

## BIOLOGICAL INDICATION OF HEAVY METAL POLLUTION IN THE AREAS OF DONJE VLASE AND CERJE (SOUTHEASTERN SERBIA) USING EPIPHYTIC LICHENS

S. S. STAMENKOVIĆ<sup>1</sup>, TATJANA LJ. MITROVIĆ<sup>1\*</sup>, V. J. CVETKOVIĆ<sup>1</sup>, N. S. KRSTIĆ<sup>2</sup>,  
RADA M. BAOŠIĆ<sup>3</sup>, MARIJA S. MARKOVIĆ<sup>1</sup>, N. D. NIKOLIĆ<sup>2</sup>, V. LJ. MARKOVIĆ<sup>4</sup> and M. V. CVIJAN<sup>5</sup>

<sup>1</sup> Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, 18000 Niš, Serbia

<sup>2</sup> Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, 18000 Niš, Serbia

<sup>3</sup> Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Serbia

<sup>4</sup> Department of Physics, Faculty of Sciences and Mathematics, University of Niš, 18000 Niš, Serbia

<sup>5</sup> Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia

**Abstract** - The performance of two epiphytic lichen species (*Evernia prunastri* (L.) Ach. and *Parmelia sulcata* Taylor) as bioindicators of heavy metal pollution in natural areas around the city of Niš (southeastern Serbia) were evaluated. The concentration of 19 heavy metals in lichen samples was measured by inductively coupled plasma-optical emission spectroscopy. For the majority of the elements the concentrations found in *Parmelia sulcata* Taylor were higher than in *Evernia prunastri* (L.) Ach. In addition, interspecific differences in heavy metal accumulation between *Evernia prunastri* (L.) Ach. and *Parmelia sulcata* Taylor are observed. *Parmelia sulcata* Taylor showed a tendency to accumulate Fe, Mn, Ni and Ti while *Evernia prunastri* (L.) Ach. preferentially concentrated Cu on both locations. A clear distinction between lithogenic (Mn-Cu-Ti) and atmospheric elements (Ni-Co-Cr-Ag-Pb-Hg) was achieved by cluster analysis.

**Key words:** Lichens, *Evernia prunastri* (L.) Ach., *Parmelia sulcata* Taylor, bioindication, air pollution, bioaccumulation, heavy metals, ICP OES, hierarchical cluster analysis.

### INTRODUCTION

The use of living organisms in the study of environmental quality is widely accepted. Numerous studies have employed lichens, mosses and vascular plants in the assessment of environmental pollution, either as bioindicators/biomonitoring organisms of air quality or as bioaccumulators of atmospheric pollutants. In general, an organism or part of an organism or a community of organisms that is used in identification and qualitative determination of environmental pollutants is referred to as a bioindicator, whereas an organism or part of an organism or a community of organisms that contains the quantitative aspects of the environ-

ment is referred to as biomonitor (Conti and Cecchetti, 2001; Markert, 2007; Markert et al., 2003; Wolterbeek, 2002). A biomonitor is always a bioindicator as well, but a bioindicator does not necessarily possess all the requirements of a biomonitor. An accumulation bioindicator/biomonitor is an organism that accumulates one or more elements and/or compounds from the environment, whereas an impact bioindicator/biomonitor is an organism that demonstrates specific or unspecific response to exposure to elements and/or compounds (Markert, 2007).

Lichens are cryptogams that occur in all terrestrial ecosystems, including extreme ones. They

represent unique life forms – a symbiosis between a fungus (mycobiont) and an alga and/or cyanobacterium (photobiont). Having a simple anatomy (without waxy cuticle, stomata and root system) and a large surface area to volume ratio, lichens rely directly on atmospheric deposition for nourishment. Lichens readily accumulate air pollutants in their tissues without significant adverse effects on their survival or growth. Lichens were recognized as potential indicators of air pollution as early as the 1860s in Britain and the rest of the Europe. Nowadays, they dominate among organisms for the evaluation of air quality (Aslan et al., 2011; Guttova et al., 2011; Klos et al., 2011; Mendil et al., 2009; Paoli et al., 2012; Vicol, 2010). Lichens, as slow-growing, long-lived organisms, are ideal for long-term surveys of air pollution (Geiser and Neitlich, 2007; Jovan and McCune, 2005; Loppi et al., 2003; Paoli et al., 2004).

Heavy metal pollution as a consequence of increased urbanization and expansion of industrial activities is a serious environmental problem. Most heavy metals are essential elements for living organisms, but in excessive amounts they are generally harmful. Being non-biodegradable, heavy metals tend to accumulate in living systems and have a long half-life in soil. In plants, through which heavy metals enter the food chain, they can lead to morphological and physiological changes (high affinity for the –SH group of some enzyme systems, free radical production, etc.) (Goyer and Clasen, 1995). The accumulation of heavy metals in plants depends upon many factors, such as the availability of metals, plant characteristics, climate conditions, etc. The specificities of lichen anatomy and their dependence on mineral nutrients from wet atmospheric deposition (precipitation and occult precipitation such as fog and dew) and dry atmospheric deposition (sedimentation, impaction and gaseous absorption) allows heavy metals to be absorbed over the whole thallus surface (Knops et al., 1991; Nash and Gries, 1995). Furthermore, intracellular spaces of the lichen thallus can accumulate and retain heavy metals by trapping insoluble particles, extracellular ion exchange processes, adsorption and active uptake (Sloof, 1995; Richardson, 1995). Lichens tolerate high concentra-

tions of heavy metals by sequestering them extracellularly as oxalate crystals or lichen acid complexes (Richardson, 1995). The concentrations of heavy metals in lichen thalli are directly correlated with their atmospheric concentrations or depositions (Brunialti et al., 2002; Purvis, 1996; van Dobben et al., 2001). Thus, lichens are exploited for the monitoring of the spatial and/or temporal deposition patterns of heavy metals (Loppi et al., 2004). Generally, foliose (leaf-like) and fructose (shrub-like) lichens are chosen for heavy metal deposition studies over crustose (crust-forming) lichens since they are easily separated from the substrate. The most commonly used are two foliose lichen species from the family Parmeliaceae - *Parmelia sulcata* Taylor and *Flavoparmelia caperata* (L.) Hale (*synonym: Parmelia caperata* (L.) Ach.) (Szczepaniak and Biziuk, 2003). Numerous studies are based on these two *Parmelia* species, using them as biomonitors of heavy metal pollution in the atmosphere either by sampling of the organisms *in situ* or by using the transplantation technique (Boamponsem et al., 2010; Frigoli and Quartieri, 1999; Horvat et al., 2000; Koz, Celik and Celik, 2010; Loppi and Bonini, 2000; Loppi and Pirintsos, 2003; Loppi et al., 2004; Marques et al., 2004; Marques et al., 2005; Mendil et al., 2009; Nimis, Andreussi and Pitao, 2001; Paoli et al., 2012; Suetjoviene, 2010). The other epiphytic lichen frequently used in bioindicator/biomonitoring studies is the widely spread fructose species *Evernia prunastri* (L.) Ach. (Cansaran-Duman, Atakol and Aras, 2011; Cercasov et al., 2002; Conte et al., 2004; Jovan and McCune, 2005; Loppi et al., 1998; Paoli and Loppi, 2008; Pacheco et al., 2008). Some studies included *Parmelia sulcata* and *Evernia prunastri* together with other lichen species (Conti and Cecchetti, 2001; Egilli et al., 2003; Geiser and Neitlich, 2007; Guttova et al., 2011; Stamenković and Cvijan, 2003; Vokou, Pirintsos and Loppi, 1999). In addition, Nimis and Pittao (2001), by analyzing the performance of two lichen species (*Parmelia caperata* (L.) Ach. and *Xanthoria parietina* (L.) Th. Fr.) demonstrated intra- and interspecific variability in the heavy metal accumulation in lichens.

The aim of this study was to perform biomonitoring of air quality via examination of heavy metal

concentrations in two epiphytic lichen species, *Evernia prunastri* and *Parmelia sulcata*. The study was performed at two different areas, Cerje and Donje Vlase. These natural areas were chosen because they are relatively close to the biggest urban and industrial center of southeastern Serbia, the city of Niš, and are considered to be spared from significant anthropogenic impact (motor vehicle traffic, industry etc).

MATERIALS AND METHODS

Sample location

The study was performed in two natural areas of southeastern Serbia: Donje Vlase (350 m altitude) and Cerje (600 m altitude) (Fig. 1). The selected areas have similar vegetation, geological and climate features: deciduous forests on calcareous bedrocks are under moderate continental climate conditions. The nearest urban and industrial area is the city of Niš (approximately 350,000 inhabitants) (Fig. 1). Donje Vlase is located 15 km south of Niš, and Cerje is situated 15 km north of Niš. The climate in Niš is valley type of moderate continental, with a mean annual rainfall of 543.3 mm, a mean temperature of 11.5°C, and a mean annual relative humidity of 69%. The

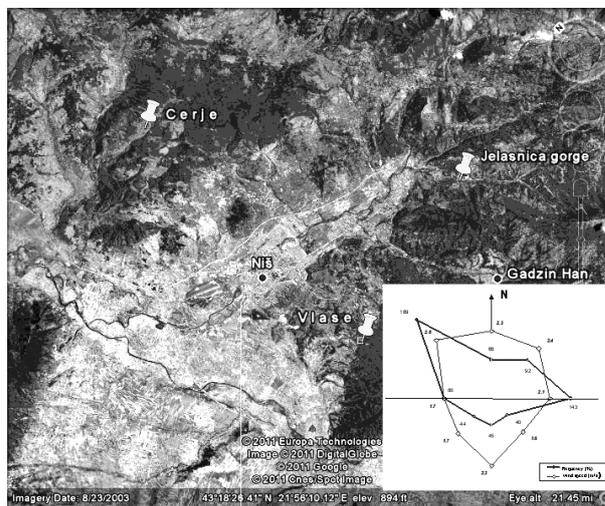


Fig. 1. Geographical location of Cerje and Donje Vlase with wind roses.

prevailing winds are northwesterly in winter, and northeasterly and easterly in summer. Wind roses are shown in Fig. 1.

Data on the average concentrations of heavy metal pollutants (Ni, Pb, Cd and Cr) at 6 different sites in the city of Niš from the Republic Hydrometeorological Service of Serbia are given in Table 1.

Table 1. Heavy metal pollution in the city of Niš (data from Republic Hydrometeorological Service of Serbia).

Site	Concentration [ $\mu\text{g}/\text{m}^2/\text{day}$ ]															
	Ni				Pb				Cd				Cr			
	X	Min	C50	Max	X	Min	C50	Max	X	Min	C50	Max	X	Min	C50	Max
1	9.0	2.5	6.5	24.26	8.3	1.41	3.86	33.08	2.1	1.03	2.03	3.5	9.7	1.07	5.5	35.6
2	18.6	2.7	10.5	127.0	6.6	1.7	5.0	19.5	1.8	1.0	1.8	3.3	12.4	1.0	7.5	43.3
3	10.5	2.1	7.4	45.9	3.1	1.3	2.4	8.8	1.7	0.7	1.7	2.4	12.9	1.0	2.7	64.3
4	10.2	1.2	5.2	37.0	10.3	1.2	5.2	25.1	2.6	1.0	2.3	5.2	29.1	1.6	11.8	182.9
5	8.9	1.9	4.1	22.1	8.6	1.6	5.3	30.0	5.2	1.8	4.1	10.3	14.8	1.6	8.3	80.9
6	8.0	1.0	7.3	19.3	10.7	1.4	7.7	26.8	2.1	0.6	1.4	8.8	20.7	2.0	11.3	76.2

X – mean annual concentration  
 Min – minimal concentration  
 Max –maximal concentration

### Sample description

Fructose lichen species *Evernia prunastri* (L.) Ach. (common name: oakmoss) and foliose lichen species *Parmelia sulcata* Taylor (common name: green shield lichen) used in this study were collected in April 2009. Determination of the lichens was performed by using several standard keys (Boqueras, 2000; Dobson, 2005; Wirth, 1995). Lichen specimens were obtained from a height of 1.5-2 m above ground, on the side of the trunks of *Prunus domestica* and *Salix sp.* not affected by stemflow. The collection of many samples from 30 trunks was performed in one day at two sampling areas. The samples from the same area were washed with deionized water, mixed together and air-dried. Dried samples were homogenized in agate mortars and prepared for inductively coupled plasma-optical emission spectroscopy (ICP-OES).

### Sample preparation

One mg of each sample was burned in an oven ( $T = 300\text{-}550^\circ\text{C}$ ) to remove organic matter. The inorganic residue was demineralized with 6M HCl ( $T=80^\circ\text{C}$ , 10-12 h). The samples were then centrifuged, rinsed with distilled water and evaporated. Pellets were re-suspended with 25 mL of deionized water.

### Inductively coupled plasma-optical emission spectroscopy (ICP-OES)

The content of heavy metals in the lichen samples was analyzed by Spectroflame ICP-OES instrument using Ar as the plasma gas in the Laboratory for Physical Chemistry in the Institute of Nuclear Sciences "Vinča", Belgrade.

### Reagents and chemicals

All chemicals used in this study were analytical grade, obtained from Merck (Darmstadt, Germany). Ultra pure water was prepared by the reverse osmosis water purification system TKA-7-DEN (TKA Wasser aufbereitungs systeme GmbH, Niederelbert, Germany).

### Statistical analysis

Heavy metal concentration data were submitted to Pearson's correlation coefficient analysis; hierarchical cluster analysis was performed using PLS Toolbox version 5.2.2 (Eigenvector Research) for the MATLAB version 7.4.0.287 (R2007a) (MathWorks, Natick, MA, USA). Cluster analysis was performed on the data sets using the between-groups linkage based on correlation coefficients (Pearson coefficient) by pairwise deletion. Normalized data set was analyzed by Ward's method using squared Euclidean distances as a measure of similarity between metal concentrations.

## RESULTS AND DISCUSSION

The ubiquitous lichen species *Evernia prunastri* (L.) Ach. and *Parmelia sulcata* Taylor were collected from two natural areas, Cerje and Donje Vlase, 15 km from the city of Niš (southeastern Serbia). The concentration of 19 heavy metals in the lichen samples were determined by inductively coupled plasma-optical emission spectroscopy and are presented in Table 2.

The following heavy metals were detected in all samples: Fe, Mn, Zn, Cu, Ni, Ti, Co, Cr, Ba, Ag, Pb and Hg. For the majority of the elements the total amounts found in *Parmelia sulcata* Taylor were higher than in *Evernia prunastri* (L.) Ach. The concentration of the terrigenous element Fe was the highest determined at both locations (Cerje: 374.45  $\mu\text{g/g}$  in *Evernia prunastri* (L.) Ach. and 810.36 in *Parmelia sulcata* Taylor and Donje Vlase: 273.11  $\mu\text{g/g}$  in *Evernia prunastri* (L.) Ach. and 714.40 in *Parmelia sulcata* Taylor). The second highest values determined were concentrations of Ba in *Evernia prunastri* (L.) Ach. from Donje Vlase (262.99  $\mu\text{g/g}$ ).

In Table 2, a striking interspecific variability in Fe accumulation is displayed: *Parmelia sulcata* Taylor concentrated 2.5-fold more Fe than *Evernia prunastri* (L.) Ach. In addition, *Parmelia sulcata* Taylor showed a tendency to accumulate Mn, Ni and Ti as well. *Evernia prunastri* (L.) Ach. preferentially concentrated Cu in both areas. The existence of the in-

**Table 2.** The concentration of heavy metals in lichen samples.

Metal	Concentration [ $\mu\text{g/g}$ dry lichen] mean $\pm$ SD			
	Cerje		Donje Vlase	
	<i>Evernia prunastri</i>	<i>Parmelia sulcata</i>	<i>Evernia prunastri</i>	<i>Parmelia sulcata</i>
Fe	374.45 $\pm$ 16.41	810.36 $\pm$ 63.99	273.11 $\pm$ 23.38	714.40 $\pm$ 10.30
Mn	15.07 $\pm$ 0.53	18.27 $\pm$ 0.99	15.67 $\pm$ 0.81	40.63 $\pm$ 0.69
Zn	65.94 $\pm$ 28.39	55.01 $\pm$ 3.80	30.27 $\pm$ 0.69	54.42 $\pm$ 3.22
Cu	21.23 $\pm$ 13.90	8.41 $\pm$ 1.21	13.42 $\pm$ 1.26	8.42 $\pm$ 0.52
Ni	2.42 $\pm$ 0.18	3.12 $\pm$ 0.24	1.63 $\pm$ 0.25	5.20 $\pm$ 2.61
Ti	9.31 $\pm$ 0.57	17.70 $\pm$ 1.87	7.56 $\pm$ 0.21	17.92 $\pm$ 0.88
Co	2.74 $\pm$ 1.69	1.84 $\pm$ 0.14	1.17 $\pm$ 0.03	2.48 $\pm$ 1.38
Cr	1.70 $\pm$ 0.23	1.60 $\pm$ 0.14	1.40 $\pm$ 0.20	1.65 $\pm$ 0.38
Ba	4.13 $\pm$ 0.18	7.12 $\pm$ 0.37	262.99 $\pm$ 24.51	21.39 $\pm$ 1.43
Ag	1.63 $\pm$ 0.40	<1.20	<1.17	<1.24
Pb	1.38 $\pm$ 0.32	<1.20	<1.17	<1.24
Hg	1.38 $\pm$ 0.32	<1.20	<1.17	<1.24

Not determined in the samples: Y, Zr, Hf, Nb, W, V, Cd.

**Table 3.** The Pearson's correlation coefficients matrix for heavy metals in lichen samples.

	Fe	Mn	Zn	Cu	Ni	Ti	Co	Cr	Ba	Ag	Pb
Mn	-0.234										
Zn	0.404	-0.120									
Cu	-0.745	0.298	0.294								
Ni	0.735	-0.825	0.399	-0.573							
Ti	0.985	-0.393	0.378	-0.774	0.835						
Co	0.254	-0.445	0.920	0.338	0.534	0.293					
Cr	0.446	-0.305	0.982	0.211	0.547	0.451	0.963				
Ba	-0.673	0.197	-0.948	0.023	-0.590	-0.649	-0.839	-0.952			
Ag	-0.343	0.076	0.720	0.870	-0.163	-0.363	0.743	0.665	-0.463		
Pb	-0.213	-0.072	0.803	0.777	0.017	-0.215	0.850	0.774	-0.578	0.984	
Hg	-0.213	-0.072	0.803	0.777	0.017	-0.215	0.850	0.774	-0.578	0.984	1

\* Yellow highlights represent positive correlation while green highlights represent negative correlation.

terspecific differences in the accumulation ability of heavy metals is known from previous studies (Nimis and Pittao, 2001). However, no data were available until now on possible interspecific differences between *Evernia prunastri* and *Parmelia sulcata*. We speculate that the interspecific differences in heavy metal accumulation between *Evernia prunastri* and *Parmelia sulcata* 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 level. Concentrations of Cr, Ag, Pb and Hg were approximately the same in both species in both areas.

Furthermore, the Pearson's correlation coefficients analysis of heavy metals concentrations was performed. The obtained Pearson's correlation coefficients matrix is shown in Table 3 with significant correlation at  $p < 0.01$ .

Fe, Ni and Ti displayed significant positive correlations with each other, which indicate their association in lichens. Positive correlations were noticed between Zn, Co, Cr, Ag, Pb and Hg, as well as Cu, Ag, Pb and Hg. On the other hand, Ba and Co, Cr, Zn as well as following pairs of metals Cu-Fe, Ni-Mn, Ti-Cu, showed negative correlation with each other, meaning they are probably competing for the same cell wall exchange sites (Loppi and Pirintsos, 2003).

Finally, heavy metal concentration data were submitted to cluster analysis using the correlation coefficient as similarity measure and the weighted pair group as clustering algorithm. Cluster analysis was performed on all heavy metals except Fe and Ba (Fig. 2). In order to determine the presence of outliers among the heavy metals and to overview the data for similarities and dissimilarities, PCA was applied to the entire data set. Fe and Ba were observed outside the Hotelling  $T^2$  ellipse, suggesting they are the outliers. Therefore, Fe and Ba were excluded from further analysis. No other *outliers* were detected after this *exclusion*.

Three clusters were obtained: (1) Mn-Cu-Ti, (2) Ni-Co-Cr-Ag-Pb-Hg, and (3) Zn (Fig. 2). Some

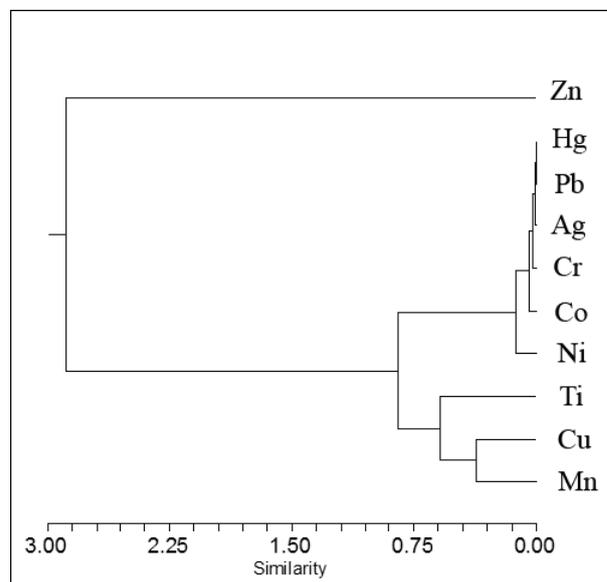


Fig. 2. Cluster analysis of heavy metals in lichen samples

authors suggest that these findings reflect possible common sources for each group of elements (Adamo et al., 2003; Loppi and Pirintsos, 2003; Boamponsem et al., 2010). Determination of the sources of heavy metal pollution is more complicated in practice. Some heavy elements originate from two or more sources. The first cluster (Mn-Cu-Ti) reflects particulates of soil origin (i.e. lithogenic elements) (Fig. 2). Mn and Cu are partly terrigenous and partly associated with farming (fertilizers and manures) (de Vries et al., 2002). 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 and other plants. Dongarra et al. (2003) defined roadway dust as a mixture of 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. He suggested that Pb and Cu from anthropogenic emissions and Br from maritime-derived aerosol are emitted in a gaseous form which condenses on suspended particulate matter. Paoli et al. (2012) confirmed that the high concentrations of lithogenic elements in lichens are associated with high levels of depositions from airborne soil dust (Fe, Cd, Cr, Ni) and decreased lichen diversity around solid waste

landfills in Italy, Mexico and Greece. The increase in lithogenic elements in lichens in this study is connected to excavation works on the enlargement of a dumping area and hyperproduction of airborne emissions. Aslan et al. (2011) showed contamination of Cu, Pb, Cr, Cd and Ni in roadside soils from traffic activities.

The second cluster (Ni-Co-Cr-Ag-Pb-Hg) in our cluster analysis indicated anthropogenic influence and long-distance atmospheric transport of particulates from vehicle exhaust emissions (Fig. 2). Mendil et al. (2009) reported the maximum concentrations of these heavy metals near the Sivats/Tokat motorway (Ni 79.6 µg/g, Cr 3.8 µg/g, Pb 6.5 µg/g, Cu 25.6 µg/g). Ni is ubiquitous in nature and occurs mainly in the form of sulfide, oxide and silicate minerals. Higher concentrations of Ni were observed near petrochemical refineries where crude oil is enriched with Ni. Apart from petrol and abrasion of vehicle parts, contamination with Ni could originate from industrial complexes for electroplating, steel and alloy production (Ng, Tan and Obbard, 2005). Cr pollution is caused by the erosion of vehicle engine and body, extensive road marking with yellow lead chromate paint and some industrial activities (Kord and Kord, 2011). Pb is a well-known pollutant derived mostly from emissions from motor vehicles using leaded petrol and also from the manufacture of batteries, metal products, paints, ceramic products (Ng, Tan and Obbard, 2005).

The third cluster (Zn) in our analysis (Fig. 2) could be considered a unique category, combining an atmospheric transportable element from vehicle exhaust emissions and agricultural chemicals (fertilizers and pesticides) on one side, and natural leaching from the supporting tree on the other side (Adamo et al., 2003; Dongarra et al., 2003; Kord and Kord, 2011; Loppi et al., 1998; Loppi and Pirintsos, 2003; Nriagu 1979; ).

## CONCLUSIONS

Epiphytic lichens are valuable bioindicators/biomonitors of air quality. Ours is a preliminary study for

the assessment of atmospheric pollution in south-eastern Serbia. The comparison of the heavy metal accumulation of two lichen species *Evernia prunastri* (L.) Ach. and *Parmelia sulcata* Taylor revealed inter-specific variability which could be assigned to their morphological, anatomical and physiological differences. *Evernia prunastri* preferentially concentrated Cu and *Parmelia sulcata* showed a tendency to accumulate Fe, Mn, Ni and Ti in both study areas. Our study revealed higher concentrations of Fe and Zn in the lichen samples from Cerje and higher concentrations of Ba those from Donje Vlase. Heavy metal pollutants probably originated from wind-transported particulate emissions from local farming (applied pesticides and fertilizers), traffic, landfills and industries near the city of Niš and from the city itself. Our goal is long-term monitoring at local and regional levels using the lichen species tested.

*Acknowledgments* - This research was supported by the Ministry of Science and Education of the Republic Serbia, projects III41018, OI 171025, 172017 and III41017.

## REFERENCES

- Adamo, P., Giordano, S., Vingiani, S., Castaldo Cobiانchi, R. and P. Violante (2003). Trace elements accumulation by moss and lichen exposed in bags in the city of Naples (Italy). *Environ. Pollut.* **122**, 91-103.
- Aslan, A., Cicek, A., Yazici, K., Karagoz, Y., Turan, M., Akkus, F. and O.S. Yildirim (2011). The assessment of lichens as bioindicator of heavy metal pollution from motor vehicles activities. *Afr. J. Agr. Res.* **6**, 1698-1706.
- Boamponsem, L.K., Adam, J.I., Dampare, S.B., Nyarko, B.J.B. and D.K. Essumang (2010). Assessment of atmospheric heavy metal deposition in the Tarkwa gold mining area of Ghana using epiphytic lichens. *Nucl. Instrum. Meth. B.* **268**, 1492-1501.
- Boqueras, M. (2000). Líquens Epífits i Fongs Liquenícules del Sud de Catalunya: Flora i Comunitats. Institut d'Estudis Catalans, Barcelona.
- Brunialti, G., Giordani, P., Isocrono, D. and S. Loppi (2002). Evaluation of data quality in lichen biomonitoring studies: the Italian experience. *Environ. Monit. Assess.* **75**, 271-280.
- Cansaran-Duman, D., Atakol, O. and S. Aras (2011). Assessment of air pollution genotoxicity by RAPD in *Evernia prunastri* L. ach. from around iron-steel factory in Karabuk, Turkey. *J. Environ. Sci.* **23**, 1171-1178.

- Cercasov, V., Pantelica, A., Salagean, M., Caniglia, G. and A. Scarlat (2002). Comparative study of the suitability of three lichen species to trace element air monitoring. *Environ. Pollut.* **119**, 129-139.
- Conti, M.E. and G. Cecchetti (2001). Biological monitoring: lichens as bioindicators of air pollution assessment – a review. *Environ. Pollut.* **114**, 471-492.
- Conti, M.E., Tudino, M., Stripeikis, J. and G. Cecchetti (2004). Heavy metal accumulation in the lichen *Evernia prunastri* transplanted at urban, rural and industrial sites in Central Italy. *J. Atmos. Chem.* **49**, 83-94.
- De Vries, W., Romkens, P.F.A.M., van Leeuwen, T. and T. Brodwijk (2002). Heavy metals in agriculture, hydrology and water quality. CAB Int.
- Dobson, F.S. (2005). Lichens: an Illustrated Guide to the British and Irish Species. The Richmond Publishing, Richmond.
- Dongarra, G., Sabatino, G., Triscari, M. and D. Varrica (2003). The effects of anthropogenic particulate emissions on roadway dust and *Nerium oleander* leaves in Messina (Sicily, Italy). *J. Environ. Monit.* **5**, 766-773.
- Egilli (Olmez), E., Topcuoglu, S., Kut, D., Kirbasoglu, C. and N. Esen (2003). Heavy metals and radionuclides in lichen and mosses in Thrace, Turkey. *B. Environ. Contam. Tox.* **70**, 502-508.
- Frigoli, G. and M. Quartieri (1999). Biomonitoring of atmospheric deposition of heavy metals by means of the lichen *Parmelia sulcata* in an urbanized area. *Annali Di Chimica (Rome)*. **89**, 711-719.
- Geiser, L.H. and P.N. Neitlich (2007). Air-pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. *Environ. Pollut.* **145**, 203-218.
- Goyer, R.A. and C.D. Clasen (1995). Metal toxicology, 31-45. Academic Press, San Diego.
- Guttova, A., Lackovičova, A., Pišut, I. and P. Pišut (2011). Decrease in air pollution load in urban environment of Bratislava (Slovakia) inferred from accumulation of metal elements in lichens. *Environ. Monit. Assess.* **182**, 361-373.
- Horvat, M., Jeran, Z., Špirič, Z., Jačimović, R. and V. Miklavčić (2000). Mercury and other elements in lichens near INA Naftaplin gas treatment plant, Molve, Croatia. *J. Environ. Monit.* **2**, 139-144.
- Jovan S. and B. McCune (2005). Air quality bioindication in the greater Central Valley of California, with epiphytic macrolichen communities. *Ecol. Appl.* **15**, 1712-1726.
- Klos, A., Rajfur, M., Šramek, I. and M. Waclawek (2011). Use of lichen and moss in assessment of forest contamination with heavy metals in Praded and Glacensis Euroregions (Poland and Czech Republic). *Water Air Soil Pollut.* **222**, 367-376.
- Knops, J.M.H., Nash III, T.H., Boucher, V.L. and W.L. Schlesinger (1991). Mineral cycling and epiphytic lichens: implications at the ecosystem level. *Lichenologist.* **23**, 309-321.
- Kord, B. and B. Kord (2011). Heavy metal levels in pine (*Pinus eldarica* Medw.) tree barks as indicators of atmospheric pollutions. *BioResources.* **6**, 927-935.
- Koz, B., Celik, N. and U. Celik (2010). Biomonitoring of heavy metals by epiphytic lichen species in Black Sea region of Turkey. *Ecol. Indic.* **10**, 762-765.
- Loppi, S. and I. Bonini (2000). Lichens and mosses as biomonitors of trace elements in areas with thermal springs and fumarole activity (Mt. Amiata, central Italy). *Chemosphere.* **41**, 1333-1336.
- Loppi, S. and S.A. Pirintsos (2003). Epiphytic lichens as sentinels for heavy metal pollution of forest ecosystems (central Italy). *Environ. Pollut.* **121**, 327-332.
- Loppi, S., Fratti, L., Paoli, V., Bigagli, V., Rossetti, C., Bruscoli, C. and A. Corsini (2004). Biodiversity of epiphytic lichens and heavy metal contents of *Flavoparmelia caperata* thalli as indicators of temporal variations of air pollution in the town of Montecatini Terme (central Italy). *Sci. Total Environ.* **326**, 113-122.
- Loppi, S., Pacioni, G., Olivieri, N. and F. Di Giacomo (1998). Accumulation of trace metals in the lichen *Evernia prunastri* transplanted at biomonitoring sites in Central Italy. *The Bryologist.* **101**, 451-454.
- Markert, B. (2007). Definitions and principles for bioindication and biomonitoring of trace metals in the environment. *J. Trace Elem. Med. Bio.* **21**, 77-82.
- Markert, B., Breure, A., and H. Zechmeister (2003). Bioindicators & biomonitors: Principles, concepts and applications. Elsevier, Amsterdam.
- Marques, A.P., Freitas, M.C., Reis, M.A., Wolterbeek, H.T., Verburg, T. and J.J.M. De Goeij (2004). Lichen – transplant biomonitoring in the assessment of dispersion of atmospheric trace-element pollutants: effects of orientation towards the wind direction. *J. Atmos. Chem.* **49**, 211-222.
- Marques, A.P., Freitas, M.C., Wolterbeek, H.T., Steinebach, O. M., Verburg, T. and J.J.M. De Goeij (2005). Cell-membrane damage and element leaching in transplanted *Parmelia sulcata* lichen related to ambient SO<sub>2</sub>, temperature and precipitation. *Environ. Sci. Technol.* **39**, 2624-2630.
- Mendil, D., Celik, F., Tuzen, M. and M. Soyolak (2009). Assessment of trace metal levels in some moss and lichen samples collected from near the motorway in Turkey. *J. Hazard. Mater.* **166**, 1344-1350.

- Nash III, T.H. and C. Gries (1995). The use of lichens in atmospheric deposition studies with an emphasis on the Arctic. *Sci. Total Environ.* **160**, 729-736.
- Ng, O.H., Tan, B.C. and J.P. Obbard (2005). Lichens as bioindicators of atmospheric heavy metal pollution in Singapore. *Environ. Monit. Assess* **123**, 63-74.
- Nimis, P.L. and S.A. Pittao (2001). The performance of two lichen species as bioaccumulators of trace metals. *Sci. Total Environ.* **275**, 43-51.
- Nriagu, J.O. (1979). Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Nature.* **279**, 409-411.
- Ozturk, S., Oran, S., Guvenc, S. and N. Dalkiran (2010). Analysis of the distribution of epiphytic lichens in the oriental beech (*Fagus orientalis* Lipsky) forests an altitudinal gradient in Uldag mountain, Bursa-Turkey. *Pak. J. Bot.* **42**, 2661-2670.
- Pacheco, A.M.G., Freitas, M.C., Baptista, M.S., Vasconcelos, M.T.S.D. and J.P. Cabral (2008). Elemental levels in tree-bark and epiphytic-lichen transplants at a mixed environmental in mainland Portugal, and comparisons with an *in situ* lichen. *Environ. Pollut.* **151**, 326-333.
- Paoli, L. and S. Loppi (2008) A biological method to monitor early effects of the air pollution caused by the industrial exploitation of geothermal energy. *Environ. Pollut.* **155**, 383-388.
- Paoli, L., Corsini, A., Bigagli, V., Vannini, J., Bruscoli, C. and S. Loppi (2012). Long-term biological monitoring of environmental quality around a solid waste landfill assessed with lichens. *Environ. Pollut.* **161**, 70-75.
- Paoli, L., Pisani, T., Guttova, A., Sardella, G. and S. Loppi (2011). Physiological and chemical response of lichens transplanted in and around an industrial area of south Italy: relationship with the lichen diversity. *Ecotox. Environ. Safe.* **74**, 650-657.
- Purvis, O.W. (1996). Interactions of lichens with metals. *Sci. Prog.* **79**, 283-309.
- Richardson, D.H.S. (1995). Metal uptake in lichens. *Symbiosis.* **18**, 119-127.
- Sloof, J.E. (1995). Lichens as quantitative biomonitors for atmospheric trace element depositions using transplants. *Atmos. Environ.* **29**, 11-20.
- Stamenković, S. and M. Cvijan (2003). Bioindication of air pollution in Niš by using epiphytic lichens. *Arch. Biol. Sci. Belgrade.* **55**, 130-140.
- Sujetoviene, G. (2010). Road traffic pollution effects on epiphytic lichens. *Ekologija.* **56**, 64-71.
- Szczepaniak, K. and M. Biziuk (2003). Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environ. Res.* **93**, 221-230.
- Van Dobben, H.F., Wolterbeek, H.T.H., Wamelink, G.W.W. and C.J.F. Ter Braak (2001). Relationship between epiphytic lichens, trace elements and gaseous atmospheric pollutants. *Environ. Pollut.* **112**, 163-169.
- Vicol, I. (2011). Preliminary study using lichen species diversity as an indicator of local environmental quality within Nature reserves from Romania. *Analele Universitatii din Oradea – Fascicula Biologie*, **18**, 53-58.
- Vokou, D., Pirentos, S.A. and S. Loppi (1999). Lichens as bioindicators of temporal variations in air quality around Thessaloniki, northern Greece. *Ecol. Res.* **14**, 89-96.
- Wirth, V. (1995). Die Flechten Baden-Württembergs. Teil 1&2. Eugen Ulmer GmbH & Co., Stuttgart.
- Wolterbeek, H. Th. (2002). Biomonitoring of trace element air pollution: principles, possibilities, and perspectives. *Environ. Pollut.* **120**, 11-21.