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Epiphytic lichen *Flavoparmelia caperata* as a sentinel for trace metal pollution

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Abstract: The widely spread lichen specie *Flavoparmelia caperata* was used in a biomonitoring study for atmospheric trace metal pollution in natural ecosystems in south-eastern Serbia. The concentration and distribution pattern of 21 metals in lichens were determined by inductively coupled plasma atomic emission spectrometry. The difference observed between metal deposition in the peripheral and central parts of lichen thalli reflected air quality changes in the last and previous years. These findings were confirmed with principal component analysis. The study demonstrated the accumulation of Ba, K, Mg, Na, Tl and Zn in the peripheral parts of thalli, while As, B, Cd, Cr, Cu, Fe, Ga, In, Li, Ni, Pb and Se were concentrated in the central parts of thalli.

Keywords: lichen; *Flavoparmelia caperata*; trace metals; sentinel; biomonitoring.

INTRODUCTION

Biological monitoring is an effective way of detecting early changes of the environment.¹ Species capable of accumulating pollutants are considered as sentinels. Accumulation of trace metals causes chronic changes to ecosystems and permanent damage to the health of biota and thus must be carefully monitored. Urban, industrial and traffic heavy metal pollution has had a serious impact on distance natural oasis, such as National Parks and Special Nature Reserves.

Lichens represent unique life forms – symbioses between fungi (mycobionts) and algae and/or cyanobacteria (photobionts). The lack of a waxy cuticle and

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long life span result in the absorption of pollutants across the entire thalli surface. Epyphitic lichens as rootless plants show no substantial metal uptake from substrates and thus their metal content is obtained from aerial supplies only (*i.e.*, wet and dry atmospheric deposition).² Metal accumulation in lichens is in correlation with their environmental levels^{3–5} and spatial- and/or temporal-deposition patterns were demonstrated.⁶ Additionally, intra- and inter-specific variability in metal accumulation in lichens and a relationship between the metal content and the age of the thalli was observed.^{7,8}

In this study, the potential of a widely distributed lichen species, *Flavoparmelia caperata* (L.) Hale, as a bioindicator and bioaccumulator of trace metal pollutants was investigated. The study was performed at 3 different natural ecosystems: north-western (near the village Cerje), south-eastern (near the village Vlase) and north-eastern (Jelašnička Gorge) from the biggest urban and industrial centre of south-eastern Serbia Niš (approximately 350.000 inhabitants). The importance of the last location, Jelašnička Gorge, which has the status of a Special Nature Reserve, should be emphasized. This study is the first attempt to evaluate the anthropogenic impact on this protected area. Lichen samples were analyzed for their trace metal contents. The composition data were submitted to principal component analysis and interpreted.

EXPERIMENTAL

Sampling and sample preparation

The study was performed in natural ecosystems of south-eastern Serbia. The collection sites were: Jelašnička Gorge (330 m altitude), Vlase (350 m altitude) and Cerje (600 m altitude) in the vicinity of a road (2, 500 and 2000 m, respectively, Fig. 1).

The nearest urban and industrial area is the city of Niš (approximately 350000 inhabitants). The climate is a moderate continental with a mean annual rainfall of 543.3 mm, a mean annual temperature of 11.5 °C, and a mean annual relative humidity of approximately 69 %. The prevailing winds are north-westerly in winter and north-easterly and easterly in summer. The wind rose is shown in Fig. 1.

Foliose lichen *Flavoparmelia caperata* (L.) Hale (syn. *Parmelia caperata* (L.) Ach.; common name: greenshield lichen) was collected in April 2009. Determination of lichen was performed using several standard method.^{9–11} The lichen material was sorted into two samples: peripheral and central, corresponding to peripheral and central parts of the thalli. The peripheral samples contained the outermost 3–4 mm of the thalli, distinguishable by a paler colour, absence of rhizinae and easy separation from the bark. These parts of the thalli are the most active physiologically and have a known age of approximately 1 year.⁵ The central samples included the inner, older parts of thalli. The samples were air-dried, homogenized and further analyzed.

Analysis of trace metals

Lichen samples (0.5 g) were digested with a mixture of 7 mL of concentrated HNO₃ and 1 mL of 30 % H₂O₂ in an Advanced Microwave Digestion System (Ethos 1, Milestone, Italy) under the following programme: heated up to 200 °C in 10 min and held for 10 min at that temperature. The digested samples were transferred into a volumetric flask (25 mL).

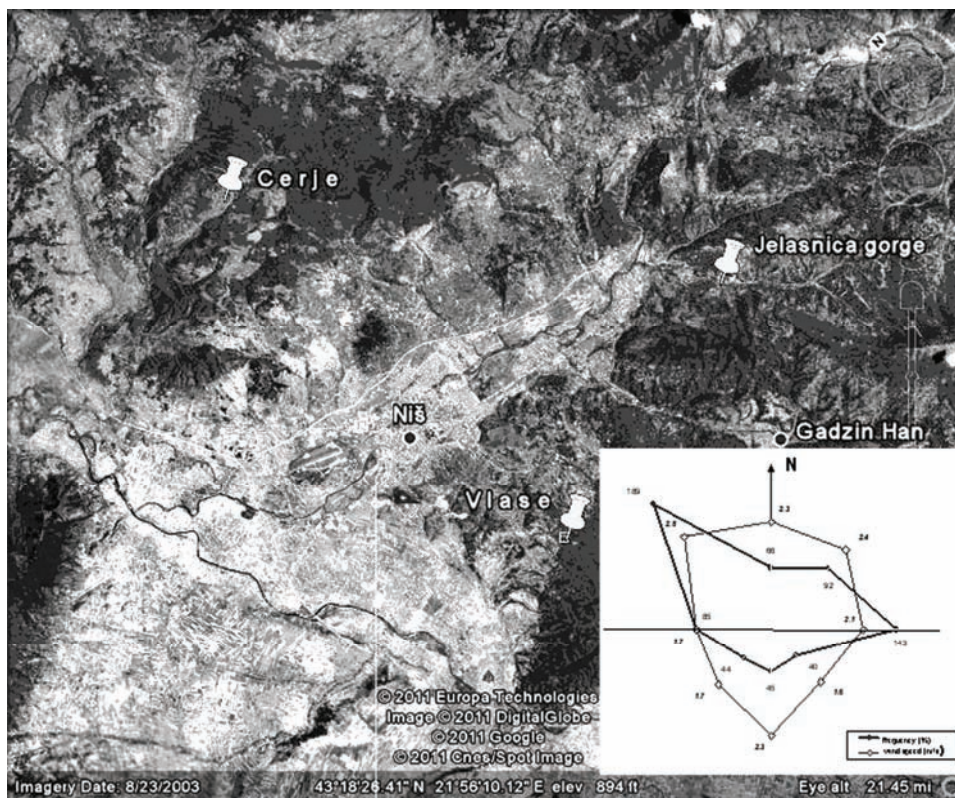


Fig. 1. Study area (Jelašnica Gorge, Vlasce and Cerje with respect to the city of Niš) with the wind rose for the area of Niš (data from the Republic Hydrometeorological Service of Serbia).

The contents of the metals in the samples were determined using an inductively coupled atomic emission spectrometer model 6500 Duo (Thermo Scientific, United Kingdom) equipped with a CID86 chip detector. The system was equipped with an integrated unit for hydride generation. This instrument operates sequentially with both radial and axial torch configurations. The entire system was controlled with Iteva software. The instrument operating conditions for the determination of the heavy metals in the lichen samples and selected emission lines are shown in Tables I and II, respectively.

To identify the relationship among the metals in samples and their possible sources, Pearson's correlation coefficient analysis and principal component analysis (PCA) were performed using PLS Toolbox, version 5.2.2 (Eigenvector Research), for MATLAB version 7.4.0.287 (R2007a) (MathWorks, Natick, MA, USA). The principal component analysis was performed using Varimax Normalized rotation.

RESULTS AND DISCUSSION

The bioaccumulation capacity for trace metals of lichen species *F. caperata* was determined by inductively coupled atomic emission spectrometry, Pearson's

correlation coefficient analysis and principal component analysis. The analyses of 21 metals in the three different ecosystems are given in Table III.

TABLE I. Instrument operating conditions for the determination of the concentration of heavy metals in the lichen samples

Spectrometer	ICAP 6500 (Thermo Scientific)
Nebulizer	Concentric
Spray chamber	Cyclonic
Radio frequency power, W	1150
Principal argon flow rate, L min ⁻¹	12
Auxiliary argon flow rate, L min ⁻¹	0.5
Nebulizer flow rate, L min ⁻¹	0.5
Sample flow rate, mL min ⁻¹	1.0
Detector	CID86

TABLE II. Selected emission lines

Element	λ / nm
Ag	328.0
As	193.7
B	249.6
Ba	455.4
Cd	228.8
Co	228.6
Cr	283.5
Cu	324.7
Fe	259.9
Ga	294.3
In	230.6
K	766.4
Li	670.7
Mg	280.2
Mn	257.8
Na	588.9
Ni	231.6
Pb	220.3
Sr	215.0
Tl	276.7
Zn	213.8
Hg	253.6
Se	196.0

Two types of lichen samples were used in this study: central and peripheral. The central samples represented the inner, older parts of the thalli that had been exposed longer to pollutants. The peripheral samples consisted of the outermost 3–4 mm of the thalli, with a maximum age of 1 year.^{12,13} Thus, the peripheral

samples reflected recent changes of the environment. The obtained data indicated differences in metal content of the central and peripheral samples of lichens.

TABLE III. Concentration of metals in central and peripheral parts of the thalli of *F. caperata* ($\mu\text{g g}^{-1}$ dry weight)

Element	Location					
	Jelašnička Gorge		Cerje		Vlase	
	Centre (sample 1)	Periphery (sample 2)	Centre (sample 3)	Periphery (sample 4)	Centre (sample 5)	Periphery (sample 6)
As	0.0037	0.0038	0.0036	0.0033	0.0031	0.0027
B	19.4303	9.5051	6.1341	6.9682	5.8576	6.7637
Ba	27.2333	21.5575	9.5615	24.5084	13.8145	15.2969
Cd	0.2558	0.1942	0.1681	0.1864	0.1310	0.1546
Co	0.1512	0.0822	1.0894	2.1865	0.0970	0.0856
Cr	1.9119	1.4572	3.7140	1.4670	1.7550	1.4879
Cu	8.3806	5.9573	15.3721	5.7307	6.1412	6.5179
Fe	476.6209	374.5863	645.2168	397.9597	493.2096	399.2086
Ga	0.1069	0.0000	0.0413	0.0000	0.0346	0.0177
In	0.0000	0.0000	0.0802	0.0341	0.0810	0.0000
K	2608.8040	2852.2720	2300.1670	2706.2540	2506.7160	3156.7050
Li	0.6932	0.5035	0.7566	0.4607	0.5617	0.4582
Mg	251.8673	315.6654	274.1127	313.7536	287.8818	373.2304
Mn	14.1271	13.5242	13.6761	12.4049	13.9091	15.6287
Na	71.1085	98.1346	89.5684	103.6953	78.8976	110.4994
Ni	1.2703	1.0977	1.5682	1.1916	1.4227	1.3011
Pb	22.3011	10.0742	12.0318	7.3955	9.8771	7.4223
Se	0.0043	0.0045	0.0042	0.0039	0.0036	0.0031
Sr	31.4610	23.8918	16.8445	11.3996	40.6104	32.6951
Tl	0.0000	0.6093	0.0000	0.9164	0.0000	1.5415
Zn	17.6053	17.0019	18.0832	19.5534	20.7641	24.2578

Furthermore, the trace metal content data were submitted to Pearson's correlation coefficients analysis (Table IV).

The correlation matrix was created from the values of the variables for all 21 metals in 6 samples. The Pair-Wise method was employed for the missing values. The results showed that these metals were strongly interrelated ($p < 0.01$), with correlation coefficients ranging from -0.790 to 0.990 at the 99 % confidence level. B, Cd, Ga and Pb evidently displayed significant positive correlations with each other (Table IV), which indicated their association in the analyzed samples. Other metals, such as Cr, Cu, Fe, Li and Ni, also showed significant correlations. The exceptions were the element pairs As–Zn and Se–Zn, with significant negative correlations and As, Mn and Zn without any observed correlations.

In order to better describe the relationship among the metals and/or samples, principal component analysis (PCA) was performed. The analytical data were represented in a multidimensional space with variables defining the axes, and

TABLE IV. Pearson correlations coefficient matrix for the metals concentrations

	B	Ba	Cd	Co	Cr	Cu	Fe	Ga	In	K	Li	Mg	Mn	Na	Ni	Pb	Sr	Tl	Zn	As
Ba	0.717																			
Cd	0.921	0.792																		
Co	-0.303	0.123	0.002																	
Cr	-0.135	-0.605	-0.068	0.186																
Cu	-0.046	-0.542	0.033	0.161	0.988															
Fe	-0.094	-0.615	-0.125	0.099	0.953	0.916														
Ga	0.795	0.232	0.603	-0.329	0.293	0.340	0.432													
In	-0.558	-0.684	-0.596	0.290	0.614	0.489	0.734	-0.048												
K	-0.019	0.271	-0.031	-0.282	-0.735	-0.658	-0.828	-0.390	-0.776											
Li	0.371	-0.246	0.324	-0.076	0.841	0.849	0.881	0.728	0.408	-0.786										
Mg	-0.523	-0.166	-0.479	-0.050	-0.487	-0.457	-0.601	-0.708	-0.376	0.854	-0.809									
Mn	0.073	-0.328	-0.175	-0.726	-0.064	0.001	-0.032	0.233	-0.310	0.511	-0.028	0.432								
Na	-0.591	-0.135	-0.379	0.317	-0.276	-0.243	-0.478	-0.824	-0.288	0.669	-0.706	0.896	0.100							
Ni	-0.311	-0.789	-0.423	0.020	0.819	0.767	0.916	0.313	0.789	-0.641	0.670	-0.332	0.201	-0.328						
Pb	0.915	0.427	0.796	-0.309	0.222	0.282	0.303	0.943	-0.211	-0.363	0.698	-0.760	0.067	-0.807	0.075					
Sr	0.179	-0.147	-0.210	-0.848	-0.299	-0.322	-0.082	0.366	-0.036	0.187	-0.025	0.022	0.663	-0.420	0.130	0.218				
Tl	-0.322	0.089	-0.219	0.109	-0.540	-0.478	-0.678	-0.600	-0.547	0.892	-0.802	0.945	0.341	0.900	-0.465	-0.620	-0.139			
Zn	-0.453	-0.353	-0.600	-0.134	-0.312	-0.311	-0.246	-0.273	-0.034	0.577	-0.522	0.745	0.640	0.494	0.149	-0.526	0.394	0.671		
As	0.504	0.359	0.651	0.062	0.315	0.333	0.227	0.298	-0.063	-0.504	0.533	-0.702	-0.547	-0.461	-0.175	0.561	-0.380	-0.621	-0.990	
Se	0.47	0.373	0.633	0.097	0.284	0.299	0.188	0.240	-0.066	-0.485	0.484	-0.672	-0.587	-0.419	-0.213	0.513	-0.414	-0.591	-0.992	0.998

projected into a few principal components (PCs) that were linear combinations of the original variables and described the maximum variation within the data.¹⁴ The obtained results clearly showed that Co, Mn and Sr had no impact. This was in accordance with the results of the determinations of the metals (Table III) as small differences in their values in different samples were found. Therefore, the PC analysis was realised without these metals, which resulted in a three-component model explaining 91.78 % of the data variation. The first PC comprised 49.75 % of the total data variability, and the cumulative variance explained by the first two components was 81.09 %. The addition of more PCs did not significantly change the classification of the analates described below. The score values for the samples, *i.e.*, their mutual projections, for the first two PCs are shown in Fig. 2.

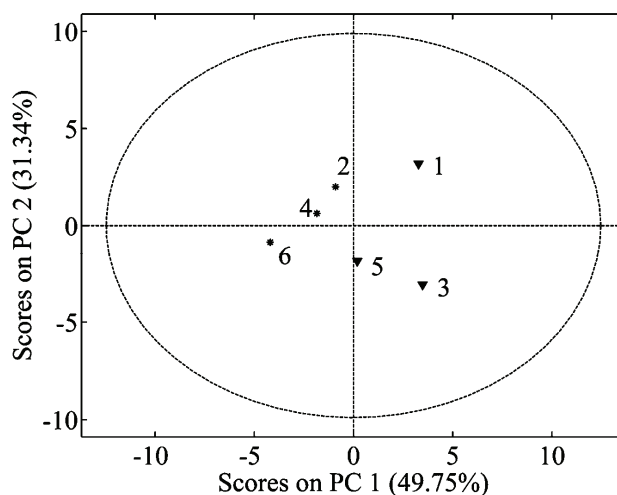


Fig. 2. Score values of the first and the second PC for the samples.

Additionally, the score values for the samples and metals for the first two PCs are shown in Fig. 3.

The first PC distinguished two separate groups of samples according to metals content and the age of the sampled lichen thalli. The first group was formed from samples 2, 4 and 6, obtained from the peripheral (younger) thalli parts, with a characteristic accumulation of Ba, K, Mg, Na, Tl and Zn. The second group contained samples 1, 3 and 5, and belonged to the central (older) thalli parts with higher concentrations of As, B, Cd, Cr, Cu, Fe, Ga, In, Li, Ni, Pb and Se. These findings revealed differences in the air quality in the last and previous years.

Compared to the location of sampling, it is obvious that the Special Nature Reserve, Jelašnička Gorge, from which sample 1 and 2 originated, is becoming polluted. The deposition of As, B, Ba, Cd, Ga, Pb and Se in sample 1 reflected

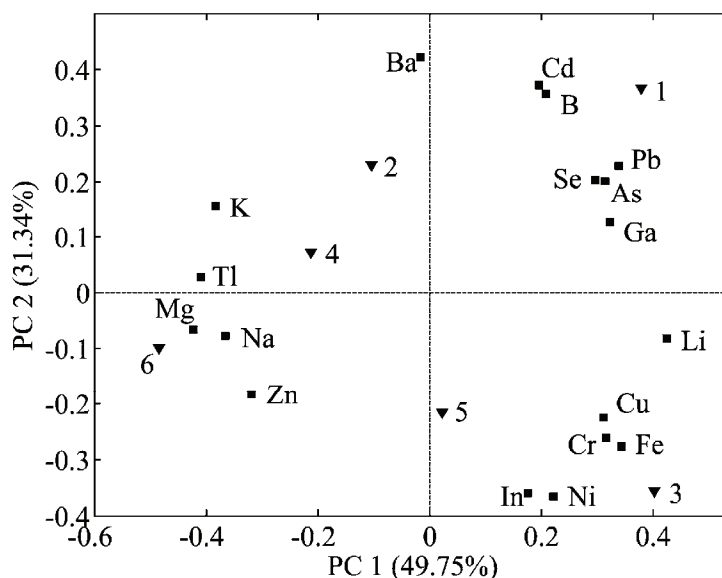


Fig. 3. Biplot of the first and the second PC for the samples and metals.

the vicinity of the road (2 m) and the towns Niš and Niška Spa at 15 and 3 km, respectively. The values of Pb in this sample were higher than the values reported for *F. caperata* near a motorway in Turkey in the study of Mendil *et al.* (2009).¹⁵ The high concentration of Ba, K and Tl in sample 2 could be considered as being due to a probable contribution of particles from roadway dust to the soil material, as previously suggested in some studies. The other two locations, Cerje and Vlase, with samples 3–6, showed presence of trace metals of crustal and anthropogenic origin. An accumulation of Cr, Cu, Fe, In, Li and Ni was noticed in samples 3 and 5. Higher concentrations of Ba, K and Tl were determined in sample 4, as in sample 2. Finally, increased concentrations of Mg, Na and Zn were observed in sample 6. The deposition of trace metals of crustal origin, such as Cr, Cu, Fe, In, K, Li, Mg and Na, implied a specific composition of the geological substrate, as well as the influence of vegetation, *i.e.*, the substrate from which the lichen samples were collected. Anthropogenic emission of trace metals (Ni, Cd, Cr, Cu, Pb, Zn) from motor vehicles is mainly from the combustion of the fossil fuels or abrasion of vehicle parts (tyres, paint, greases and catalysts).^{17,18} Although vehicles using unleaded petrol prevail, a high density of old vehicles using leaded petrol or diesel oil is still present on the Serbian roads. Diesel soot is the main source of emission of Zn and Cu in urban areas.¹⁶ Besides its utilization as an oil additive, Zn is well-known, together with Cd, as a rubber additive.¹⁶ Both Zn and Cd are atmophile elements subjected to a long-distance transport.¹⁹ Moreover, a part of Zn could be obtained from a supporting tree since higher

plants are known to release 20 % of the total Zn from natural sources.²⁰ Among lichen species, *F. caperata* is famous for its higher capacity for Zn and Cd uptake.⁷

Bearing in mind the low frequency of traffic on the road in close range of the location of lichen sampling, the high concentrations of Cu, Pb and B detected are surprising. This is probably due the specific wind rose (see Fig. 1) which put this area under indirect impact of huge metal complexes, situated 10–15 km north-westerly (at the periphery of the city of Niš). Further research and monitoring should be performed in order to obtain the correct conclusions.

CONCLUSION

This study is the first biomonitoring study of atmospheric trace metal pollution in natural ecosystems in Serbia. The lichen species *Flavoparmelia caperata* was chosen because of its wide distribution and proven sentinel functions. Its peripheral parts were used as sensors of annual changes in the environment, while the central, older parts of the thalli were treasurers of the pollutants from the past. The deposition pattern of trace metals in the studied samples probably reflected the volume of traffic and the types of engines on the nearby roads, the activity of industrial complexes, soil and substrate compositions and the prevailing wind directions. This study is evidence that there is a need for continuous biomonitoring surveys of the studied areas, especially, the Special Nature Reserve Jelašnička Gorge.

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ИЗВОД

ЕПИФИТНИ ЛИШАЈ *Flavoparmelia caperata* КАО ИНДИКАТОР ЗАГАЂЕЊА МЕТАЛИМА У ТРАГОВИМА

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Широко распрострањена врста лишајева *Flavoparmelia caperata* је коришћена у биомониторингу атмосферског загађења металима у траговима у природним екосистемима на југоистоку Србије. Концентрација и дистрибуција 21 метала у лишајевима је одређена атомско-емисионом спектрометријом са индуктивно спрегнутом плазмом. Примећена разлика у нагомилавању метала између периферних и централних делова талуса лишајева је приписана разликама у квалитету ваздуха последње и претходних година. Ови налази су потврђени анализом главних компоненти (РСА). Истраживање је пока-

зало акумулирање Ba, K, Mg, Na, Tl и Zn у периферним деловима талуса, односно As, B, Cd, Cr, Cu, Fe, Ga, In, Li, Ni, Pb и Se у централним деловима талуса.

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