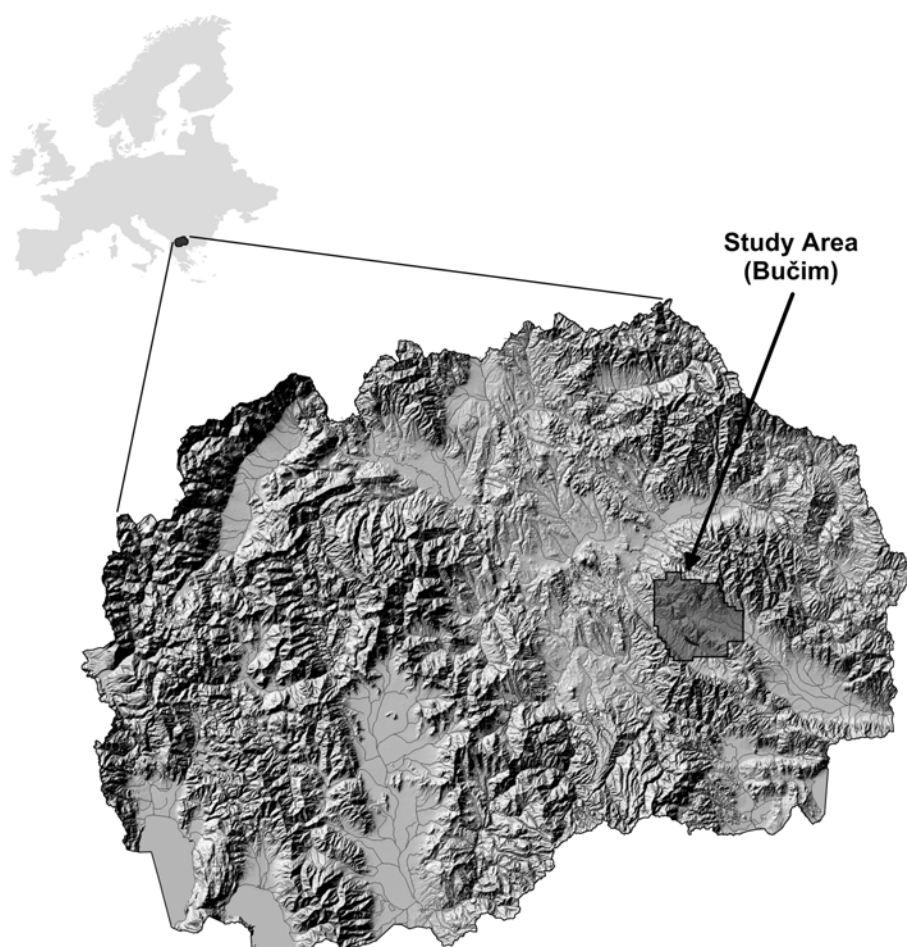
The Republic of Macedonia does not deviate from the global framework of air pollution with heavy metals. The first systematic study for atmospheric pollution with heavy metals on the territory of the Republic of Macedonia was undertaken using moss samples (Barandovski *et al.*, 2006; 2008). Significant emission sources that contribute to atmospheric pollution with heavy metals in the territory of the Republic of Macedonia appear to be all the mines, drainage systems and smelters near the cities of Veles, Kavadarci and Tetovo (Barandovski *et al.*, 2006, 2008; Stafilov *et al.*, 2008, 2010, 2010a). In the eastern part of the country the appearance of some metals (Au, Mg, Al, Sc, Ti, V, Cu) in the air is related to the presence of a copper

mine and flotation plant, “Bučim”, near to the Radoviš town (Stafilov *et al.*, 2010b; Balabanova *et al.*, 2010; Balabanova *et al.*, 2011). In this area an influence from the former iron mine, Damjan, has also been determined (Serafimovski *et al.*, 2005). The main aim of this study was to assess the atmospheric pollution with heavy metals due to copper mining activities near the mine using lichens collected in the investigated region.

## MATERIAL & METHODS

The study area is located in the eastern part of the Republic of Macedonia with a surface area of 20 km (W–E) × 20 km (S–N), a total surface of 400 km<sup>2</sup>, limited by the coordinates N: 41°32' – 41°44' and E: 22°15' – 22°30' (Fig. 1). The region is characterised by a moderate continental climate (Lazarevski, 1993). The altitude varies between 350 and 1000 m. The average annual temperature is around 10°C. The average annual rainfall amounts to 563 mm with a large variations from year to year. The most frequent winds in the region are those from the west with a frequency of 199 ‰ and speed of 2.7 m/s, and winds from the east with a frequency of 124 ‰ and speed of 2.0 m/s (Fig. 3).

The Bučim copper mine is located in the north-west part of the studied area. The Bučim mine territorially and administratively belongs to the municipality of Radoviš, and is located 14 km from the town. In the close vicinity of the mine there are two settlements, the villages Bučim and Topolnica. Mine activities cover 7 km<sup>2</sup> of total mine surfaces, 4 km<sup>2</sup> for the placement of ore tailings and the rest of the land belongs to the open ore pit and to the plant for ore processing. The main ore contents are: 0.3 % Cu, 0.3 g/ton Au, 1 g/ton Ag, 13 g/ton Mo, and 1–4 % pyrite; the igneous rocks have been altered to clays and micas. The unique copper mineralization of porphyry type is occurring in the gneisses to their contact with the andesites. The mineral content decreases gradually with increasing distance from the contact and occurs principally as filings and coatings on fracture plans. Andesites are barren in general; however, copper mineralization associated with fractures and joining is found in the andesites as well. The important metallic minerals are chalcopyrite, pyrite, and bornite, with small amounts of galena, sphalerite, magnetite and hematite (Serafimovski *et al.*, 1995; Alderton *et al.*, 2005; Balabanova *et al.*, 2011). The “Bučim” mine and the ore processing plant have been functioning since 1979 and copper ore was mined by open pit method from a total ore reserve of about 85 Mt. The basic ore proceeding process includes: drilling and blasting, than blasted ore is transported towards primary crushing while the tailings on the mine disposal. The mineral processing and ore concentration processes cover the



**Fig. 1. Location of the study area**

following technological operations: primary, secondary and tertiary crushing, screening and storing, grinding and classification, flotation and finally the tailing removal in tailing dump. The annual values for some technological parameters are: mined ore ~4 Mt/y; Cu content ~0.25 %; Cu concentrate ~40,000 t/y; Cu-recovery of 88 %; Cu in tailings 0.03 %. The values for these parameters do not vary significantly over the years (Hadži Jordanov *et al.*, 2006). The ore tailings are dropped out by the dampers from the open ore pit at an open site near to the mine. The ore tailings deposit occupies a surface of 0.80 km<sup>2</sup>, located southwest of the open ore pit, near the regional road Štip-Strumica. The ore tailings deposit has about 130 million tons of ore tailings. Exposure of this great mass of ore tailings to constant air flow and wind leads to the distribution of fine dust in the air. The flotation tailings are drained and disposed of on a dump near the mine. The location of this dump is east of the flotation plant, 2.2 km to the dump. The Bucim mine also was listed among the major “environmental hot spots” in the Republic of Macedonia by the United Nations in 2000 (UNEP, 2000).

The investigated area represents a part of the Vardar structural zone, separated from the other structural zones during the Caledonian, and subjected to strong tectonic processes, during the Herzynian orogenesis. The structural relations were further complicated by the Alpine orogenesis (Rakićević *et al.*, 1969). At the study area the following main geotectonic structural units have been identified: (1) the Kriva Lakavica basin (2) the Smrdeš-Gabreš syncline, (3) the Radoviš basin, (4) the Radoviš anticline divided to (4a) the Štip Block and (4b) the Bučim Block (Map. 2). The Radoviš anticline represents the eastern boundary of the Vardar zone towards the Serbian-Macedonian mass. These two large structural units are separated by a deep NW-SE fault (Hristov *et al.*, 1965).

The Bučim-Damjan-Borov Dol area is divided to two tectonic blocks. The Bučim tectonic block and the southern tectonic block Damjan are a part of the Vardar zone. The blocks are divided by a fault of first order in the SE direction. Despite the disposition into two different tectonic blocks, the metallogenic area is

unified based on the similarities of Tertiary magmatism and the analogous ore mineralisations. The Bučim copper-porphyry deposit with additional gold mineralisation is found in the northern block (Stefanova *et al.*, 2004).

Fifty samples of lichen species *Hypogymnia physodes* (Nyl.) and *Evernia prunastri* (Ach.) were collected from the whole study area. These lichen species are characteristic for the flora of the Republic of Macedonia and can be used as biomonitors. Depending on the conditions and the accessibility of the locations, the lichen species available and typical for the region were collected.

The lichen sampling protocol was performed according to the set standard rules for collection of such samples and was performed in the following order: one sampling spot was formed by collecting five sub-spots in an area of 50 x 50 m<sup>2</sup>. Every spot of the sampling network must be at a minimum distance of 300 m from main roads, 100 m from local roads, and 200 m from villages. Lichen samples were collected using polyethylene gloves how to prevent any further sample

contamination. The collected material was stored in paper bags. After it was cleaned from other plant species and soil, individual plant samples were separated and air dried for several days (Tuba *et al.*, 2007). Lichen samples were collected at 50 localities, given in Fig. 2. Locations of the lichen samples were previously defined by means of a GPS device.

Sample preparation was performed in this order: lichen taluses were cleaned from soil and other plant species remains, after that were air-dried and digested using acid mixture. For digestion of lichen samples, the microwave digestion system (CEM, model Mars) was applied. The precisely measured mass (0.5 g) of each lichen sample was measured in teflon digestion vessels. Plant tissues were treated with 5 mL concentrated nitric acid, HNO<sub>3</sub> and 2 mL hydrogen peroxide, H<sub>2</sub>O<sub>2</sub> (30 %, m/V), added in teflon's. Lichen samples were digested using the following programme: 1 step: temperature 180 °C, 5 min ramp time, with power of 500 W and 20 bar pressure; 2 step: temperature 180 °C, 5 min hold time, with power of 500 W and 20 bar pressure. The digestion was carried out in the microwave oven. Finally, the vessels were cooled,

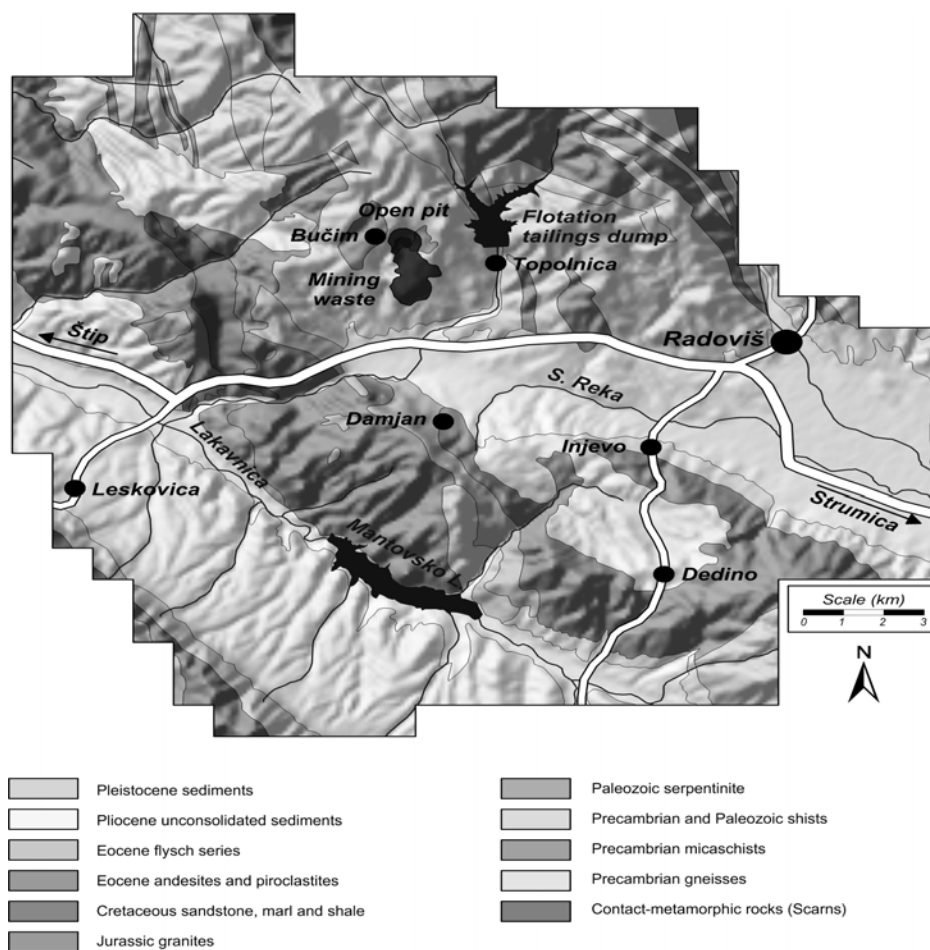


Fig. 2. Generalised geology map of study area

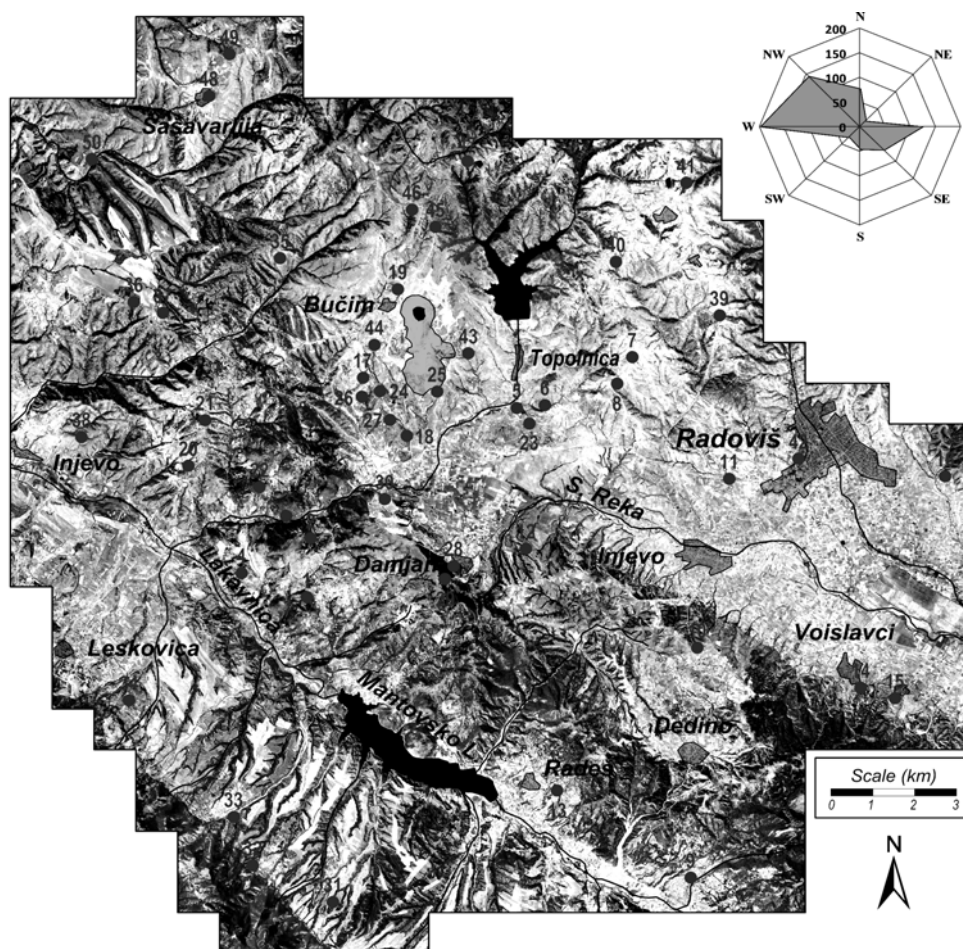
carefully opened, and digests quantitatively transferred to 25 mL calibrated flasks (Rusu, 2002).

For this study reagents with analytical grade or better were used: nitric acid, trace pure (Merck, Germany) and hydrogen peroxide, p.a. (Merck, Germany). Redistilled water was used for the preparation of all solutions. Standard solutions of metals were prepared by dilution of 1000 mg/L solutions (11355-ICP multi Element Standard). The analyses of the digest samples of 18 elements (Al, Ba, Ca, Cr, Cu, Li, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, V and Zn) were performed with an atomic emission spectrometry with inductively coupled plasma, ICP-AES, Varian 715-ES. The operating conditions for the applied technique

are given in Table 1. As and Cd were analysed by ETAAS. Optimisation of instrumental conditions has been previously determined (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 between 98.5–101.2 % and for ETAAS, between 96.9 % – 103.2 %. The sensitivity in regard to the lower limit of detection for the analysed elements is given in Table 1. Quality control was also ensured by standard moss reference materials M2 and M3, which are prepared for the European Moss Survey (Steiness *et al.*, 1997). The measured concentrations were in good agreement with the recommended values.

**Table 1. Detection limit for analysed elements**

Elements	Detection limit
Cd	0.05 mg/kg
As, Ba	0.1 mg/kg
Cu, Mn, V	0.5 mg/kg
Cr, Li, Fe, Mg, Na, Ni, Sr, Zn	1 mg/kg
Al, K, Pb	2 mg/kg
Ca	10 mg/kg



**Fig. 3. Lichen sample locations**

For the statistical analysis of data parametric and nonparametric statistical methods were used (Hollander and Wolfe, 1999). The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics (Table 2). Data distribution was examined with the application of the normality test.

The application of the bivariate statistics showed the correlation between the content of the investigated elements in the lichen samples. In order to analyse this, the linear coefficient of correlation was used, and the absolute values  $r > 0.50$  indicated a good association between variables (Table 3).

Multivariate statistical methods (cluster and R-mode factor analyses) were used to reveal the associations of the chemical elements (Fig. 4; Table 4). Clustering multivariate method was applied in order to verify the accuracy and precision of the results obtained by factor analyses. Cluster analysis encompasses a number of different classification algorithms that can be used to develop similarities between grouped elements. The factor analysis was performed on variables standardised to zero mean and unit standard deviation (Reimann *et al.*, 2002; Filzmoser *et al.*, 2005; Šajn, 2006; Žibret and Šajn, 2010). As a measure of similarity between variables, the product-moment correlation coefficient ( $r$ ) was applied. For orthogonal rotation the varimax method was used. In the final multivariate factor solution 13 elements were retained (Table 4). The elements As, Ba, Mg, Mn and Na were excluded because of the inability to form significant associations with other chemical elements or because of their low communalities.

The universal kriging method with linear variogram interpolation was applied for the construction of spatial distribution maps of each factor score (F1–F4 and distribution of Cu) obtained from lichen samples (Figs 5–9). Seven classes of the following percentile values were selected: 0–10, 10–25, 25–40, 40–60, 60–75, 75–90 and 90–100.

## RESULTS & DISCUSSION

Based on the normality tests and distribution histograms of the independent variables, the normal distribution for nickel was determined. For the rest of the elements skewed distributions were confirmed by relatively high values of standard deviation, measured the degree of data deviation (dispersions) around the mean. On the other hand, significant differences between mean and median value were not obtained for the variables (Table 2).

Three geogenic (F1, F2, F3) and one anthropogenic (F4) associations were established on the basis of:

visually indicated similarity of spatial distribution of elemental patterns; correlation coefficient matrix (Table 3); and results of cluster (Fig. 4) and factor analyses (Table 4).

Factor 1, the first geogenic association, consists of Al, Cr, Fe, Li and V, elements that are only slightly affected by anthropogenic activities. This group characterizes high values of correlation coefficients between chemical elements within (Table 3). The existence of the group is also confirmed by results of cluster (Fig. 4) and factor analysis (Table 4). Factor 1 explains 35% of total variability within the data. Their sources are mainly natural phenomena, such as rock weathering and soil surface dusting. In addition, the areal distribution of mentioned elements is closely dependent on the lithology. Highest contents were found in areas of Precambrian micaschists and Precambrian and Paleozoic shists (Figs 2 and 5). The spatial distribution of these elements is due to the presence of clay, which is the product of disintegration of primary rocks.

Factor 2 associates Ca, Cd, K and Zn, based on the similarity of areal distribution and characteristic associations indicated by correlation analysis, represents the group of geochemical association. The geochemical association is also indicated by cluster analysis (Fig. 4) and by the R-mode factor analyses (Table 4). Factor 2 is the second strongest factor, explaining 21% of total elemental variability. These elements are contained in the environment as a biogenic elements and essential trace elements in lichen plant tissue (except Cd) (Adamo *et al.*, 2007). On the other hand, high values for factor loadings were probably related to the geology of the study area, with remnants of Pleistocene unconsolidated sediments (Figs 2 and 4.). Geological background is not the only factor that controls the distribution of these chemical elements. For them, a statistically significant correlation with altitude was found. Higher concentrations, especially of Cd and Zn, were present in the higher elevation sites. Similar conditions have also been identified in the area of Kavadarci, Macedonia (Stafilov *et al.*, 2008).

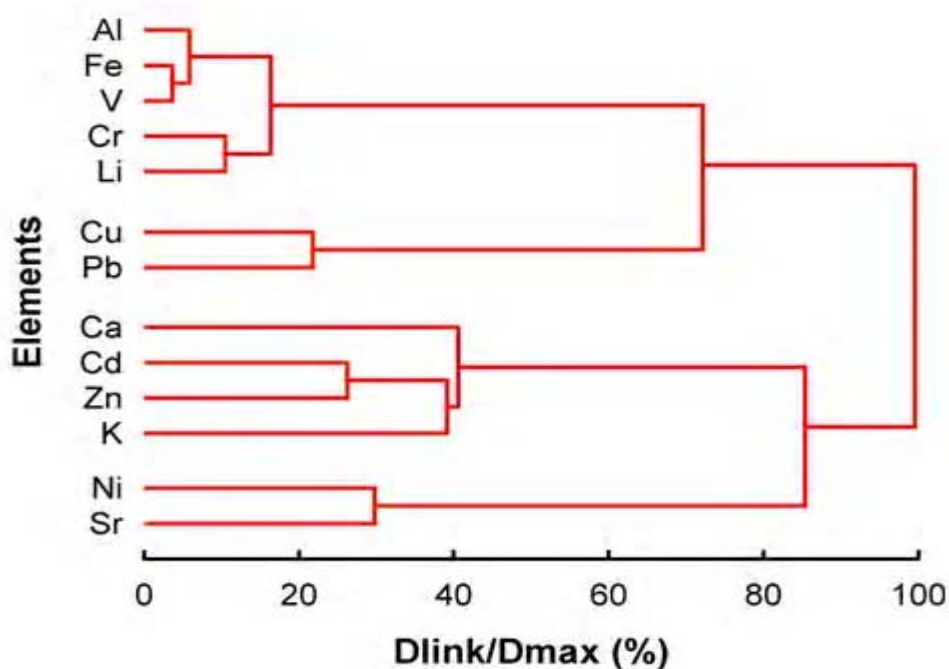
Factor 3 presents the third naturally association of elements, consisting of Ni and Sr (Table 4 - F3). Association is naturally found in the living environment. Their origin is mainly due to certain natural phenomena and the geology of the ground. The highest content of these elements is found in the area of Neogene dacites, andesites and pyroclastites and the residues from Proterozoic micashist (Figs 2 and 7).

Factor 4 demonstrates a geochemical anomaly caused by the Cu mining (Table 4). The group comprises Cu and Pb, chemical elements that were

**Table 2. Descriptive statistics for elements content values in lichen samples (n= 50, content values are given in mg/kg)**

Element	X <sub>a</sub>	X <sub>g</sub>	Md	min	max	s	s <sub>x</sub>	CV	A	E
Al	580	480	440	150	2500	420	60	73	2.54	9.08
As	0.89	0.74	0.87	0.10	3.8	0.54	0.077	61	2.93	15.67
Ba	14	13	14	3.8	30	6.0	0.84	42	0.60	-0.03
Ca	6500	5600	5700	1300	20000	3700	530	58	1.69	3.52
Cd	0.12	0.11	0.11	0.05	0.38	0.054	0.008	46	2.82	11.45
Cr	2.3	2.1	1.9	1.0	6.9	1.1	0.16	50	1.63	3.55
Cu	12	7.9	6.8	1.5	130	19	2.7	159	5.47	34.30
Li	710	560	520	190	4500	660	93	93	4.14	22.69
Fe	2400	2300	2500	1200	3700	460	65	19	-0.07	0.79
K	0.48	0.42	0.37	0.17	1.1	0.27	0.038	55	0.94	-0.13
Mg	740	710	750	280	1600	210	30	28	1.50	5.53
Mn	57	51	49	14	150	29	4.2	52	1.49	2.26
Na	71	57	66	16	250	47	6.6	65	1.44	3.49
Ni	2.8	2.6	2.5	1.5	10	1.5	0.21	53	2.80	11.45
Pb	6.7	4.3	4.2	0.61	120	17	2.4	253	6.98	49.08
Sr	14	13	12	2.9	37	7.8	1.1	55	1.62	2.30
V	2.3	1.8	1.7	0.58	11	1.7	0.25	76	2.49	9.38
Zn	21	20	20	10	39	5.4	0.77	26	1.52	3.32

X<sub>a</sub> – mean; X<sub>g</sub> – geometrical mean; Md–median; min – minimum; max – maximum; s -standard deviation; s<sub>x</sub> – standard error of mean; CV – coefficient of variation; A – skewness; E – kurtosis



**Fig. 4. Dendrogram of cluster analyses**

Table 3. Matrix of correlation coefficients

Al	1.00																						
As	<b>0.69</b>	1.00																					
Ba	0.32	0.15	1.00																				
Ca	0.21	0.28	0.33	1.00																			
Cd	<b>0.57</b>	<b>0.54</b>	0.29	<b>0.68</b>	1.00																		
Cr	<b>0.90</b>	<b>0.56</b>	0.21	0.22	<b>0.51</b>	1.00																	
Cu	<b>0.65</b>	<b>0.76</b>	0.07	0.31	<b>0.67</b>	<b>0.57</b>	1.00																
Li	<b>0.85</b>	0.44	0.25	0.06	0.27	<b>0.86</b>	0.30	1.00															
Fe	<b>0.95</b>	0.77	0.26	0.28	<b>0.68</b>	<b>0.86</b>	<b>0.83</b>	<b>0.70</b>	1.00														
K	0.02	0.24	0.14	<b>0.52</b>	0.45	0.05	0.19	-0.15	0.09	1.00													
Mg	<b>0.67</b>	0.48	<b>0.60</b>	0.43	<b>0.52</b>	<b>0.65</b>	<b>0.55</b>	0.48	<b>0.72</b>	0.21	1.00												
Mn	0.24	0.01	<b>0.61</b>	0.26	0.24	0.24	-0.01	0.22	0.16	0.22	0.33	1.00											
Na	<b>0.61</b>	<b>0.50</b>	0.15	0.28	0.47	0.49	<b>0.54</b>	0.46	<b>0.60</b>	-0.04	0.31	0.05	1.00										
Ni	0.21	-0.09	0.22	0.20	0.32	0.37	0.05	0.27	0.19	0.01	0.27	0.14	1.00										
Pb	0.70	0.78	0.15	0.36	<b>0.74</b>	<b>0.61</b>	<b>0.93</b>	0.36	<b>0.86</b>	0.11	<b>0.60</b>	0.07	<b>0.55</b>	1.00									
Sr	0.21	0.11	0.42	0.49	<b>0.55</b>	0.22	0.31	0.01	0.30	0.14	0.48	0.35	<b>0.61</b>	0.43	1.00								
V	<b>0.96</b>	<b>0.69</b>	0.31	0.19	<b>0.57</b>	<b>0.92</b>	<b>0.71</b>	<b>0.79</b>	<b>0.96</b>	0.08	<b>0.68</b>	0.21	<b>0.55</b>	0.22	<b>0.72</b>	1.00							
Zn	0.39	0.41	0.02	0.51	0.73	0.40	<b>0.54</b>	0.18	0.48	<b>0.50</b>	0.20	0.07	0.41	0.11	<b>0.51</b>	0.25	1.00						
	Al	As	Ba	Ca	Cd	Cr	Cu	Li	Fe	K	Mg	Mn	Na	Ni	Pb	Sr	V	Zn					



Table 4. Matrix of rotated factor loadings

Element	F1	F2	F3	F4	Com
Li	<b>0.96</b>	-0.08	0.01	-0.06	94
Al	<b>0.96</b>	0.05	-0.02	0.16	95
Cr	<b>0.93</b>	0.07	0.13	0.13	90
V	<b>0.91</b>	0.08	0.02	0.31	94
Fe	<b>0.89</b>	0.10	-0.01	0.39	96
K	-0.10	<b>0.84</b>	-0.11	0.05	72
Zn	0.07	<b>0.80</b>	-0.06	0.24	71
Cd	0.22	<b>0.80</b>	0.34	0.23	86
Ca	0.04	<b>0.80</b>	0.31	-0.07	74
Sr	-0.12	0.17	<b>0.90</b>	0.05	86
Ni	0.18	0.03	<b>0.88</b>	-0.09	81
Cu	0.21	0.16	-0.17	<b>0.89</b>	89
Pb	0.29	0.16	0.13	<b>0.86</b>	87
Var	35	21	14	15	86

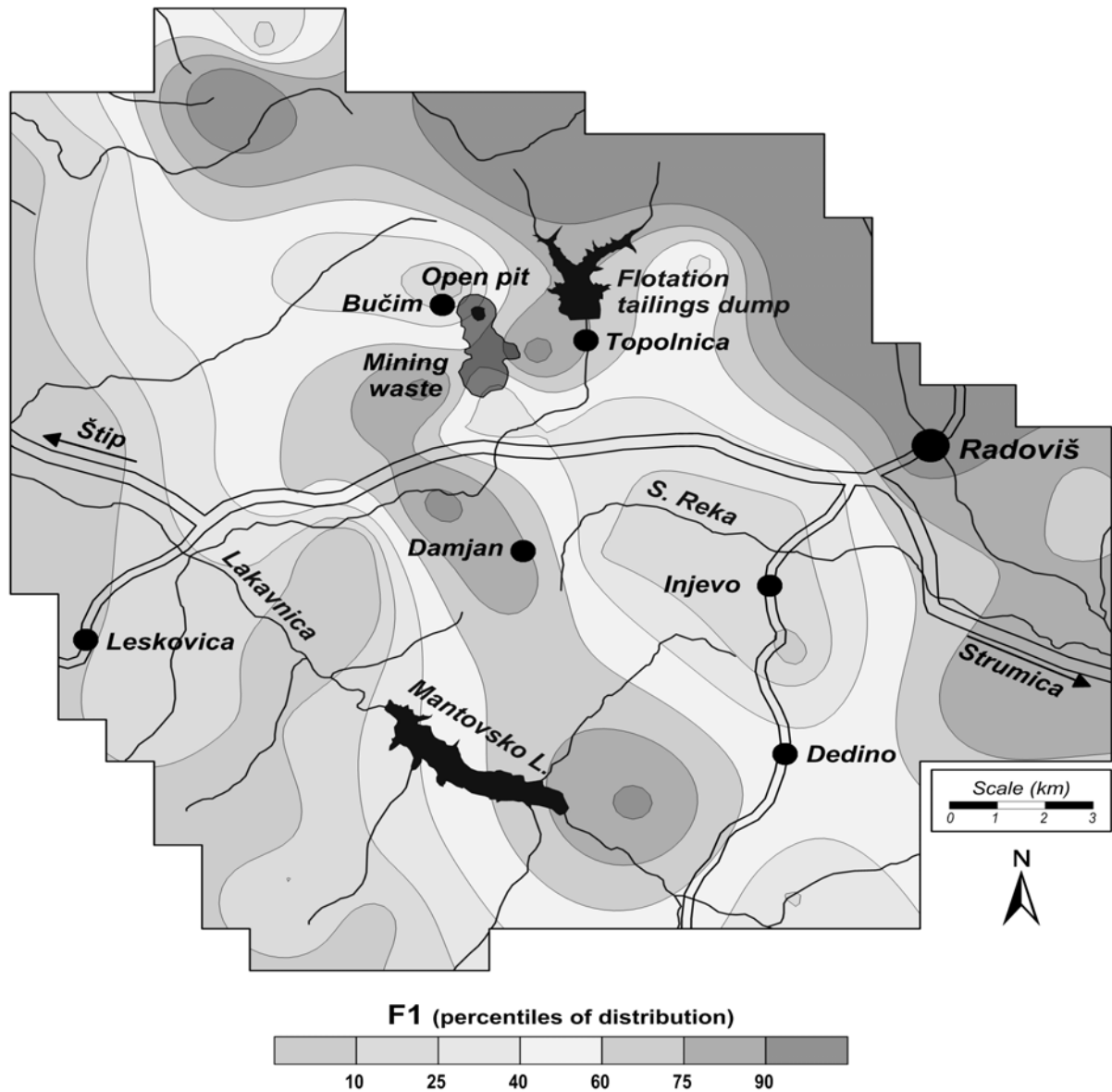


Fig. 5. Spatial distribution of Factor 1 scores (Al, Cr, Fe, Li and V)

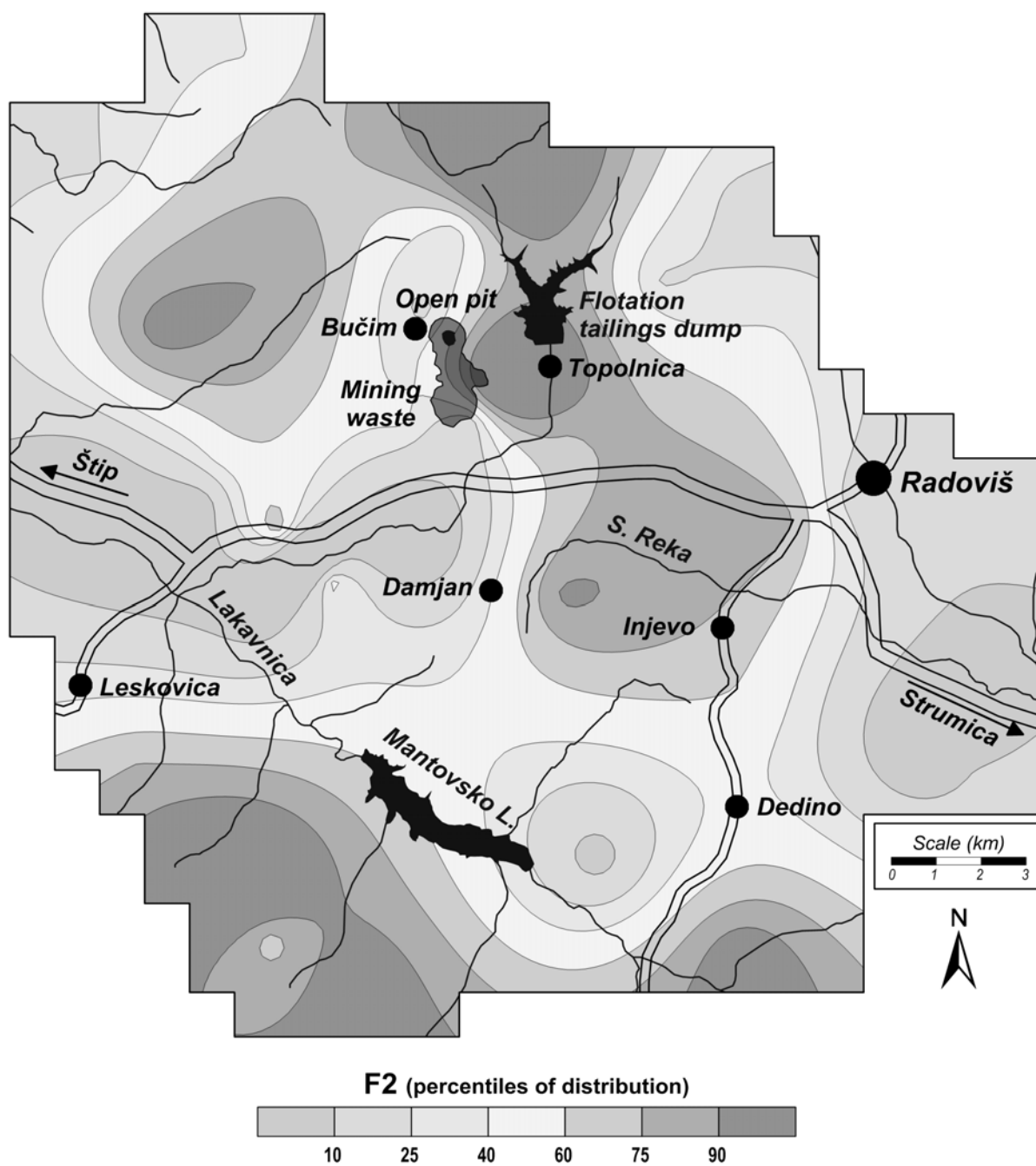


Fig. 6. Spatial distribution of Factor 2 scores (Ca, Cd, K and Zn)

Table 5. Comparative analysis of median and range values between moss and lichen element content

Element	Lichen samples Present study		Moss samples Balabanova et al., 2010		Correlation coefficients moss:lichen
	Median	Range	Median	Range	
Cu	6.84	1.50-130	10	2.1-198	0.86
Pb	4.20	0.61-120	6.8	2.7-40.2	0.68

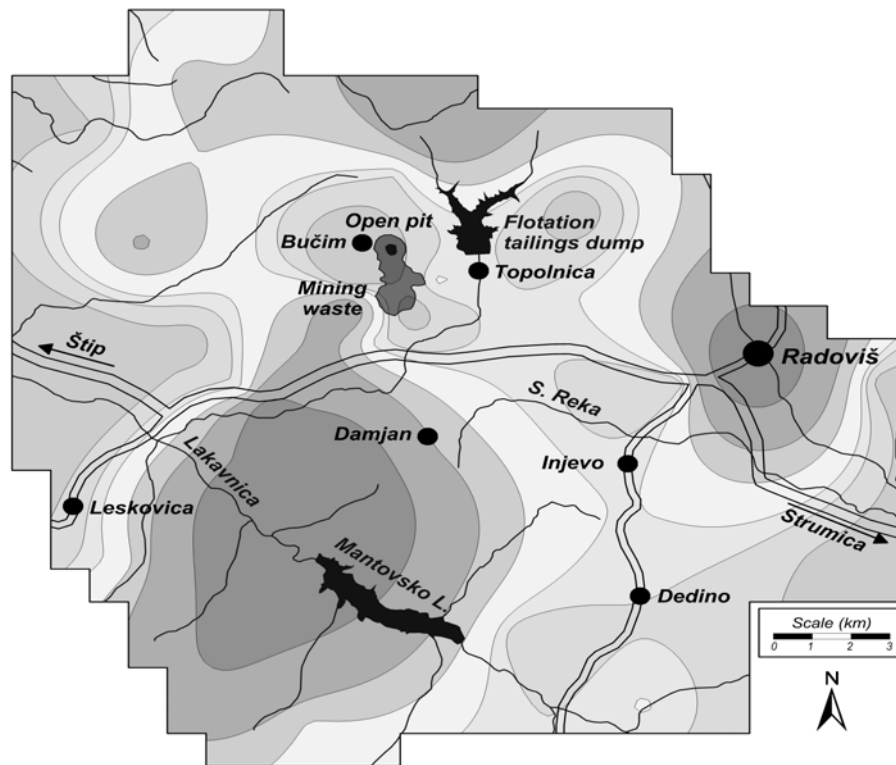


Fig. 7. Spatial distribution of Factor 3 scores (Ni and Sr)

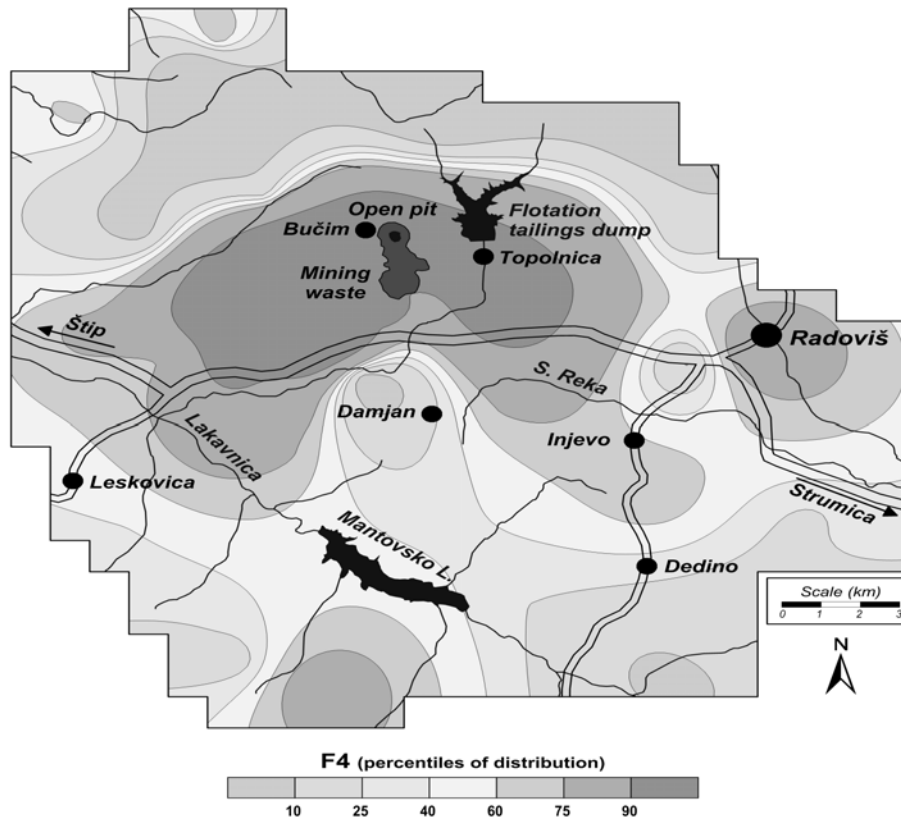


Fig. 8. Spatial distribution of Factor 4 scores (Cu and Pb)

introduced into the environment through anthropogenic activities (copper mine). Their relatively high correlation coefficients within sampling materials are typical (Table 2). The group's existence is also confirmed by the results of multivariate statistical analyses (Fig. 4, Table 4).

Appearance of this anthropogenic factor was expected because of the presence of copper open pits. These elements usually appear in higher content due to mining activities (Williamson *et al.*, 2004). Fine dust fractions are easily emitted from the open ore pit and minerals. The exposure of ore and flotation tailings at the open, under the continuous influence of the erosive effects of winds and moisture allows distribution of the finest particles from the surface. Fine dust with a higher content of these elements is constantly carried by the winds and so distributed in the air and spread to larger distances away from the mine. The spatial distribution of this factor (Fig. 8) clearly shows that high contents of these elements are found in close vicinity of the mine following the wind rose for these regions (Fig. 3). Distant areas are not affected by higher values of their contents.

The lichen samples, taken from the close vicinity of the mine, showed high values for the copper content and contents of the other anthropogenic elements. The median value of copper is not of a worrisome aspect (6.8 mg/kg), but the median value of lichen samples collected from the vicinity of the mine, is showing a significantly higher value of approximately 25 mg/kg (increased by a factor of 4 times). The highest value for copper (130 mg/kg) was obtained from a lichen sample collected very close to the mine. Lead shows a strong correlation with the copper content ( $r=0.93$ ), with a low median value (4.2 mg/kg), but very high median value from lichen samples from close vicinity of the mine (20 mg/kg), increased by a factor of 5 times and maximum value of 120 mg/kg.

One comparison of the median and range values for the anthropogenic elements obtained from lichen samples with the corresponding values obtained from the moss samples (species: *Hyloconium splendens* and *Pleurozium schreberii*) collected from the same study area (Balabanova *et al.*, 2010), is presented in Table 5. The values for the contents of the both elements is highly similar ( $r=0.86$  and  $r=0.68$ ), which of course is due to the similar modes of accumulation and binding of these elements in mosses and lichen plant tissue. On the other hand, lichen showed a strong tolerance to high contents of these heavy metals ( $>0.01\%$ ). However, the ultimate effect is that lichen species (*Hypogymnia physodes* and *Evernia prunastri*) reflect the real atmospheric distribution, not only for the

anthropogenic elements, but also for those elements that are little affected by anthropogenic activities from the copper mine (Al, Ca, Cd, Cr, Fe, Li, K, Ni, Sr, Zn and V).

## CONCLUSION

Lichen monitoring has proved to be a very effective way to monitor the distribution of heavy metals in a potentially contaminated environment (copper mine environ). Species *Hypogymnia physodes* and *Evernia prunastri* have proved to be very suitable monitors compared with certain types of moss from the same environ. These lichen species proved to be very tolerant to high contents of Cu and Pb. The presence of an open copper ore pit and ore processing and flotation plant (Bučim mine) in the eastern region of the Republic of Macedonia leads to increased deposition of copper in its surroundings (max. value 130 mg/kg). Apart from the copper, increased atmospheric deposition of Pb (120 mg/kg) was also found, singled out as anthropogenic association with copper. The distribution of the anthropogenic association of elements (Cu and Pb) at distant regions was not determined. The processes in the Bučim mine do not have an effect on the distant region distribution of Cu and Pb.

However, it is worrying that in the close vicinity of the mine there are two settlements - the villages of Bučim and Topolnica. The population in these villages is continuously exposed to a high content of copper and lead, which has a negative effect on human health and toxic effects on the environment.

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