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Elemental composition of selected lichen species growing on the Balkan Peninsula

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Abstract: The amount of nineteen elements in eleven different lichen species, six fruticose (*Bryoria capillaris*, *Bryoria fuscescens*, *Cladonia rangiformis*, *Ramalina capitata*, *Usnea chaetophora* and *Evernia prunastri*) and five foliose (*Hypogymnia tubulosa*, *Lobaria pulmonaria*, *Peltigera horizontalis*, *Umbilicaria cylindrica* and *Umbilicaria crustulosa*) from five natural areas of the Balkan Peninsula (Serbia and Bulgaria) were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES). Among macronutrients, the highest content was observed for Ca and K, while Mg and Na were represented in the smallest amount. Fe, Mn, Zn and Ba were the most abundant trace elements in contrast to Cd and Co, whose concentrationc were the lowest.

Keywords: heavy metals; ICP-OES determination.

INTRODUCTION

Lichens occur in all terrestrial ecosystems, including extreme ones. They represent the symbiotic association of fungi and algae with a simple anatomy, without a waxy cuticle layer, stomata and root system. An alga as a photobiont performs photosynthesis and provides the lichen with nutrients while a fungus as a mycobiont absorbs water with nutrients from the substrate. As a result, lichens are autotrophic and can grow on rocks, roofs, tree trunks, leaves of plants in the tropics and even on porcelain, glass, paper, cloth, resin, bones, charcoal, *etc*. They are long-lived perennial organisms and because of weak photosynthesis they grow very slowly.[1,2]

Lichens are used as medicines, food, fodder, dyes perfume, spice and for miscellaneous purposes. More than one thousand primary and secondary metabolites

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with some biological activities such as antioxidant, antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, *etc*., are known in lichens.[3]

The accumulation of metals in lichens depends on many factors, such as the availability of metals, lichen characteristics, climate conditions, *etc*. The changes in elemental bioavailability (essential and toxic to metabolic processes) depend on factors such as a rapid increase of population, urbanization, industrialization; the use of chemicals in the industry, nuclear energy, and thermic power stations; decreasing green areas, the destruction of forests and erosion; changes in climate *etc*. Air, water and soil pollution are related to these factors. Being non-biodegradable, heavy metals tend to accumulate in living systems and have a long half-life in soil. Fungi, mosses, lichens and plants have been used to detect the deposition, accumulation and distribution of metal pollution either as the bioindicators/biomonitors of air quality or as the bioaccumulators of atmospheric pollutants.

Lichens depend on mineral nutrients from a wet atmospheric deposition (precipitation, fog, dew) and a dry atmospheric deposition (sedimentation, gaseous absorption). Intracellular spaces of the lichen thallus can accumulate and retain metals by trapping insoluble particles, extracellular ion exchange processes, adsorption and active uptake. Lichens tolerate high concentrations of heavy metals by sequestering them as oxalate crystals or lichen acid complexes. As slow-growing and long-living organisms, lichens offer long-term knowledge about levels of heavy metals and other pollutants in the atmosphere.[2]

Lichens have cation exchange properties and take up rapidly soluble elements over the entire thallus surface. Slower and more selective uptake mechanisms also exist. Soil particles and aerosols adsorbed to the surface also contribute to the total element concentrations in the thalli. The exchangeable fraction of metals decreases when the thalli become air-dried. In humid regions, a high correlation exists between the levels of air pollutants and lichen physiology. They can easily lose water but can just as easily compensate it. Because of their high surface/volume ratio they are under the strong influence of atmospheric deposition which is the reason of their use for the biomonitoring purposes. Lichens and mosses have a higher capacity for metal accumulation and are probably most frequently used for biomonitoring. Lichens were found to be more sensitive than mosses to emissions of S and atmophile elements (Hg, Cd, Pb, Cu, V, Zn), while mosses showed a higher concentration of lithophile elements and dust (Al, Cr, Fe, Mn, Ni, Ti). Also, epiphytic and corticolous lichens were preferred to prevent particles coming from the ground.[4]

Lichens as species with wide geographical ranges are widely used for the determination of sulfur dioxide, trace metals, organochlorines, radionuclides. When the amount of harmful substances becomes toxic, lichens die, which is the reason of their absence in heavily polluted areas, but they are nevertheless used for biomonitoring in these areas as well by using so-called transplant techniques.

One of these techniques consists in exposing bags containing lichens to the atmospheric deposition in the studied area to measure the concentrations of the contaminants affecting the samples.[5]

Transportation, industrial activities, fossil fuels, agriculture and other human activities may cause environmental pollution. Biological material is often used for pollutants' monitoring. A high sensitivity of lichens to pollutants, particularly airborne pollutants, is well-known. Metal content of lichens vary mostly with the changes in metal amounts in the atmosphere. The metal content of lichens is usually inversely proportional to the distance from pollution sources. Particulate emissions from local farming (pesticides and fertilizers), traffic, landfills and industries can be transported by the wind. The direction of the dominant wind is important for the removal of atmospheric pollutants and the metal composition of lichen species.[6]

The wind is a driving force that resuspends natural soil material into a fine mixture of light particulates (dust) transportable over a long distance and precipitable on lichens.[2] Dongara *et al.*[7] defined roadway dust as a mixture of different particulates from the Earth's crust (quartz, calcite, feldspars, gypsum, Al, Fe, Ti, Sc, V, Cu, Mn, K, Mg, Na, P, S) and particulates of anthropogenic origin. Paoli *et al.*[8] confirmed that the high concentration of lithogenic elements in lichens is associated with high levels of depositions from airborne soil dust (Fe, Cd, Cr, Ni).

The aim of the present work was to determine the elemental composition of different lichen species from different locations and substrates and to perform the relevant conclusions and/or assumptions about the main factors which may affect this composition, such as lichen taxonomy and live forms and the substrate on which they grow (soil, rocks, tree bark).

EXPERIMENTAL

Reagents

Multi-element standard solutions for ICP, III and IV and single standard solutions of Si and P (TraceCERT, Fluka Analytical, Switzerland) were used as a stock solution for calibration. A nitric acid (Merck, Darmstadt, Germany) was used for complete mineralization of analyzed samples. Certified Reference Material Strawberry Leaves (LGC7162) was used for accuracy testing.

Instrumentation

iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) which combines an Echelle optical design and a charge injection device (CID) solid state detector was used, under the optimal operating conditions, for elements determination. iTEVA operating software was used to control all functions of the instrument. The Mettler Toledo analytical balance (Switzerland) was used to measure the mass. High purity water (conductivity, 0.05 μS cm^{-1}) was obtained using the MicroMed high purity water system, Thermo Electron LED GmbH (Germany).

Operating conditions of ICP-OES. All of measurements were done under the next operating conditions of instrument: flush pump rate 100 rpm, analysis pump rate 50 rpm, RF power 1150 W, nebulizer gas flow rate 0.7 L min^{-1}, coolant gas flow rate 12 L min^{-1}, auxiliary gas flow rate 0.5 L min^{-1}, dual (axial/radial) viewed plasma mode and sample uptake delay 30 s.

Method validation. The calibration lines were constructed at four wavelengths for each element (Table I).

TABLE I. Analytical lines, correlation coefficients (*r*), limits of detection (*LOD*), ratio of the slopes of the external calibration lines and slopes of the lines where the prepared sample solutions have been added to the calibration standards ($Slope_{cal}/Slope_{sam}$) for determined elements in analyzed lichen species and results of methods accuracy; ND – not determined, Recovery = 100(Found amount/Certified amount)

Element	Wavelength nm	*r*	*LOD* ppm	$Slope_{cal}/Slope_{sam}$	Amount, mg kg^{-1}		Recovery %
					Certified	Found	
Al	396.152	0.999873	0.001762	0.98	1000[a]	890±8	89.0
B	249.773	0.999971	0.000747	0.97	ND	32±2	–
Ba	455.403	1.000000	0.000052	1.12	107±10	97±4	90.6
Ca	393.366	0.999929	0.000101	1.03	15300±700	13800±800	90.2
Cd	214.438	0.999759	0.000100	0.97	0.17±0.04	0.18±0.03	105.9
Co	228.616	0.999986	0.000348	0.96	0.47±0.11	0.42±0.04	89.4
Cr	357.969	0.999888	0.001692	0.97	2.15±0.34	2.25±0.02	104.6
Cu	324.754	0.999948	0.000584	1.08	10[a]	11.2±0.2	112.0
Fe	259.940	0.999999	0.000514	1.06	818±48	750±20	91.7
K	766.490	0.980135	0.034503	0.99	19600±1000	18000±1000	91.8
Mg	279.553	0.999843	0.000132	0.98	3770±170	3900±200	103.4
Mn	257.610	0.999924	0.000094	1.04	171±10	180±5	105.3
Na	589.592	0.999992	0.000537	1.02	210[a]	188±8	89.5
Ni	231.604	0.999924	0.000475	0.97	2.6±0.7	2.9±0.6	111.5
P	213.618	0.998743	0.004315	1.06	2600±230	2400±200	92.3
Pb	220.353	0.999952	0.002293	0.96	1.8±0.4	2.0±0.2	111.1
Si	288.158	0.999803	0.002780	1.06	ND	770±2	–
V	292.402	0.999945	0.000522	1.05	1.8[a]	1.68±0.02	93.3
Zn	213.856	0.999909	0.000115	0.98	24±5	22.4±0.2	93.3

[a]Non-certified concentration

The selection of the analytical emission line was made based on the sensitivity of the analytical lines, the correlation coefficients, as well as based upon the tables of known interferences, baseline shifts and the background correction (the highest signal-to-background ratio) which was manually selected for the quantitative measurements. The possible matrix interferences were examined by comparing the calibration slopes of the so-called external calibration lines ($Slope_{cal}$) to slope of the standard addition method lines where the prepared sample solutions have been added to the standards for calibration ($Slope_{sam}$). Values of ratio $Slope_{cal}/Slope_{sam}$ closer to 1 indicate a negligible matrix effect at selected wavelengths. In order to test the accuracy of the methods, certified reference material strawberry leaves (LGC716) was used.[9]

Samples

Eleven lichen species were collected from five different locations on the Balkan Peninsula (Serbia and Bulgaria) and were identified and stored in the herbarium at the Department of Biology, Faculty of Sciences and Mathematics, University of Niš. The lichen species, family, genus, sampling sites, substrate and sampling year are given in Table II. A composite sample of several thalli of each investigated lichen were collected using plastic gloves and a stainless-steel knife. The soil and rock particles and the old parts of lichens were removed. The samples were dried at 40 °C to a constant weight and then ground in a mill. One gram of the obtained powder was weighed and treated with 30 ml of conc. HNO_3, left overnight, and heated on a hot plate until the nitrogen vapor stopped forming or until a clear solution was obtained. After complete mineralization, the obtained solutions were filtered and made up to 50 ml with deionized water.[10]

TABLE II. Descriptions of analyzed lichens and collecting sites

Species	Family	Genus	Thallus type	Sampling sites	Habitat type	Voucher number
Usnea chaetophora Stirt.	Parmeliaceae	*Usnea*	Fruticose	Bulgaria, Pirin, Bansko	Bark of the *Pinus spp.*	9377
Bryoria fuscenscens (Gyel) Brodo et Hawksw.	Parmeliaceae	*Bryoria*	Fruticose			9378
Bryoria capillaris (Ach.) Brodo et Hawkisw.	Parmeliaceae	*Bryoria*	Fruticose			9376
Ramalina capitata (Ach.) Nyl.	Ramalinaceae	*Ramalina*	Fruticose	Serbia, Stara Planina, Babin zub	Siliceous rocks	9374
Umbilicaria crustulosa (Ach.) Frey	Umbilicariacea	*Umbilicaria*	Foliose			9373
Umbilicaria cylindrica (L.) Duby	Umbilicariacea	*Umbilicaria*	Foliose			9375
Hypogymnia tubulosa (Schaer.) Hav.	Parmeliaceae	*Hypogymnia*	Foliose	Serbia, Seličevica, Donje Vlase, Grčke pojate	Bark of the *Pinus spp.*	10355
Cladonia rangiformis Hoffm.	Cladoniaceae	*Cladonia*	Fruticose-squamulose	Serbia, Suva planina, Duga Poljana	On the ground below *Quercus cerris*	10889
Peltigera horizontalis (Huds.) Baumg.	Peltigeraceae	*Peltigera*	Foliose			10890
Evernia prunastri (L.) Ach.	Parmeliaceae	*Evernia*	Fruticose	Serbia, Vlasinska visoravan, Vlasina Rid	Bark of the *Pinus spp.*	10892
Lobaria pulmonaria L. (Hoffm.)	Lobariaceae	*Lobaria*	Foliose	Serbia, Stara Planina, Babin zub	Beech trunks, basal parts	10891

Statistical analysis

All measurements were carried out in triplicate and presented as the mean ± standard deviation (*SD*). Shapiro-Wilk test of normality, ANOVA with Tukey's post hoc test, the Pearson's correlation study and hierarchical cluster analysis were done using a statistical package IBM SPSS 20, US.

RESULTS AND DISCUSSION

The contents of determined elements in eleven samples of lichens shown as mean values of triplicate determination with standard deviations are presented in Tables III and IV. A one-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to establish which metals significantly differed in the mean content between the all analysed samples and the results of this statistical method are also given in these tables.

Table III. Content of determined elements±*SD*, mg kg^{-1}, in analyzed samples of fruticose lichens (standard deviation for triplicate determination; mean values in the same row with the same letters indicate no significant differences ($p < 0.05$); a is always lower than b and b is always lower than c, *etc.*

Element	Lichen					
	Usnea chaetophora	*Bryoria fuscescens*	*Bryoria capillaris*	*Ramalina capitata*	*Cladonia rangiformis*	*Evernia prunastri*
Al	366±4^{a,c}	261±3	180±2	1665±8	2103±11	356±5^{b,c}
B	5.35±0.08^{b}	6.42±0.04^{c}	17.49±0.09	4.16±0.09^{a}	5.8±0.1^{b,c}	8.6±0.2
Ba	11.24±0.06^{a}	6.73±0.05^{b}	34.8±0.1	10.87±0.02^{a}	32.5±0.2	6.33±0.08^{b,c}
Ca	3570±36^{e}	2234±7^{a}	3345±17^{c}	3152±23^{d}	2223±26^{a}	3643±18^{e}
Cd	0.174±0.014^{a,e,f}	0.148±0.005^{e}	0.078±0.001	0.444±0.001	0.405±0.008	0.179±0.015^{a,d}
Co	0.189±0.028^{a,c,d}	0.781±0.01	0.011.0±0.0001	0.663±0.026	1.167±0.016	0.238±0.006^{d,e,f}
Cr	1.16±0.02^{c}	0.63±0.07^{a}	0.50±0.02^{a}	4.27±0.07^{b}	4.18±0.08^{b}	2.78±0.08^{d}
Cu	6.18±0.09^{a}	5.68±0.03	7.6±0.1	10.04±0.02	8.29±0.06^{b}	6.2±0.1^{a}
Fe	319±4	254±2	166.9±0.8	2089±8	2358±11	471±4^{a,b}
K	2239±6^{a,b}	2376±42	2250±22^{a,c}	1199±13	1489±18	1639±9
Mg	580±6^{c}	475±9^{a}	524±5^{b}	612±7	746±2	480±2^{a}
Mn	77.5±0.8	45.0±0.5	48.1±0.2	37.74±0.05	252±2	32.1±0.2^{a}
Na	9.75±0.07^{b}	9.7±0.2^{b}	15.4±0.2	7.7±0.2^{c}	3.66±0.03^{a}	3.68±0.04^{a}
Ni	1.12±0.04^{a}	1.17±0.03^{a}	0.78±0.02	2.66±0.05	3.71±0.08	5.01±0.06
P	991±34^{a,e}	1167±15	1074±7^{c}	616±3^{d}	1020±21^{b,e,f}	589±5^{d}
Pb	3.4±0.2^{a,c,f}	6.53±0.05^{h}	3.5±0.4^{a,e,g}	18.10±0.07	3.12±0.07^{b,c,e}	4.4±0.2
Si	63±7	45.7±0.6	150.3±0.9	94.3±0.3	24.7±0.2^{a}	27.1±0.6^{a}
V	0.778±0.005	0.49±0.03	0.38±0.04	4.70±0.02	5.34±0.05	0.93±0.06
Zn	28±1	33.4±0.4	42.6±0.3^{b}	37.2±0.2^{a}	24.8±0.2	19.1±0.2

It is evident from the obtained results that the contents of the investigated macroelements follow this decreasing order: Ca > K > P > Mg > Na in six samples and K > Ca > P > Mg > Na in three samples. The order is quite similar in the rest of the samples – the most abundant are Ca and K and the least abundant were Mg and Na. The regularity observed in the case of macroelements is considerably less pronounced in other tested elements. Fe, Mn, Zn and Ba are the most abundant trace elements while Cd and Co are present in the lowest content. *P. horizontalis* has the highest content of most elements (Al, Ba, Co, Cu, Fe, K, Mn, Ni, P). *U. cylindrica* is the next in the sequence with the highest content of Cd, Cr, Mg, Pb, V. Unlike them, *B. capillaries* contains the smallest quantities of most elements (Al, Cd, Co, Cr, Fe, Ni, V). The slightest variations are in the content of macro elements and among them the lowest are in Ca while they are the highest in the case of Co, Mn, Cr, Fe, Si even up to two orders of magnitude. It is evident, from the results of the One-way Anova, that the investigated species show the smallest differences in the content of Pb, P, Cd, Co and Ca and the biggest in the content of V, Si, Ni, Mn and Zn.

TABLE IV. Content of determined elements±*SD*, mg kg^{-1}, in analyzed samples of foliose lichens; *SD* – standard deviation for triplicate determination; mean values in the same row with the same letters indicate no significant differences ($p < 0.05$); a is always lower than b and b is always lower than c, *etc*.

Element	Lichen				
	Umbilicaria crustulosa	*Umbilicaria cylindrica*	*Hypogymnia tubulosa*	*Peltigera horizontalis*	*Lobaria pulmonaria*
Al	421±4	1914±8	578±4	2627±13	365±4^{a,b}
B	22.6±0.7	4.4±0.3^{a}	13.3±0.2	9.5±0.6	12.4±0.2
Ba	5.72±0.04^{c}	23.8±0.2	19.15±0.07	83.8±0.8	30.7±0.2
Ca	1293±29^{b}	1309±10^{b}	13843±82	3220±34^{d}	3314±43^{c}
Cd	0.202±0.001^{b,d,f}	0.567±0.01^{c}	0.214±0.005^{b}	0.560±0.013^{c}	0.369±0.022
Co	0.144±0.022^{b,c}	2.290±0.01	0.285±0.01^{e}	2.492±0.022	0.179±0.014^{a,b,f}
Cr	0.99±0.08^{c}	11.90±0.08	1.88±0.09	5.77±0.09	3.02±0.02^{d}
Cu	8.15±0.04^{b}	9.30±0.09^{c}	8.61±0.06	17.1±0.8	9.03±0.04^{c}
Fe	477±4^{a,c}	2293±9	766±6	3413±35	466±5^{b,c}
K	3138±11	1763±13	2238±9^{b,c}	6458±88	2631±6
Mg	715±5	1793±11	570±8^{c}	1419±10	531±2^{b}
Mn	18.23±0.09	58.4±0.3	23.64±0.07	729±12	30.1±0.2^{a}
Na	11.62±0.09	10.60±0.06	7.56±0.04^{c}	4.71±0.03	6.61±0.02
Ni	0.967±0.007	4.74±0.04	5.52±0.05	7.87±0.08	2.31±0.02
P	1572±4	1224±10	1004±7^{a,b}	2063±32	1055±10^{c,f}
Pb	14.55±0.05^{d}	22.4±0.4	14.3±0.2^{d}	7.02±0.09^{h}	3.03±0.2^{b,f,g}
Si	245±2	86.1±0.4	123±3	17.3±0.1	73.0±0.5
V	1.47±0.02	8.28±0.04	1.94±0.01	7.76±0.08	1.28±0.04
Zn	128.9±0.3	56.2±0.1	43.39±0.06^{b}	37.2±0.8^{a}	26.34±0.07

Bargagli *et al.*[10] determined the total concentration of macro- and trace elements in thalli of epilithic *U. decussate* from thirty-seven sites of ice-free areas from continental Antarctica in a period of ten years. No impact of human activities was detected, and the investigated time period did not show significant variations in this terrestrial ecosystem. They concluded that the ad/absorption of soil and rock dust particles (atmospheric deposition) is the main source of macro- and trace elements. The higher average contents of lithophilic elements (Al, Fe, and Mn) are probably due to a greater amount of dust particles in lichens' thalli than to changes in the environmental bioavailability of metals. The average concentration of Pb, Cu, Zn, Cr, Mn, and Fe were among the lowest ever reported for lichens of genus *Umbilicaria.* The higher content of the most elements was found in two types of lichens from genus *Umbilicaria* (*U. crustulosa* and *U. cylindrica*) analyzed in our work compared to the average contents of the examined species in the work of Bargagli *et al.*[10] Icel and Cobanoglu[4] analysed ten air pollutants in eight naturally growing lichens sampled at ten urban and suburban sites in the city of Istanbul using atomic absorption spectrometry and the concentrations are as follows: Mn > Zn > Pb > Cu > Al > Ni > Cr > Fe > Co > Cd. Some obtained average values are much smaller than in our study (Al and Fe); some are higher (Cu, Mn, Zn, Ni, Pb and Cr) and some are the same (Cd and Co). The behavior is the same if the same species tested in both studies are compared (*Cladonia sp.*, *H. tubulosa*, *E. prunastri*). Pb in one sample reaches a value of 119 μg g^{-1}, which is 5 times more than the maximum determined in our study. Icel and Cobanoglu[4] also used reference material IAEA 336 lichen (*E. prunastri*) as a control material and based on the obtained contents concluded that quite high levels of the elemental pollution in the area, especially for Pb, Cr and Cu, exist. Compared to the values in this reference material, the results in our study show some significantly increased levels in: *R. capitata* (Al, Fe, Pb); *U. cylindrica* (Al, Fe, Cr, Pb, Co); *C. rangiformis* (Al, Fe, Co, Mn); *P. horizontalis* (Al, Fe, Co, Cu, Mn); *U. crustulosa* (Pb, Zn); *H. tubulosa* (Fe, Pb). Dogrul *et al.*[11] found the following amounts of some heavy metals as pollutants in seven types of lichens: Cu (137.94 μg g^{-1}); Mn (466.67 μg g^{-1}); Cd (3.22 μg g^{-1}); Zn (1037.56 μg g^{-1}); Pb (225.85 μg g^{-1}); Cr (28.90 μg g^{-1}); Co (1.60 μg g^{-1}) and Ni (11.40 μg g^{-1}) in the industrial regions which are greater than in the investigated samples from the Balkan Peninsula even up to 10 times for Pb, 8 times for Zn and Cu and 6 times for Cd which indicates the importance of the air pollution impact.

Aslan *et al.*[12] determined the contents of six elements (Fe, Ba, Sr, K, Ca and Ti) in eight lichen species from rural and subrural sites of different regions in Turkey as well as in their substrates-soil, rocks, and bark of *Pinus Silvestre* and noticed that lichens may reach high levels of some metals even when these plants are growing in rural and isolated sites. The most abundant elements are Ca, K and Fe which is in accordance with the results of this work but the determined contents are higher (1.595–13.984 % for Ca; 2.615–9.190 % for K and 0.920––6.705 % for Fe). The same examined species, *R. capitata*, contains 36 times more potassium, 10 times more calcium and 5 times more iron compared to our research which is probably the consequence of different atmospheric depositions caused by different substrate compositions and weather conditions. The concentrations are as follows: 2.615–9.190 % K; 1.595–13.984 % Ca; 0.041–0.205 % Ti; 0.920–6.705 % Fe; n.d.–0.021 % Sr and n.d.–0.023 % Ba. They noticed that two crustose species (*R. melanophthalma* and *R. chrysoleuca*) which were collected from the same region and the same substrata accumulated a slightly different amount of Ca and Fe which is a probably consequence of different metal-

-accumulating capability while some other crustose species (*L. muralis*) which are from a different habitat had a different amount. Because of this, they concluded that lichens do not get any metals from their substrata, they accumulate only atmospheric depositions and distinct metal/accumulating capabilities of lichen species exist. They explained some greater metal contents by the foliose structure of this species as an additional factor which was noticed also in our work. All the largest certain contents, except sodium, are in foliose species.

Aslan *et al.*[6] determined the following concentrations in six lichen species: 3.707–5.210 % K; 1.410–6.563 % Ca; n.d.–0.069 % Ti; n.d.–2.022 % Fe and n.d.–0.021 % Ba. They noticed that some samples have similar levels of metal concentration while some have higher or lower levels which supports the hypothesis that lichens get very little or no metals from their substrates. The different element concentrations in these species are a consequence of a different thallus structure as well as different conditions of collection stations. The higher values of some elements may be the result of a large surface area, larger intercellular space in medulla and cortex of some species, while the higher value of potassium is explained by using some potassium containing fertilizers in these areas. The higher values in some fruticose as compared to some others indicate the importance of station selection in determining metal content in lichens. One of the species, *E. prunastri, was* examined in both studies. The contents of the investigated elements determined in the research of Aslan *et al.*[6] are higher: 20 times for K, 6 times for Fe and 4 times for Ca compared to our research. The situation is the same when comparing contents in species that belong to the same genus: *U. florida* and *U. longissimi* with *U. chaetophora* and *H. physodes* with *H. tubulosa.* These facts also support the above mentioned: the great influence of the different conditions at sampling sites.

Stamenković *et al.*[2] determined the content of 19 heavy metals in two epiphytic lichen species *Evernia prunastri* (L.) Ach. as fructose and *Parmelia sulcata* Taylor as foliose from two southeast places in Serbia and found that *P. sulcata* contains higher amounts of most of the elements which is in accordance with the higher affinity for metal uptake by folio species in our study. Comparing the content of the tested metals, a higher content of Fe, Mn, Ni, Cr, Ba and especially Pb in *E. prunastri* is observed in our study which confirms the significant influence of various factors on the amount of metals in the lichen in addition to the dominant influence of the type of lichen.[2] The concentration of the terrigenous element Fe was the highest determined at both locations, from 273.11 to 810.36 μg g^{-1} of dry lichens followed by Zn, Mn, Cu, Ba, Ti. The elevated values of Co and Cr were present, 1.17–2.74 and 1.40–1.70 μg g^{-1}, respectively. They also detected some amounts of Ag, Pb and Hg in some samples (1.63, 1.38, 1.38 μg g^{-1}, respectively). The results of this indicated that *P. sulcata* showed a tendency to accumulate Fe, Ni, and Ti despite the *E. prunastri* which preferentially concentrated Cu in both areas unlike the results in our research which show a slightly increased affinity *E. prunastri* towards the accumulation of Ni in the group with other fruticose species. These facts indicate that the interspecific differences in heavy metal accumulation are related to their morphological, anatomical, and physiological features, such as metal-absorbing abilities, surface area, size of intercellular space, permeability of cell membrane and *pH* values. The concentration of potential toxic heavy metals (Cr, Ag, Pb and Hg) were approximately the same in both species in both areas. Fe, Ni and Ti displayed a significant and Cu, Ag, Pb and Ag slight positive correlation with each other which indicates their association in lichens, while Ba with Co, Cr and Zn; Cu with Fe; Ni with Mn and Ti with Cu showed a slight negative.[13]

The element concentration data were submitted to the Pearson's correlation coefficient analysis, and the coefficients matrix is shown in Table S-I (available as Supplementary material) with a significant correlation at $p < 0.01$ and $p < 0.05$. It is evident that a good correlation exists between the elements which are naturally present in the soil and rocks, *i.e.*, alkali and alkaline earth metals, iron, silicon, aluminium, vanadium, copper, cobalt, chromium, nickel, *etc*. Pb does not correlate with any element, Zn correlates only with Si, and Cd only with Al, and they are assumed to be of dominantly anthropogenic origin, especially Pb. Stamenković *et al.*[2] noticed that some of the elements showed a negative correlation with each other, meaning they are probably competing for the same cell wall exchange sites. Bargagli *et al.*[10] found that the relationships between lithophilic elements from soil and rock dust particles were the most significant.

The hierarchical cluster analysis of the samples (Fig. 1) based on the content of all of the investigated elements shows the existence of several clusters and sub clusters which include different lichen species, family and genus; different sampling sites, thallus types and habitat types. A cluster analysis was performed on the data sets using Ward's method with squared Euclidean distances as a measure of similarity between the element concentrations.

Regardless of the obvious involvement of all these factors on the content of minerals, the following observations are evident. The greatest similarity exists between the samples belonging to the same family. All five members of the *Parmeliaceae* family are in the same cluster, in two close sub clusters. Also, all the species in this cluster which belong to the epiphytic lichens grow on the barks as same substrate. The remaining species are classified in the other two clusters and their common features are also substrate, soil, or rocks. It can be concluded that the species of lichens and at the same time type of habitant are the main factors which influence the mineral content. The hierarchical cluster analysis of the samples (Fig. 2) based on the content of heavy metals only shows a greater similarity between different types of samples, from different locations and substrates, which may indicate the absence of some pronounced pollution.

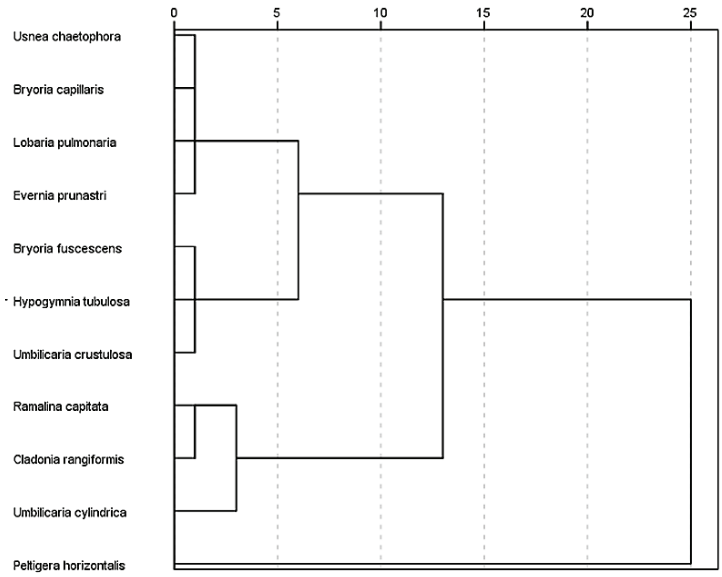

Fig. 1. Hierarchical dendrogram for investigated lichens based on all metals' contents.

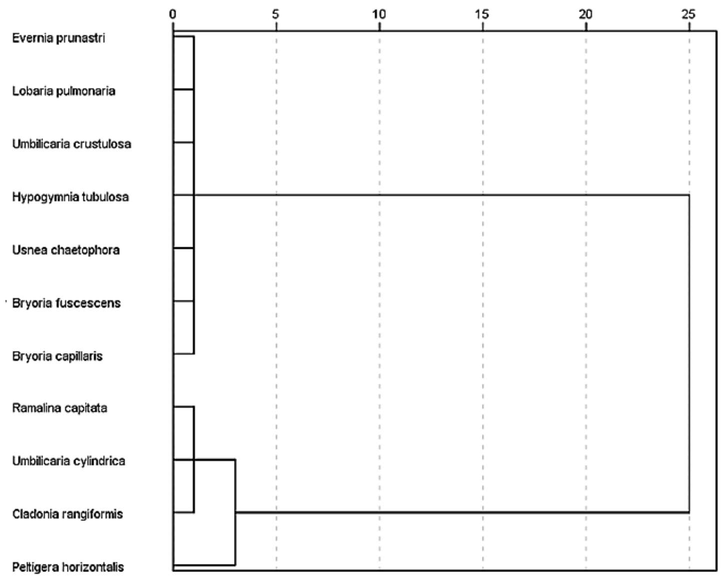

Fig. 2. Hierarchical dendrogram for investigated lichens based on heavy metals' contents.

Stamenković *et al.*[2] found the distinction between the soil origin, *i.e.*, lithogenic (Mn, Cu and Ti) and the atmospheric elements (Ni, Co, Cr, Ag, Pg and Hg) under the anthropogenic influence and the long-distance atmospheric transport of particulates from vehicle exhaust emissions using cluster analysis. Mn and Cu can partly come from fertilizers and manures.

CONCLUSION

The obtained results show that the most common macroelements are calcium and potassium while iron, manganese, zinc, and barium are the most abundant trace elements. Also, *P. horizontalis* has the highest content of most elements while *B. capillaries* contains the smallest quantities of most elements. The results of this research as well as the results of other authors' research point to the fact that many factors influence the content of elements in lichen samples such as: species, family, genus, thallus type, metal bio-absorbing capabilities of lichens, sampling site, habitat type, weather conditions, state of environmental especially pollutants present in atmosphere. Based on the results of the hierarchical cluster analysis, it can be concluded that the species of lichens, and at the same time, the type of habitant, *i.e.*, substrate (soil, rocks, barks) are the main factors which influence the mineral content of the investigated lichens.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11647, or from the corresponding author on request.

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ИЗВОД

ЕЛЕМЕНТАЛНИ САСТАВ ОДАБРАНИХ ВРСТА ЛИШАЈЕВА КОЈИ РАСТУ НА БАЛКАНСКОМ ПОЛУОСТРВУ

СНЕЖАНА ТОШИЋ, АЛЕКСАНДРА ПАВЛОВИЋ, ИВАНА ДИМИТРИЈЕВИЋ, ИВАНА ЗЛАТАНОВИЋ, ВИОЛЕТА МИТИЋ и ГОРДАНА СТОЈАНОВИЋ

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Коришћењем оптичке емисионе спектрометрије са индуктивно спрегнутом плазмом (ICP-OES) одређен је садржај 19 елемената у 11 различитих врста лишајева, шест фрутикозних (*Bryoria capillaris*, *Bryoria fuscescens*, *Cladonia rangiformis*, *Ramalina capitata*, *Usnea chaetophora* и *Evernia prunastri*) и пет фолиозних (*Hypogymnia tubulosa*, *Lobaria pulmonaria*, *Peltigera horizontalis*, *Umbilicaria cylindrica* и *Umbilicaria crustulosa*), сакупљених са пет природних станишта на Балканском полуострву (Србија и Бугарска). Међу макронутријентима, највећи садржај је забележен за Ca и K, док су Mg и Na заступљени у најмањој количини. Fe, Mn, Zn и Ba су били најзаступљенији елементи у траговима за разлику од Cd и Co чија је концентрација била најнижа.

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