Biologia, Bratislava, **59**/1: 39—45, 2004

Using EDX-microanalysis and X-ray mapping to demonstrate metal uptake by lichens

Martin Bačkor[1,2]* & Dianne Fahselt[2]

[1] *Institute of Biology and Ecology, Department of Botany, Šafárik University, Mánesova 23, SK-04167 Košice, Slovakia; e-mail: mbackor@kosice.upjs.sk*
[2] *Department of Biology, University of Western Ontario, London, Ontario, N6A 5B7, Canada*

Bačkor, M., Using EDX-microanalysis and X-ray mapping to demonstrate metal uptake by lichens. Biologia, Bratislava, **59:** 39—45, 2003; ISSN 0006-3088.

Energy-dispersive X-ray microanalysis and X-ray mapping were used to study metal uptake by the lichens *Lecidea lithophila* and *Rhizocarpon oederi* growing on centuries old copper mine spoil heaps in Špania Dolina, Slovakia. Mineral waste derived from historical extraction of copper was still rich in the heavy metals iron and aluminum, while copper was not detectable. In apothecia of both species there was, as expected, significantly more C than in the substrate but the same levels of O, Na and Mg. The elements Al, Si, and K were present in apothecia of both lichen species, but in significantly lower concentrations than in the substrate. The weight percent of Fe for *L. lithophila* ascocarps was also significantly lower than the substrate, but Cu concentrations were higher. Elemental composition of the vegetative part of thalli, determined only in *L. lithophila*, did not differ significantly from that of the substrate.

Key words: copper, elemental content, lichens, heavy metals, mining.

Introduction

Lichens are environmentally sensitive symbiotic associations, which have been used for biomonitoring of sulfur dioxide (Seaward & Richardson, 1989) and heavy metals (Garty, 2001, 2002). The atmosphere is usually considered to be the main source of heavy metals in lichens, and studies on uptake and accumulation of elements from the atmosphere are extensive (Garty, 2001, 2002; Conti & Cecchetti, 2001). Long-living lichens lacking a protective outer cuticle are used to assess principal sources of local air pollution, as they absorb elements over their entire surface. Less information is available on the metal content of lichens in relation to substrate.

Within Europe and North America specific lichen communities are known to occur on rocks and soils derived from metal mining (Nash, 1989; Purvis & Halls, 1996). Some lichens associated with heavy metal rich substrata are common species, which tolerate metals although they may be found in both polluted and unpolluted situations. Other species, however, are restricted to heavy metal rich substrata and thus may have disjunctive distributions reflecting availability of these substrates (Nash, 1989).

The majority lichens specific to metal-rich substrates belong to the crustose genera, e.g. *Acarospora*, *Aspicilia*, *Lecanora*, *Lecidea*, *Porpidia*, *Rhizocarpon* or *Tremolecia.* Ecophysiological studies of crustose lichens are usually difficult due to

* Corresponding author

the relatively low mass of thallus material and close attachment to substrate. Energy-dispersive X-ray (EDX) microanalysis, however, is an effective method for studying metal accumulation in small samples, as it is simple, requires minimal preparation and a minimal amount of material (TAKALA et al., 1990; GARTY et al., 1993; TREMBLEY et al., 1997; WILLIAMSON et al., 1998). This technique is relatively sensitive with detection limits sometimes less than 0.1% w/w.

The main objectives were to determine the utility of EDX-microanalysis and X-ray mapping for demonstrating the elemental content of crustose lichens and associated metal rich substrata and to determine if mine waste derived from historical mining retains important heavy metals after a few centuries. The next objective was to assess whether the elemental concentrations in lichens tend to parallel those in underlying rock and to demonstrate visually elemental distribution between mineral substrate and closely associated lichens.

Material and methods

Lichens chosen for this study were previously known to colonize substrata derived from metal mining (e.g. PURVIS & HALLS, 1996; FOX, 1999). Five samples each of *Lecidea lithophila* (ACH.) ACH. and *Rhizocarpon oederi* (WEBER) KÖRB., were collected randomly on June 10^{th} 2001 within about 1,500 m^2 of rock substrata from a mine-spoil heaps probably 200-300 years old in the village of Špania Dolina (Slovakia) at approximately 780 m above sea level and stored in a clean, dry condition prior to preparation and analysis.

Rocks and lichens samples, as well as handmade transverse sections of rock and lichen interfaces, were mounted onto aluminum stubs with adhesive carbon discs (12 mm, Soquelec Ltd.) and sputter-coated with a thin film of gold for 5–8 minutes on a Hummer VI Sputter Coater.

An ISI-DS130 conventional scanning electron microscope (SEM), equipped with a Gresham light element detector and a Quartz Xone energy dispersive X-ray (EDX) analysis system, was used to quantify the elemental content in rocks and lichens and to show spatial distribution of elements in the substrate and attached lichen. Specimens were examined at 20 keV.

The significance of differences in elemental concentrations between substrate and lichen thalli was evaluated by one-way analysis of variance and Tukey's pairwise comparisons (MINITAB software).

Results

Elemental concentrations in substrate, lichen thallus and apothecial surface of *Lecidea lithophila* are shown in Fig. 1 and those of the substrate and apothecial surface of *Rhizocarpon oederi* in Fig. 2. Elemental concentrations measured in vegetative parts of *L. lithophila* did not differ significantly from those in the substrate. However, significantly higher than substrate concentrations of C were found in apothecia of both analyzed lichens. A number of elements were found in the same concentrations in apothecia and substrate, i.e., O, Na and Mg in both species of lichens and Ca, Ti and Fe in *R. oederi*. Other metals present in the substrate were also found in apothecia of both species, but in significantly lower concentrations, i.e., Al, Si, and K. In *L. lithophila* Fe also fell into this category. Surprisingly, Cu concentration in *L. lithophila*, while relatively low, was significantly higher in apothecia than in the rock substrate.

X-ray maps confirming the distribution of selected elements in a cross section through the rock-lichen *Lecidea lithophila* interface are shown in Fig. 3. Clearly evident in these micrographs was the higher concentration of C in *L. lithophila* than in rocks and the greater localization of Al, Si, K, and Fe in mineral substrate than in the lichen. Copper levels were too low to permit a visual distinction to be made between amounts in thallus and substrate. Oxygen appeared to be evenly distributed between the two.

Discussion

The earliest archeologically confirmed copper mining in the village of Špania Dolina, dates from about 4000 years ago. In the 16^{th} century the Thurzo-Fugger Mining Company, the world's largest producer of copper, exported copper mainly from Špania Dolina and the associated village Staré Hory, Slovakia. However, in the 18th century copper mining declined and stopped completely in 1888. Mine spoil heaps are thus now between more 100 and 300 year old, usually toward the high end of the range.

Higher plants are ineffective colonizers of mine heap substrates due to high concentrations of some highly toxic elements. On the spoil heap slopes in Špania Dolina the dominant trees species were *Betula pendula* (syn. *B. verrucosa*), *Populus tremula* and *Picea excelsa*, and important understorey herbs species included *Agrostis stolonifera* ssp. *prorepens*, *Asplenium septentrionalis*, *Melandrium rubrum*, *Rumex acetosella* and *Silene inflata* (BANÁSOVÁ, 1976). While vascular plants were few in number, the site had a characteristic high diversity and abundance of lichens, including one species endangered in Slovakia, *Lecanora*

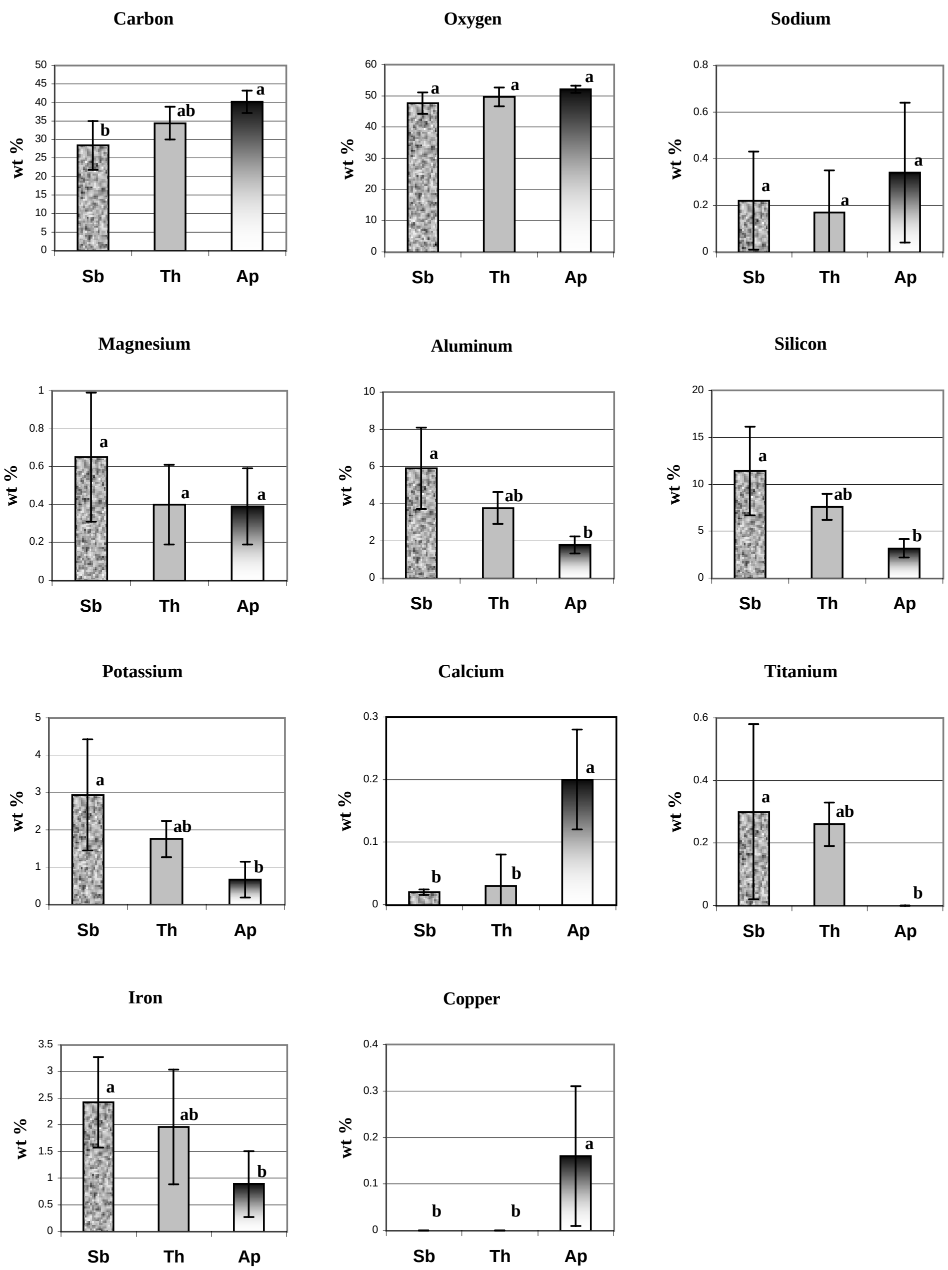

Fig. 1. Elemental concentration (wt %) in lichen *Lecidea lithophila*; substrate (Sb), thallus (Th) and apothecial surface (Ap) measured by EDX – microanalysis (n = 5, values followed by the same letter do not differ significantly at $P < 0.05$ by Tukey's pairwise comparisons).

gisleriana, as well as a number of vulnerable species, *Acarospora peliscypha*, *Lecanora handelii, L. subaurea*, *Lecidea inops*, *Rhizocarpon lecanorinum*, *R. oederi*, *Stereocaulon dactylophyllum* and *S. nanodes* (Banásová, 1976; Pišút et al., 1998; Bačkor & Bodnárová, 2002). Other lichens from mine spoil heaps in Špania Dolina included *Cladonia mitis*, *C. fimbriata*, *C. furcata*, *C. coniocraea*, *C. cariosa*, *Cetraria islandica*, *Lecanora muralis*, *L. polytropa*, *Lecidea lithophila, Physcia*

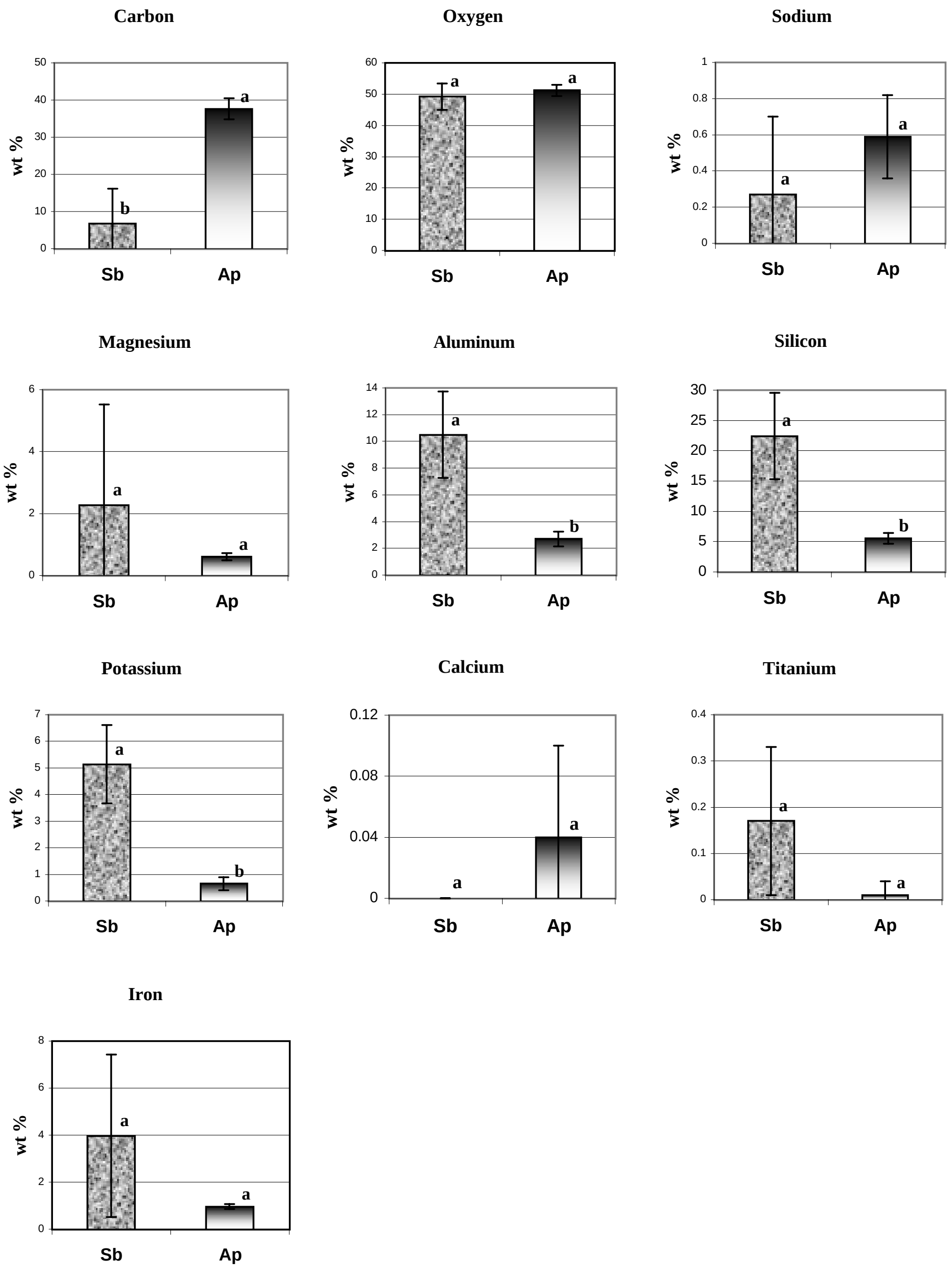

Fig. 2. Elemental concentration (wt %) in lichen *Rhizocarpon oederi*; substrate (Sb) and apothecial surface (Ap) measured by EDX – microanalysis ($n = 5$, values followed by the same letter do not differ significantly at $P < 0.05$ by Tukey's pairwise comparisons).

caesia, P. dubia, *Protoparmelia badia*, *Rhizocarpon distinctum*, *R. macrosporum* (PIŠÚT 1972, 1974; BANÁSOVÁ, 1976; BAČKOR & BODNÁROVÁ, 2002; BAČKOR, unpublished results).

Heavy metal concentrations in this mining area have previously been found to remain high decades after mining ceased. BANÁSOVÁ & HAJDÚK (1975) studied the content of Cu, Fe and Zn in the soils and plants on mine heaps in Rychtárová and Piesky, villages in the western

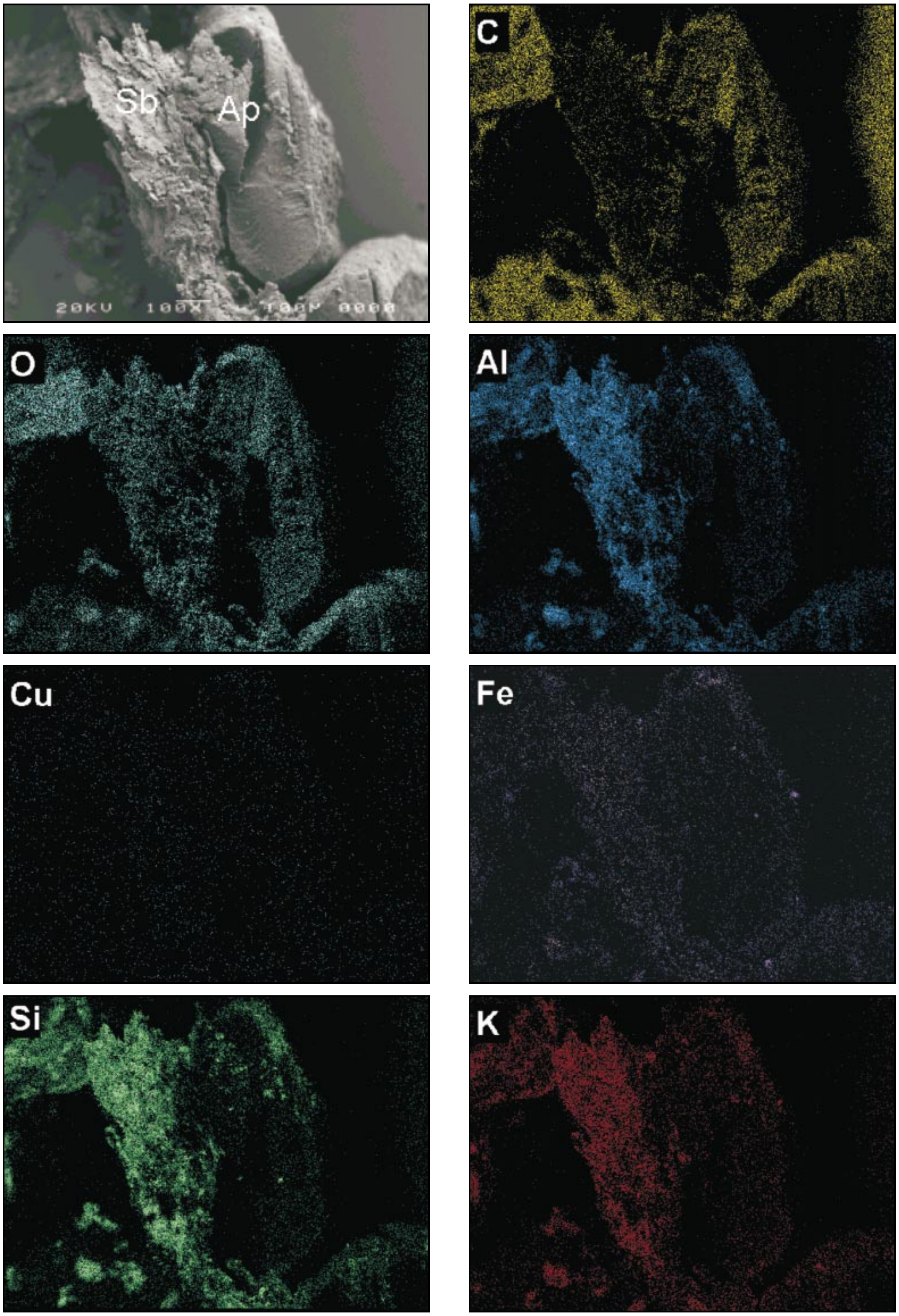

Fig. 3. Micrograph showing substrate (Sb) and apothecium (Ap) and X-ray maps of elemental distribution in the interface between rocks and the lichen *Lecidea lithophila*.

Low Tatra Mts, an area which is geologically related to Špania Dolina and they found copper and iron in the soil, approximately 1.5-2% and 1.6-10% (w/w) respectively. However, in Špania Dolina BANÁSOVÁ (1976) found that concentrations of copper and iron in the soil were lower, approximately 0.1 to 0.3% and 1.5-2.2% respectively. Our results for iron content were in the same range or even higher than those reported by BANÁSOVÁ, and on copper concentrations could also be similar, as they were near the detection limits of EDX microanalysis for copper (about 0.1% w/w). LACKOVIČOVÁ et al. (1977) demonstrated that spoil heaps in eastern Slovakia (region of Gelnica and Slovinky) also contained relatively high amount of copper. However, we found unexpectedly high amounts of aluminum and potassium. Any discrepancies between the results could be related to the fact that mine spoil heaps are characteristically chemically heterogenous. A wide range of primary minerals, containing mainly iron and copper, e.g. siderite, tetraedrite, chalcopyrite, or ankerite, and secondary minerals, e.g. malachite, devilline, celestine, aragonite, may be disposed of in varying proportions.

So far, there has been only one published paper dealing with elemental analysis of lichens from mine spoil heaps in Špania Dolina. In this work *Cladonia mitis* was reported to contain 0.002818% (w/w) copper, 0.000088% mercury and 0.000185% cadmium (BAČKOR et al., 1998). Similarly, copper levels for *C. mitis* collected in the geologically related locality, Rychtárová, was 0.00623% (w/w) (BANÁSOVÁ & HAJDÚK, 1975). The higher substrate concentrations of copper, found on apothecial surfaces of *Lecidea lithophila* in our study, was an interesting phenomenon. Loss of Cu^{2+} from mineral substrate might be explained by leaching following weathering of chalcopyrite, because copper is more soluble under acidic conditions. At the same time dissolved copper could be taken up by lichens and accumulated by some physiological processes. Some crustose lichens (e.g., *Lecidea* sp. and *Acarospora* sp.) were in fact previously found to be hyperaccumulators of copper (PURVIS & HALLS, 1996).

The higher amount of calcium in apothecia of both lichens (significantly higher only in *L. lithophila*) was also surprising. Calcite associated with chalcopyrite mineralization helps to immobilize copper (PURVIS & HALLS, 1996), and we found that calcium can play a significant role in copper detoxification in axenic cultures of the lichen photobiont *Trebouxia erici* (BAČKOR et al., 2003). However, it is not clear if the higher calcium concentrations observed on apothecial surfaces were accumulated from the air or from the mineral substrate.

The heavy metal content in lichens has previously been found to parallel that in the underlying rocks (e.g. NASH, 1989; PURVIS & HALLS, 1996; CHETTRI et al., 1997). However, CHIARENZELLI et al. (1997) suggested that substrate was not a significant source of metals as they noted poor correlation between lichen and substrate chemistry. It appears that substrate in our study was the main source of metal pollution, as the study site was part of a protective zone of National Park of Low Tatra Mts where there is little human activity. The present population is only about 170 inhabitans. Epiphytic lichens present including several ones sensitive to sulphur dioxide (HAWKSWORTH & ROSE, 1970): *Parmelia tiliacea*, *Evernia prunastri*, *Platismatia glauca*, *Pseudevernia furfuracea*, *Usnea hirta*, *Vulpicida pinastri*. The abundance of these species (BAČKOR & BODNÁROVÁ, 2002; BAČKOR, unpublished results) suggested air purity.

Elemental concentrations in lichens were shown by EDX to parallel those in underlying rock thus confirming ion uptake from a mineral substrate, and X-ray mapping showed the distribution of several mineral elements of rock substrates also incorporated in crustose lichens.

Acknowledgements

This work was supported financially by Operating Grants from the Natural Sciences and Engineering Research Council Canada and from the Slovak Ministry of Education, and made possible by a NATO Science Fellowship to M. BAČKOR from NSERC Canada. Authors wish thank to Mr. Ross DAVIDSON for technical assistance with EDX microanalysis, Dr. Anna GUTTOVÁ for verifying the identity of *L. lithophila* and Mr. Kenneth DVORSKÝ for assistance with the design of plates.

References

BAČKOR, M. & BODNÁROVÁ, M. 2002. Additions to lichen flora of Slovak Republic I. Thaiszia – J. of Botany **12:** 173–178.

BAČKOR, M., FAHSELT, D., DAVIDSON, R. D. & WU, C. T. 2003. Effects of copper on wild and tolerant strains of the lichen photobiont *Trebouxia erici* (Chlorophyta) and possible tolerance mechanisms. Archives of Environmental Contamination and Toxicology **45:** 159–167.

BAČKOR, M., HUDÁK, J. & BAČKOROVÁ, M. 1998. Comparison between growth responses of autotrophic and heterotrophic populations of lichen photobiont *Trebouxia irregularis* (Chlorophyta) on

Cu, Hg and Cd chlorides treatment. Phyton – Annales Rei Botanicae **38:** 239–250.

BANÁSOVÁ, V. 1976. Vegetácia medených a antimónových háld. Veda, Bratislava.

BANÁSOVÁ, V. & HAJDÚK, J. 1975. Gehalt an Cu, Zn, As und andere Elemente in einigen Pflanzen und haldeböden sowie in Gebieten mit Exhalatquellen. Biológia, Bratislava, **30:** 293–301.

CHETTRI, M. K., SAWIDIS, T. & KARATAGLIS, S. 1997. Lichens as a tool for biogeochemical prospecting. Ecotoxicology and Environmental Safety **38:** 322–335.

CHIARENZELLI, J. R., ASPLER, L. B., OZARKO, D. L., HALL, G. E. M., POWIS, K. B., DONALDSON, J. A. 1997. Heavy metals in lichens, southern District of Keewatin, northwest territories, Canada. Chemosphere **35:** 1329–1341.

CONTI, M. E. & CECCHETTI, G. 2001. Biological monitoring: lichens as bioindicators of air pollution assessment – a review. Environ. Pollut. A **114:** 471–492.

FOX, H. 1999. Lichens of three mine sites in Co. Wicklow, Ireland. Proceedings of the Royal Irish Academy Section B-Biological Geological and Chemical Science **99:** 67–71.

GARTY, J. 2001. Biomonitoring atmospheric heavy metals with lichens: theory and application. Crit. Rev. Plant Sci. **20:** 309–371.

GARTY, J. 2002. Biomonitoring heavy metal pollution with lichens, pp. 458–482. In: KRANNER, I., BECKETT, R. & VARMA, A. (eds), Protocols in Lichenology: Culturing, biochemistry, ecophysiology and use in biomonitoring. Springer, Berlin-Heidelberg.

GARTY, J., KARARY, Y., HAREL, J. & LURIE, S. 1993. Temporal and spatial fluctuations of ethylene production and concentrations of sulfur, sodium, chlorine and iron on/in the thallus cortex in the lichen *Ramalina duriaei* (DE NOT.) BAGL. Environ. Exp. Bot. **33:** 553–563.

HAWKSWORTH, D. L. & ROSE, F. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature **227:** 145–148.

LACKOVIČOVÁ, A., LIŠKA, J. & PIŠÚT, I. 1977. Lišajníky medených háld v okolí Gelnice a Sloviniek (východné Slovensko). (Lichens from the region of Gelnica and Slovinky). Múzeum, Bratislava **22:** 92–98. (in Slovak)

NASH, T. H., III. 1989. Metal tolerance in lichens, pp. 119–131. In: SHAW A. J. (ed.) Heavy metal tolerance in plants: Evolutionary Aspects, Boca Raton: CRC Press.

PIŠÚT, I. 1972. Lichenes Slovakiae Exsiccati, Editi a Museo Nationali Slovaco, Bratislava. Fasc. **IX**. (No. 201–225), 7 pp.

PIŠÚT, I. 1974. Doplnky k poznaniu lišajníkov Slovenska 7. Acta Rer. Nat. Mus. Natl. Slov., Bratislava, **20:** 37–40.

PIŠÚT, I., GUTTOVÁ, A., LACKOVIČOVÁ, A. & LISICKÁ, E. 1998. Lichenizované huby (lišajníky), pp. 229–295. In: MARHOLD, K. & HINDÁK, F. (eds), Zoznam nižších a vyšších rastlín Slovenska. Veda, Bratislava.

PURVIS, O. W. & HALLS, C. 1996. A review of lichens in metal-enriched environments. Lichenologist **28:** 571–601. doi:10.1006/lich.1996.0052

SEAWARD, M. R. D. & RICHARDSON, D. H. S. 1989. Atmospheric sources of metal pollution and effects on vegetation, pp. 75–92. In: SHAW A. J. (ed.) Heavy metal tolerance in plants: Evolutionary aspects, CRC Press, Boca Raton.

TAKALA K., OLKKONEN H., JAASKELAINEN J. & SELKAINAHO K. 1990. Total chlorine content of epiphytic and terricolous lichens and birch bark in Finland. Anales Botanici Fennici **27:** 131–137.

TREMBLEY, M. L., FAHSELT, D. & MADZIA, S. 1997. Localization of uranium in *Cladina rangiferina* and *Cladina mitis* and removal by aqueous washing. Bryologist **100:** 368–378.

WILLIAMSON, B. J., MCLEAN, J. & PURVIS, O. W. 1998. Application of X-ray Element mapping across the lichen-rock interface. J. Microsc., Oxford, **191:** 91–96.

Received Nov. 13, 2002
Accepted Oct. 22, 2003