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Lichens as Biomonitors: Global, Regional and Local Aspects*

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Lichens, a symbiosis of fungi and algae, have been widely used for biological monitoring of environmental quality. As some lichen species are virtually cosmopolitan organisms, they may be used for a survey of organic, inorganic, or radioactive contaminants on more or less a global scale, as well as for regional comparisons, and certainly at a more local scale. Some examples will be given for the use of different lichen species (*Usnea ssp*., *Lecanora muralis*, *Pseudevernia furfuracea* and *Xanthoria parietina)* in biological monitoring of trace elements in various contexts.

INTRODUCTION

Biological monitoring using indicator organisms can be carried out in various ways, from a mere phenomenological approach to detailed quantitative determination of elements, species or nuclides in the tissues of organisms or in parts of them. Active or passive monitoring, depending on the origin of the organism – transplanted or taken from its natural habitat, has been successfully applied using bryophytes and lichens for air and water monitoring.[1,2,3,4,5] The absence of any root uptake of nutrients in some mosses and virtually all lichen species renders them potentially useful as direct monitors of air pollution. Various models have been proposed to quantify mean element concentrations in air by average concentrations found in lichens.[4,6,7,8] In addition, the slow metabolic activity and slow growth rate of lichens makes them a sink and an accumulator of contaminants in the prevailing environments.[9.10]

Large differences in species specific response to air pollutants such as $S0_2$, NO_x and poly-chlorinated biphenyls (PCBs), however, restrict the use of some lichen species to rural or very remote areas where background or near-background concentrations can be expected. In addition, the preference of certain species for a particular growing substrate might prevent the application of a particular lichen species in large scale monitoring programmes. Nevertheless, some common lichen species can be found on almost every continent and might be suitable for direct comparisons of air quality in different parts of the world in terms of the analytes determined in lichen. Examples of analytical results for *Usnea sp*., *Lecanora muralis*, *Pseudevernia furfuracea* and *Xanthoria parietina* are given below.

EXPERIMENTAL

Usnea spp., shrubby lichens hanging from trees (coniferous and deciduous) in bundles of thalli are abundant on almost all continents and in remote areas of the world with moderately humid climate. This group of species are mainly found in elevated mountainous regions and are collected without

* Dedicated to the memory of the late Professor Marko Branica.

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risk of contamination from the host tree. Since lichens possess no roots, they mostly rely on nutrient uptake from air components. Samples of *Usnea* were collected in Siberia, near Lake Baikal and in Alberta, Canada, in boreal forests and in Sri Lanka in a mountainous rain forest at about 1200 m above sea level. In the Bavarian Forest, Germany, a sample was taken by the local forestry personnel, air dried and sent to the laboratory in Jülich, where all samples were milled and analysed.

All samples, including the reference material IAEA 336, were acid digested and analysed using a PE ELAN 5000 ICP-MS and a PE Optima-500 ICP-AES system. The analytical parameters and settings used are described elsewhere.[11]

Lecanora muralis, an epilithic lichen growing on rocks, was collected in August 1999 from cemetery stones in 74 cities in Northrhine-Westfalia (NRW), Germany, by carefully scratching off the biological tissue from rock materials. Several specimens from one stone were combined to make up a sample. Several such samples were further united to obtain representative samples per cemetery and those samples were taken to represent the individual cities. The material was desiccated in a freeze-dryer and milled in a centrifugal mill (ZM 1000, Retsch, Germany) at 10000 x *g* for 2 min.

Analysis was carried out following the same procedures as described above using the ICP-MS and ICP-AES instruments. Quality control of the results was done by analyses of RM IAEA-336 (lichen), GBW 7602 (bush branches and leaves), NBS 1575 (pine needles) and NIES No. 9 (sargasso).

Pseudevernia furfuracea is a shrubby lichen hanging in bundles from branches and is one of the most frequent large lichens in European mountains. About 11 kg of fresh material was collected in October 1998 in the high-altitude National Park area of the Bavarian Forest. At that time a vast bark beetle calamity destroyed the major part of spruce stands in the area and the lichen could be easily collected from collapsed trees near the ground. The material was frozen at the sampling site in the vapour phase of liquid nitrogen and milled cryogenically at the Institute in Jülich to particle sizes comparable to the current reference materials.[12] Intensive investigations are in progress to prepare this material as a candidate RM for analytical purposes. Preliminary results can be compared with the results from spruce shoots and beech leaves from the same area and collected, prepared and analysed in exactly the same way as the lichen.

The foliose lichen *Xanthoria parietina* is a rather compact species adhering to bark or rock with a few rhizines and can be hence collected easily from the substrate by careful dissection of its basal organs. Investigated specimens were collected together with the *Lecanora m.* samples during the NRW sampling campaign in August 1999. Intact specimens, about 5 to 10 cm in diameter, were investigated in detail using solid sampling AAS to study the distribution of Cr, Cd and Pb in each lichen specimen. This analytical technique is particularly suited for investigating the spatial distribution of elements because it consumes only tiny amounts of sample for analysis (0.05–2.0 mg) without prior digestion. It is a direct method and is fast and inexpensive. Further details of the method are described elsewhere.[13,14]

RESULTS AND DISCUSSION

Example 1: The Global Aspect

Apart from the difficulties in sampling lichens of the same age and from the same substrate, in a similar seasonal situation and at a comparable altitude, it is hard to identify a single species that is abundant in various parts of the world and the analytical results of which could be readily compared. The difference in accumulation patterns of different species makes it almost impossible to compare results between species.[15] The genus *Usnea* Dill. ex Adanson occurs in moist upland areas throughout the world. The species grow fast (2–10 mm per year) and can be easily collected in large amounts. Although quite susceptible to air pollution, it can still be found in remote mountainous regions, especially of Europe, North America, and Asia.

As the first attempt, *Usnea* samples collected in Siberia, Canada, Sri Lanka and Germany were analysed together and the results were compared (Figures 1–3).

Compared to the IAEA lichen (*Evernia prunastri*), concentrations in the *Usnea* samples are of similar magnitude. Both are shrubby lichens attached only at one

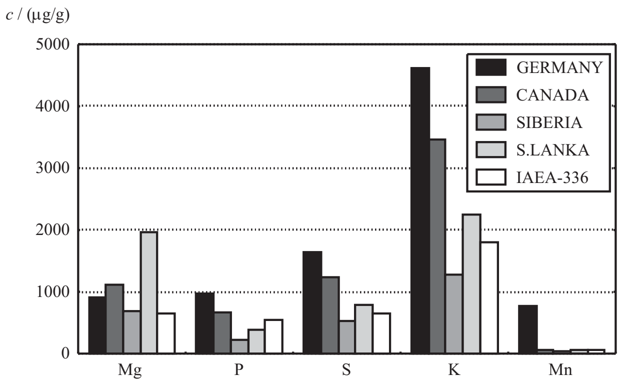

Figure 1. Concentrations of main elements in *Usnea sp.* samples from Germany, Canada, Siberia, Sri Lanka and the reference material IAEA-336.

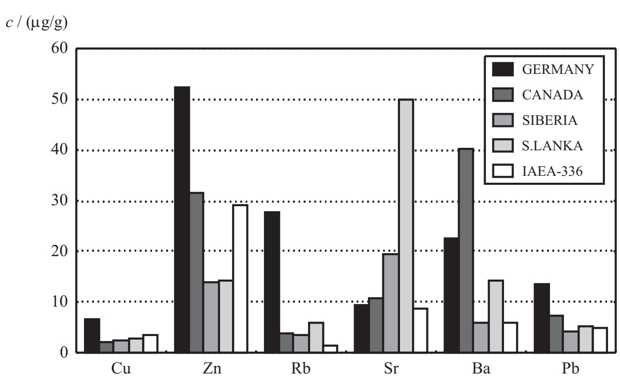

Figure 2. Concentrations of medium elements in *Usnea sp.* samples from Germany, Canada, Siberia, Sri Lanka and the reference material IAEA-336.

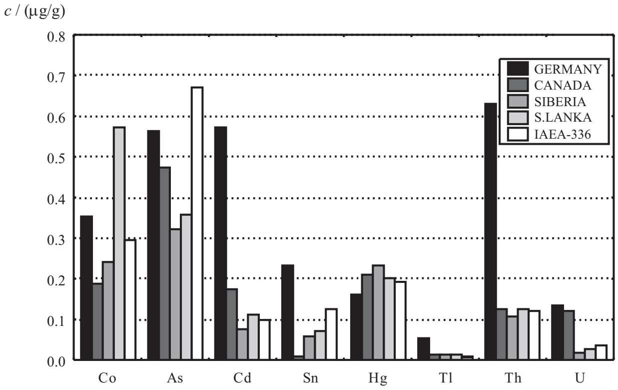

Figure 3. Concentrations of trace elements in *Usnea sp.* samples from Germany, Canada, Siberia, Sri Lanka and the reference material IAEA-336.

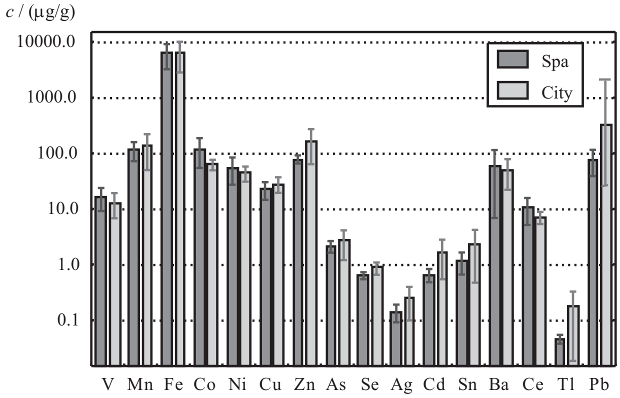

Figure 4. Comparison of the results for *Lecanora muralis* from 8 nature-reserve towns and 10 large cities in Northrhine Westfalia, Germany, collected in 1999.

point to the bark and containing large amounts of usnin acid, which might make them similarly susceptible to trace elements. Sulphur, K, Mn, Cu, Zn, Cd, Tl, and Pb show the highest concentrations in the sample from the Bavarian Forest. This is reasonable considering the prevailing east winds that expose the region to emissions from the coal mining area in the Czech Republic, and the local emissions from lead-glass manufacturers. Astonishingly, Ca, Mg, Co, Sr and rare earth elements are enriched in the sample from Sri Lanka, indicating a geogenic influence of the nearby monazite sands of southern India (Ref. 16 and unpublished data).

Rough evaluation of different environmental stress situations on a global scale seems to be feasible by using cosmopolitan lichen species and it should just be a matter of extensive sampling to improve the grid resolution. It is a challenging task, however, to enlarge the application of biomonitoring from the hitherto practiced local or regional scale to a more or less global scale. As air masses move freely around the globe dispersing emitted compounds boundlessly, long distance transport may only be monitored by such a universal approach. Results could then be directly linked with recent and subrecent works on global long-range atmospheric transport, interpreted from peat bog archives,[17,18] sediments,[19,20] and glacier ice cores.[21,22]

Example 2: The Regional Aspect

Most surveys based on lichen analysis have been carried out on a regional basis. Environmental impact assessment in some European countries is obligatory prior to undertaking new industrial or technical activities as well as some advise on the use of lichen for these surveys.[23] J. E. Sloof used *Parmelia sulcata* for a survey of The Netherlands in 1986–1987.[24] *Hypogymnia physodes*, another foliose lichen was used by Jeran *et al.*[25] to monitor trace elements in Slovenia and they came out with 9 distinct factors that explained 84 % of the total variance of the results. In a similar approach, the epilithic lichen *Lecanora muralis* was collected at 205 different sampling sites in NRW, Germany, in 1999 to evaluate the differences in atmospheric deposition in this densely populated region in the centre of Europe. Since evaluation of the results within a doctoral thesis is still in progress, only some preliminary results will be presented here. In Figure 4, some elements are displayed with their mean concentrations in the 10 largest cities of that area as compared to the means in eight health resorts (spas).

Elements of environmental importance such as Zn, Ag, Cd, Sn, Tl and Pb occur in increased concentrations in large cities. Additionally, the variance of results is generally larger within the big cities compared to the health resorts. Large concentration differences exist between certain industrial centres (*e.g.,* Duisburg, Siegen) and smaller cities at the edge of the area (*e.g.,* Aachen, Siegburg, Wesel).

Example 3: Lichen Results Compared to Other Biomonitors

It should be interesting to compare the results of lichen analyses with the results on other biomonitors collected in the same sampling area at more or less the same time. In the case of *Pseudevernia furfuracea* collection in the Bavarian Forest in October 1998, we are in the fortunate position that beech leaves (*Fagus sylvatica*) were collected in September 1998 and spruce shoots (*Picea abies*) in May 1999 on about the same location by using very similar procedures. This sampling was done within the regular sampling scheme for the Specimen Banking project of the Federal Republic of Germany.[26] Sample processing as well as analysis were carried out at the same laboratories using the same equipment. Sampling in large quantities (10 to 20 kg fresh weight) assures a representative sample for a larger area as compared to a few single specimens. In Figure 5, the results of a number of elements are displayed on logarithmic scale.

With the exception of S, Ni, and Cu, levels of all the other elements are much higher in lichen compared to

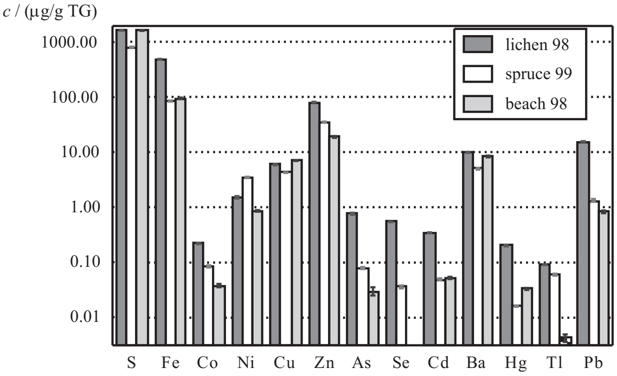

Figure 5. Comparison of the results for *Pseudevernia furfuracea* with spruce shoots (*Picea abies*) and beach leaves (*Fagus sylvatica*) from the nature-reserve area of the Bavarian Forest.

their concentrations in spruce shoots or beech leaves. This clearly reflects the longevity and exposure age of lichen compared to one-year-old spruce shoots and beech leaves of only one growing season. Additionally, lichens seem to possess a different eco-physiological accumulation pattern compared to nutrient uptake by fungi and algae. In monitoring programmes requiring high time resolution, the biomonitors of defined exposure age (spruce shoots or needles, leaves of deciduous trees, *etc.*) have a clear advantage over lichens. If the focus is on assessment of, and comparison with, other ecosystems at a given time, the analysis of lichens would be preferable from the viewpoint of the ease of analysis.

Example 4: The Local Aspect

When lichens are used as monitors for particular emission point sources such as smelters or power plants, individual specimens from the vicinity of these sites will be mainly analysed.[27,28] For better interpretation of the results, more should be known about uptake mechanisms, accumulation with age or distribution of elements within the lichen tissue. *Xanthoria parietina*, a foliose lichen with disk-shaped fruiting bodies was used for the investigation of the spatial distribution of elements. Cr, Cd and Pb were analysed using the SS-AAS approach to investigate whether an accumulating effect could be detected with the age of the tissue. This species is supposed to grow in concentric rings around a nucleus. As this bright yellow lichen is attached to the substrate with only a few rhizines or attachment discs, large specimens can be removed without a great risk of contamination and without breaking it into pieces. Several of these specimens were investigated analysing the fruiting bodies from the upper part, the lobes and the adhering black seston attached to the lower part of the lobes. As can be seen from Figure 6, the fruiting bodies from the innermost part (e) seem to have somewhat elevated concentrations compared to younger plant parts (b) but the differences are small and standard deviations of repetitive analyses overlap.

In Figure 7, concentrations of Cr, Cd and Pb in lobes and in the adhering material underneath them are displayed. Clear differences in concentration are visible for all three elements. It is obvious that this adhering material must originate from sources other than the biological tissue of the lichen. It does not seem to be of geogenic origin (substratum) but resembles soot or air particulate matter.

This particular lichen species develops 1–2 mm above the substrate rock, being attached to it with tiny rhizines.[29] These bundles of fibres (hyphae) could act as filters for air particulate matter, accumulating air dust over time. As lichens absorb elements preferably in the dissolved state, this species offers the opportunity to monitor dissolved and particle bound contributions of air-borne contaminants separately. This can be achieved by careful separation of the lichen body and the adhering black seston underneath it prior to analysis.

Matschullat *et al.*[15] report analytical results for *Xanthoria elegans* from Moose Factory, Ontario, Canada and from the alpine village of Alt St. Johann, Switzerland. In Table I their values are compared with the mean values for *X. parietina* from this study collected in Northrhine Westfalia in July 1999.

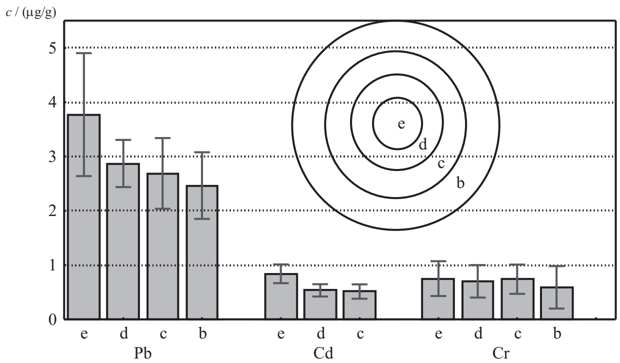

Figure 6. Concentrations of Pb, Cd and Cr in different parts of *Xanthoria parietina* from Northrhine Westfalia, Germany, collected in 1999. Results of e, d, c, and b from sections of lichen specimens as indicated in the inlay.

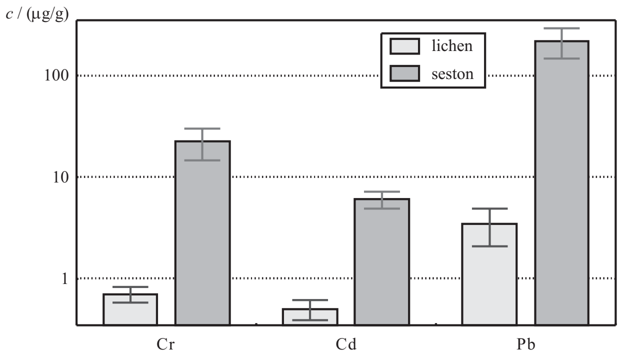

Figure 7. Concentrations of Cr, Cd and Pb in the lichen body and black adhering matter (seston) of *Xanthoria parietina* from Northrhine Westfalia, Germany.

TABLE I. Results for Cr, Cd and Pb in *Xanthoria elegans* from Canada[a] and Switzerland[a] and *X. parietina* from Germany[b]. In addition, concentrations in the underlying black material (seston) are given

Element	Canada[a]	Switzerland[a]	Germany[b]	seston
	c / (μg/g)			
Cr	3.9	8.9	0.71 ± 0.12	22.4 ± 7.7
Cd	0.37	0.27	0.51 ± 0.11	6.04 ± 1.1
Pb	17	21	3.49 ± 1.4	219 ± 72

[a] Ref. 15. [b] This study.

The results for Cr and Pb suggest that the black adhering material influenced the analysis of *Xanthoria* samples from previous measurements. Northrhine Westfalia is a densely populated area in Germany with heavy industries. As the results from the carefully prepared lichen material from Germany show Cd results comparable to the samples of remote Switzerland and Canada but much lower concentrations for Cr and Pb, it seems plausible that the lichen body should be carefully separated from the adhering black matter prior to analysis if reliable results of biomonitoring with lichen (*Xanthoria sp.*) are desired. The black matter more likely resembles soot or atmospheric dust and, hence, will bias the results of the biomonitors.

The concentration ratios in black matter and lichen material for Cr, Cd, and Pb are 31.6, 11.9, and 62.8, respectively. Cr was among the most enriched elements in urban dust compared to concentrations found in lichen from very remote areas.[16] Unfortunately, enrichment factors for Pb and Cd were not reported. However, solubility constants in the water of carbonates or hydrates for both elements differ by 3–5 orders of magnitude, confirming the higher accumulation of Cd in the biological tissue compared to Pb.

CONCLUSIONS

It has been demonstrated that various lichen species can be used profitably for environmental monitoring on different geographical scales. Long-distance transport of hazardous substances as well as point source emissions can be monitored using epiphytic and epilithic lichens. Concentrations are generally higher in lichens compared to other biomonitors, such as spruce shoots or beach leaves. This seems to reflect the longer exposure time and differences in accumulation properties of lichens compared to other biological specimens. Particular care, however, should be taken in sampling specimens attached with rhizines to the substratum. As the organisms take up elements only in dissolved form and particularly from the upper exposed side, a mixing of lichen tissue with particulate matter should be avoided to omit overestimation of pollution and to enhance the comparability with the results on other biological species. On the other hand, a differentiated picture on elemental stress in an ecosystem can be obtained using particular information about concentrations in lichen tissues and the adhering solid matter. It might be possible to specify contributions to contamination in soluble (bio-available) and insoluble (particulate) form.

(According to IUPAC the quantity used here and called "concentration, *c*" should systematically be called "mass fraction, *w*".)[30]

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SAŽETAK

Lišajevi kao biomonitori: globalni, regionalni i lokalni aspekti

Matthias Rossbach i Susanne Lambrecht

Lišajevi, simbioza gljiva i algi, bili su u prošlosti u širokoj uporabi za biološki monitoring kakvoće okoliša. Kako određene vrste lišajeva predstavljaju gotovo kozmopolitske organizme, može ih se koristiti za praćenje organskih, anorganskih i radioaktivnih onečišćivala na globalnoj razini, ali svakako i na regionalnim i lokalnim razinama. U radu se daje nekoliko primjera korištenja različitih vrsta lišajeva za biološki monitoring elemenata u tragovima u raznim sustavima.