

## Metal Uptake in Lichens

D.H.S. RICHARDSON

*Saint Mary's University, Halifax, Nova Scotia B3H 3C3, Canada.*

*Tel. +902-4205493, Fax. +902-4205261*

Received January 15, 1995; Accepted February 14, 1995

### Abstract

Lichens and free-living fungi are known to exhibit three metal uptake mechanisms: (1) an extracellular uptake via an ion exchange process, (2) intracellular accumulation, and (3) trapping of metal-rich particulates. As a result lichens have been found to be excellent biological monitors of metal deposition around industrial centers and fungi have been proposed for decontaminating effluents contaminated by metals. The mechanisms and characteristics of metal uptake by lichens and fungi are still only partially understood; this review highlights the areas of uncertainty and proposes that some of the methods used to study free-living fungi might be applied to lichens and vice versa. Such research would elucidate the uptake mechanisms and enhance the potential of these plants for industrial use or as biological monitors.

Keywords: lichens, fungi, metal uptake, ion exchange, particulates

### 1. Introduction

The remarkable ability of lichens to accumulate a range of elements, from the alkali metals and their isotopes like Cs<sup>137</sup> to the heaviest of the transition metals such as lead and mercury or even the transuranic series, has been established unequivocally in the last twenty years (Richardson, 1992). Taxonomically, the fungal components of over 90% of lichen species are related to ascomycete fungi but there are also, especially in the tropics and subtropics, lichens with basidiomycete partners. The ability of free-living fungi, of either

Presented at the Fifth Intl. Mycological Congress IMC5, Aug. 14–21, 1994, Vancouver, BC, Canada.

0334-5114/95/\$05.50 ©1995 Balaban

group, to accumulate metals has been known for a long time. The application of this for industrial purposes was suggested for uranium by Galun et al. (1983, 1984) and for other metals by Siegel et al. (1986) and Galun et al. (1987a). In recent years, the use of fungi for the removal of metals from industrial process streams has been evaluated and found effective (Muraleedharan et al., 1994; Fourest and Roux, 1992).

Certain lichens are better at accumulating metals than others; for example, *Cetraria nivalis* was consistently better at accumulating lead<sup>210</sup> than *Cladonia mitis* (Thomas et al., 1994). Interspecific calibration factors have been calculated for different lichens used in monitoring studies which have had limited applicability (Folkesson, 1979; Sloof and Wolterbeek, 1993). Similarly, the mycelium of different genera of fungi has been shown to vary in metal accumulating ability (Fourest and Roux, 1992; Mullen et al., 1992). What are the causes of the observed variation? Is it just a question of lichen morphology, with the more flattened *Cetraria nivalis* providing a greater surface for wind and rain impaction than *Cladonia mitis* (as suggested by Thomas et al., 1994), or are chemical and anatomical characteristics also involved? Little experimental work has been done to address these questions.

It has been established that lichens are effective accumulators of metals because they take up soluble metal ions very rapidly (within about one hour) via an extracellular ion exchange process (Nieboer et al., 1976). This may be accompanied by a slower, probably energy-dependent intracellular accumulation of metals. Lichens also accumulate metal-rich particulates, a process which probably begins within 24 hours, judging from studies on free-living fungi (Singleton et al., 1990) and reaches equilibrium with deposition in the environment within four months (Gailey et al., 1985). The ability of free-living fungi to accumulate metals by all these processes has been documented recently (e.g., Pumpel and Schinner, 1993; Starling and Ross, 1990; Singleton et al., 1990).

## 2. Extracellular ion exchange

It is well established that lichens accumulate soluble metal ions via an ion exchange process in which the entering ion displaces hydrogen ions or ions of other more weakly bound metals from the binding site on or in the cell walls. Studies on the characteristics of binding and metal ion affinities indicate that the fixed anionic groups in the lichens are probably carboxylic acid or hydrocarboxylic acid in nature (Puckett et al., 1973; Nieboer et al., 1976). More circumstantial evidence for this was recently deduced (Richardson et al., 1985; Dempsey et al., 1992). In free-living fungi, metal binding (or biosorption as

many mycologists term it) is greater in some fungi than in others; for example, Fourest and Roux (1992) found that metal ion uptake by *Rhizopus arrhizus* was higher under natural pH conditions than by *Penicillium chrysogenum* or *Aspergillus niger*, but at pH 7 there was virtually no difference in binding capacity. They explained this in terms of the higher proportion of chitin and chitosan in the cell walls of *Rhizopus*. However, other extracellular products such as phenolic polymers can also bind metal ions strongly (Mullen et al., 1992). In a comprehensive recent review, Gadd (1993) concluded that a variety of potential sites may be involved including carboxyl, amine, hydroxyl, phosphate and sulphhydryl groups although their relative significance is difficult to resolve. Primary interactions probably involve carboxyl and phosphate groups. Nuclear magnetic resonance (NMR) has been used to provide direct experimental evidence that the cadmium and copper binding ligands in the alga *Stichococcus bacillaris* are carboxylic acid in nature (Majidi et al., 1990; Zhang and Majidi, 1994). Another technique, electron paramagnetic resonance (EPR), has been used to show that the metal biosorption sites in the fungus *Ganoderma lucidum* are oxygen dominated with the structural polysaccharides of the fungal wall being the main sites of interaction (Muraleedharan and Venkovbchar, 1994). Similar studies are clearly needed on a range of lichens. The rhizinae of lichens such as *Peltigera* often have a greater binding capacity than the remainder of the thallus (Goyal and Seaward, 1982; Brown, 1991). It would be interesting to use NMR and EPR to determine whether there are both more binding sites per unit mass and/or perhaps different types of binding sites on these structures.

Electron microscope studies have shown that metals such as lead bind effectively to the hyphal walls of lichens (Lawrey, 1977; Jones et al., 1982) and more recently colorimetric methods have been used to show that the cortex is an effective area for adsorption of this metal (Garty and Theiss, 1990).

Modern electron microscopes with electron probes should reveal in more detail exactly where different metals bind to lichens and free-living fungi. A start on this type of research has involved studying the routes of water and organic solute translocation in *Lobaria*, *Parmelia*, *Peltigera* and *Xanthoria* (Honegger and Peter, 1994). In the moss *Hylocomium splendens*, Doyle (1981) showed, using transmission electron microscopy (TEM), that at low concentrations, uranium bound throughout the cell wall, whereas lead was restricted to the outer and innermost layers of the thick leaf cell walls in this plant. This difference may have reflected the greater binding affinity of lead for protein components in the outer layers of the moss cell wall and at the inner cell wall/cell membrane interface (Nieboer and Richardson, 1980). TEM was also used by Galun et al. (1987b) to investigate uranium uptake in the free-living fungus *Penicillium digitatum*. With relatively high uranium

concentrations in the incubation medium, they discovered conspicuous crystal-like deposits both on the surface of the hyphae and inside the cells near the cell walls. Some elements like calcium sodium and magnesium are more easily displaced from metal binding sites than others such as lead, iron and uranium and seasonal variability is often observed (Prussia and Killingbeck, 1991).

### 3. Particulate trapping

Many studies have shown the effectiveness of lichens in trapping atmospheric particulates and reflecting deposition patterns of metals in the environment (Herzig et al., 1989). However, it is still unclear exactly where the metals accumulate and how this occurs. It is generally accepted that much of the particulate material accumulates within the loose hyphal weft of the medulla region of lichens. How do the particulates reach this area? Electron microscope studies reveal pores and holes in the upper surface of some lichens but others appear to have an upper cortex which is reasonably well sealed, presumably by polymers such as lichenins and isolichenins. It is unlikely that particulates enter via the growing margins of the thallus lobes because these exhibit a lower concentration of metals (Hale and Lawrey, 1985).

Free-living fungi also take up particulates from suspensions including zinc dust, sulphur and clays, accumulating up to 60% w/w within 24 hours. The process appears independent of fungal metabolism. However, the uptake of sulphur particulates is decreased by the presence of copper ions and stimulated by magnesium ions, possibly by creating ionic bridges between the cell walls and the sulphur particles (Singleton et al., 1990). *Mucor flavus* mycelium has a net negative charge and probably attracts the positively charged edges of the clay particles. In addition, hydrogen bonding between B glucans on the outer surface of the fungal walls and the waters of hydration of the clay particles may also be a mechanism of particulate adsorption. Similar experimental studies on the ability of lichens to adsorb these types of particulates should be carried out. A parallel study using electron microscopy to discover the site of accumulation would also help to elucidate particulate trapping in lichens. Such research would augment wind tunnel and modelling studies using lichens and help determine the fate of trace element particulates in the environment (Schuepp, 1984; Milford and Davidson, 1985).

### 4. Intracellular uptake

In lichens, intracellular uptake of metals has been extensively studied by Brown and co-workers (Brown and Brown, 1991; Brown and Avalos, 1993),

particularly in respect of cadmium ions. The intracellular uptake of cadmium in *Peltigera membranacea* followed the behavior of transmembrane transport systems in other organisms and is probably energy requiring. The rate of uptake strongly correlates with the quantity of cadmium bound to the cell wall exchange sites. Calcium may be indirectly involved in the control of cadmium uptake by virtue of its role in maintaining membrane integrity and as an intracellular messenger. Cadmium uptake is not directly related to calcium transmembrane carried systems (Brown and Avalos, 1993) as has been found in mosses. Unfortunately, it is not possible as yet to produce protoplasts of lichen fungi collected from their natural habitat so that intracellular uptake can be studied in the absence of the cell wall as has been done in free-living fungi (Starling and Ross, 1990). The sequential elution techniques devised by Brown and co-workers could usefully be applied to studies on free-living fungi (Brown and Brown, 1991). It would be interesting to discover similarities and differences between symbiotic and non-symbiotic fungi using this technique.

## 5. Ecological and economic considerations

The value of lichens as biological monitors around metal-emitting industrial activities has been widely accepted (Richardson, 1992; Richardson et al., 1995). Such studies have even been made a condition of industrial waste permits for electricity generating stations. In monitoring studies, the question as to how quickly lichens reflect a change in metal deposition pattern is frequently raised. From transplant studies we know that they reflect metal deposition within about four months (Gailey et al., 1985), but how quickly do they respond to falling pollution levels? The physiological basis for mineral cycling in lichens has been recently reviewed by Brown and Brown (1991) and Knops et al. (1991). Experimental data on the temporal changes following closure of an industrial complex in south-west Louisiana, USA, has started to provide reliable answers to this question (Walther et al., 1990). They discovered that the mean residence time of metallic elements in the lichens *Parmotrema* and *Ramalina* ranged from 1.7 years for aluminium to 3.5 years for zinc. The collected lichen thalli were much older than this, so the question arises as to what factors cause the residence time to be short.

While some elements, such as potassium, can be easily washed from lichens when dry lichens are suddenly soaked by rain (Prussia and Killingbeck, 1991), it is difficult to understand how other metals such as Fe, originally accumulated as particulates, are lost. Are such metal particulates dissolved by the lichen compounds, or are they somehow displaced by freshly deposited particulates with higher electrostatic charge? If so, does this mean that the

bulk of particulates are adsorbed to the surface layers of the lichen rather than being located in the thallus interior? These are aspects which urgently need experimental study.

The effectiveness of lichens and fungi as biosorbants for metal ions and metal particulates has recently been demonstrated. Waste mycelium of *Rhizopus arrizus* grown by the fermentation industry to produce lipase has been shown to have commercial potential for removing metals from contaminated effluents. Non-viable granular yeast biomass is also capable of adsorbing over 99% of zinc, chromium and copper from electroplating waste water, although only about 50% of chromium was removed from tannery effluent (Brady et al., 1994). *Penicillium* pellets, grown on a mixture of sewage wastes and glucose, have been used directly to pack columns that provide excellent scavengers for waters containing low metal concentrations (Pumpel and Schinner, 1993). Similarly, the lichens *Bryoria* and *Letharia*, when immobilized in silica gel, concentrate dissolved metals tenfold from drinking water and compare favorably with commercial chelating resins (Ramelow et al., 1993). Lichens have also been shown to have useful applications for modifying carbon paste electrodes for the determination of metal ions in multi-element and speciation studies (Dempsey et al., 1992). Such applications can best be realized if we extend fundamental research to provide a better understanding of the basis of metal uptake by lichens and fungi. It is hoped that this review draws attention to areas that urgently need additional study.

### Acknowledgements

I would like to acknowledge the helpful comments made on a draft of this manuscript by Professors M.R.D. Seaward and M. Galun.

### REFERENCES

- Brady, D., Stoll, A., and Duncan, J.R. 1994. Biosorption of heavy metal cations by non-viable yeast biomass. *Environmental Technology* **15**: 429–438.
- Brown, D.H. 1991. Lichen mineral studies – currently clarified or confused. *Symbiosis* **11**: 207–223.
- Brown, D.H. and Avalos, A. 1993. The role of calcium in intracellular cadmium uptake by the lichen *Peltigera membranacea*. *Annals of Botany* **71**: 467–473.
- Brown, D.H. and Brown, R.M. 1991. Mineral cycling and lichens: the physiological basis. *Lichenologist* **23**: 293–309.

- Dempsey, E., Smyth, M.R., and Richardson, D.H.S. 1992. Application of lichen-modified carbon paste electrodes to the voltammetric determination of metal ions in multi-element and speciation studies. *Analyst* **117**: 1467-1470.
- Doyle, H.M. 1981. *An investigation into the uptake of lead and uranium in Hylocomium splendens*. Honours Thesis, School of Botany, Trinity College, Dublin.
- Folkesson, L. 1979. Interspecies calibration of heavy metal concentrations in nine mosses and lichens: applicability to deposition measurements. *Water, Air and Soil Pollution* **11**: 253-260.
- Fourest, E. and Roux, J.-C. 1992. Heavy metal biosorption by fungal mycelial by-products mechanisms and influence of pH. *Applied Microbiology and Biotechnology* **37**: 339-403.
- Gadd, G.M. 1993. Tansley Review No. 47: Interactions of fungi with toxic metals. *New Phytologist* **124**: 25-60.
- Gailey, F.A.Y., Smith, G.H., Rintoul, L.J., and Lloyd, O.L. 1985. Metal deposition patterns in central Scotland as determined by lichen transplants. *Environmental Monitoring and Assessment* **5**: 291-309.
- Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S.M., and Siegel, B.Z. 1983. Removal of uranium (VI) from solution by fungal biomass and fungal wall-related biopolymers. *Science* **219**: 285-286.
- Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S., and Siegel, B. 1984. Removal of uranium (VI) from solution by fungal biomass: inhibition by iron. *Water, Air, and Soil Pollution* **21**: 411-414.
- Galun, M., Galun, E., Siegel, B.Z., Keller, P., Lehr, H., and Siegel, S.M. 1987a. Removal of metal ions from aqueous solutions by *Penicillium* biomass: kinetics and uptake parameters. *Water, Air and Soil Pollution* **33**: 359-371.
- Galun, M., Siegel, S.M., Cannon, M.I., Ziegel, B.Z., and Galun, E. 1987b. Ultrastructural localization of uranium biosorption in *Penicillium digitatum* by stem X-ray microanalysis. *Environmental Pollution* **43**: 209-218.
- Garty, J. and Theiss, H.-B. 1990. The localization of lead in the lichen *Ramalina duriaei* (De Not.) Bagl. *Botanica Acta* **103**: 311-314.
- Goyal, R. and Seaward, M.R.D. 1982. Metal uptake in terricolous lichens. II. Effects on the morphology of *Peltigera canina* and *Peltigera rufescens*. *New Phytologist* **90**: 73-84.
- Hale, M.E. and Lawrey, J.D. 1985. Annual rate of lead accumulation in the lichen *Pseudoparmelia baltimorensis*. *Bryologist* **88**: 5-7.
- Herzig, R., Liebendorfer, L., Urech, M., Amman, K., Cuecheva, M., and Landolt, W. 1989. Passive biomonitoring with lichens as part of an integrated biological measuring system for monitoring air pollution in Switzerland. *International Journal of Environmental and Analytical Chemistry* **35**: 43-57.
- Honegger, R. and Peter, M. 1994. Routes of solute translocation and the location of water in heteromorous lichens visualized with cryotechniques in light and electron microscopy. *Symbiosis* **16**: 167-186.
- Jones, D., Wilson, M.J., and Laundon, J.R. 1982. Observations on the location and form of lead in *Stereocaulon vesuvianum*. *Lichenologist* **14**: 281-286.

- Knops, J.H.M., Nash, T.H., Boucher, V.L., and Schlesinger, W.L. 1991. Mineral cycling and epiphytic lichens: implications at the ecosystem level. *Lichenologist* **23**: 309–320.
- Lawrey, J.D. 1977. X-ray emission microanalysis of *Cladonia cristatella* from a coal strip-mining area in Ohio. *Mycologia* **69**: 855–860.
- Milford, J.B. and Davidson, C.I. 1985. The sizes of particulate trace elements in the atmosphere – a review. *Air Pollution Control Association Journal* **35**: 1249–1260.
- Majidi, V., Laude, D.A., and Holcombe, J.A. 1990. Investigation of the metal algae binding site with nuclear magnetic resonance. *Environmental Science and Technology* **24**: 1310–1315.
- Mullen, M.D., Wolf, D.C., Beveridge, T.J., and Bailey, G.W. 1992. Sorption of heavy metals by the soil fungi *Aspergillus niger* and *Mucor rouxii*. *Soil Biology and Biochemistry* **24**: 129–135.
- Muraleedharan, T.R. and Venkobachar, L.I. 1994. Further insight into the mechanism of biosorption of heavy metals by *Ganoderma lucidum*. *Environmental Technology* **15**: 1015–1027.
- Muraleedharan, T.R., Venkobachar, C., and Iyengar, L. 1994. Investigations of fungal fruiting bodies as biosorbents for the removal of heavy metals from industrial processing streams. *Separation Science and Technology* **29**: 1893–1903.
- Nieboer, E., Puckett, K.J., and Grace, B. 1976. The uptake of nickel by *Umbilicaria muhlenbergii*: a physicochemical process. *Canadian Journal of Botany* **54**: 724–733.
- Nieboer, E. and Richardson, D.H.S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environmental Pollution (Series B)* **1**: 3–26.
- Puckett, K.J., Nieboer, E., Gorzynski, M.J., and Richardson, D.H.S. 1973. The uptake of metal ions by lichens: a modified ion exchange process. *New Phytologist* **72**: 329–342.
- Pumpel, T. and Schinner, F. 1993. Native fungal pellets as a biosorbent for heavy metals. *FEMS Microbiology Reviews* **11**: 159–164.
- Prussia, C.M. and Killingbeck, K.T. 1991. Concentrations of ten elements in two common foliose lichens: leachability, seasonality, and the influence of rock and tree bark substrates. *Bryologist* **94**: 135–142.
- Ramelow, G.J., Liu, L., Himel, C., Fralick, D., Zhao, Y., and Tong, C. 1993. The analysis of dissolved metals in natural waters after preconcentration on biosorbents of immobilized lichen and seaweed biomass in silica. *International Journal of Analytical Chemistry* **53**: 219–232.
- Richardson, D.H.S. 1992. *Pollution Monitoring with Lichens*. Richmond Publishing, Slough.
- Richardson, D.H.S., Kiang, S., Ahmadjian, V., and Nieboer, E. 1985. Lead and uranium uptake by lichens. In: *Lichen Physiology and Cell Biology*, D.H. Brown, ed., Plenum Press, London.
- Richardson, D.H.S., Shore, M., Hartree, R., and Richardson, R.M. 1995. The use of X-ray fluorescence spectrometry for the analysis of plants, especially lichens, employed in biological monitoring. *Science of the Total Environment*, in press.



- Schuepp, P.H. 1984. Observations on the use of analytical and numerical models for the description of transfer to porous surface vegetation such as lichen. *Boundary Layer Meteorology* **29**: 59-73.
- Siegel, S., Keller, P., Galun, M., Lehr, H., Siegel, B., and Galun, E. 1986. Biosorption of lead and chromium by *Penicillium* preparations. *Water, Air and Soil Pollution* **27**: 69-75.
- Singleton, I. Wainwright, M., and Edyvean, R.G.J. 1990. Some factors influencing the adsorption of particulates by fungal mycelium. *Biorecovery* **1**: 271-289.
- Sloof, J.E. and Wolterbeek, B.T. 1993. Interspecies comparisons of lichens as biomonitors of trace-element air pollution. *Environmental Monitoring and Assessment* **25**: 149-157.
- Starling, A.P. and Ross, I.S. 1990. Uptake of manganese by *Penicillium notatum*. *Microbios* **63**: 93-100.
- Thomas, P.A., Sheard, J.W., and Swanson, S. 1994. Transfer of  $Po^{210}$  and  $Pb^{210}$  through the lichen-caribou-wolf food chain in Northern Canada. *Health Physics* **66**: 666-677.
- Walther, D.A., Ramelow, G.J., Beck, J.N., Young, J.C., Callahan, J.D., and Marcon, M.F. 1990. Temporal changes in metal levels of the lichens *Parmotrema prasorediosum* and *Ramalina stenospora*, Southwest Louisiana. *Water, Air and Soil Pollution* **53**: 189-200.
- Zhang, W. and Majidi, V. 1994. Monitoring the cellular responses of *Stichococcus bacillaris* to exposure to different metals using in vivo phosphorus NMR and other spectroscopic techniques. *Environmental Science and Technology* **20**: 1577-1582.