

UPTAKE, RETENTION AND TOXICITY OF HEAVY METALS IN LICHENS

A Brief Review

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(Received May 6, 1988; revised April 4, 1989)

Abstract. Literature on metals, particularly heavy metals, in lichens is reviewed including mechanisms of metal uptake, retention, toxicity and tolerance. Interspecies differences in sensitivity are discussed as well as the development and nature of extreme tolerance encountered in certain taxa.

1. Introduction

Lichens are symbiotic duplicate organisms, essentially fungi united only in having a functional co-existence with algae. There are at least 15 000 to 20 000 'species' spread all over the world in a wide range of ecosystems. Lichens are the first colonizers of exposed rocks in almost all climatic regions and particularly characteristic of dry or nutrient poor habitats in boreal and subarctic areas. They usually constitute the main vegetation under extreme alpine and arctic conditions.

Their mode of growth may be crustose (crust-forming), foliose (leaf-like) or fructose (shrub-like), including various intermediates. As with bryophytes they may be epigeic (growing on soil), epilithic (growing on rocks) or epiphytic (growing on higher plants, usually trees).

The fungal partner (mycobiont) of the lichen symbiosis is usually an ascomycete (a few basidiomycetes and some Fungi Imperfecti are known) and mostly unable to develop without an algal partner (phycobiont). As a contrast, the alga mostly belongs to genera or species which occur in a free-living state as well. Green algae (*Chlorophyceae*), predominate, but blue-green 'algae' (*Cyanobacteria*) are the phycobionts in some lichen genera. The green algae are mainly unicellular forms. *Nostoc* is the most common cyanobacterium.

Like bryophytes lichens are ectohydric; they absorb water over the entire surface of the thallus and easily lose it to the atmosphere in dry weather. Most lichens endure long periods of drought and some are evidently favored by more or less extensive periods in an air-dry condition.

Mineral nutrients are derived from ions dissolved in rainwater or throughfall but the substrate may also be an important source in many forms. Lichens produce lichen 'acids', substances unknown elsewhere in the biosphere, probably of importance in competition and possibly in the uptake of nutrients and immobilization of toxic elements.

The objectives of this paper are to give a brief review of the literature on uptake,

retention and toxicity of heavy metals in lichens, including some notes on possible tolerance mechanisms. The use of lichens as monitors of atmospheric deposition will not be treated here (cf., e.g., Nieboer and Richardson, 1981). Species names are given according to the references cited.

2. Mechanisms of Metal Uptake and Retention

The lichen thallus is characterized by cation exchange properties, just as in bryophytes (Tuominen, 1967) and the uptake of alkali and alkaline metal ions is mainly or partly a process of passive ion exchange. Also heavy metal cations are absorbed by exchange mechanisms. Metals absorbed from solution are in a completely exchangeable form initially. However, the exchangeable fraction decreases if the thalli are air-dried. In field material collected from a smelter area the proportion of exchangeable heavy metal varied between 13% in Fe and 83% in Zn (Nieboer *et al.*, 1972).

From a study of the cellular location of Pb and K in *Cladonia*, Brown and Slingsby (1972) concluded that the bulk of the K was present intracellularly in a freely diffusible form normally contained by the permeability barriers of the cell. Lead, however, whether taken up naturally or artificially, was bound to insoluble anionic sites in an exchangeable form. Competitive uptake studies by Puckett *et al.* (1973) revealed the selectivity sequence Fe (III) \gg Pb $>$ Cu \gg Ni, Zn $>$ Co, similar to the one demonstrated for moss by Rühling and Tyler (1970). The sequence is in accord with a cation uptake mechanism involving ion exchange modified by metal-complex formation.

Kinetic studies by Wainwright and Beckett (1975) on the binding of Zn ions by *Usnea florida* revealed the classical Langmuir adsorption isotherm. The Zn-binding was dependent on pH, explainable as competitive inhibition of Zn-binding by H⁺. The mass balance of the exchange of Ni for Sr, Sr for Tl and Sr for H⁺ was consistent with a cation exchange mechanism of metal-ion uptake in *Umbilicaria muhlenbergii* (Nieboer *et al.*, 1976b). Both the kinetics and the thermodynamics of Ni absorption in *Umbilicaria muhlenbergii* are governed by physicochemical processes. Metabolic inhibitors caused no significant decrease in Ni-uptake, and dead thalli accumulated Ni to a slightly greater extent than did live thalli (Nieboer *et al.*, 1976a). However, the major anions are likely accumulated against an electrochemical potential gradient and thus actively sorbed (Nieboer *et al.*, 1978).

The nature of the binding sites in lichens is still insufficiently known. As about 90% of the biomass usually is made up by the mycobiont, most of the cation exchange capacity is probably localized to the fungal cell walls, but consistent data to document this seems to be lacking. In an experiment using *Umbilicaria muhlenbergii* 66 and 34%, respectively, of the Ni²⁺ taken up by the thallus was associated with fungal and algal zones (Flora and Nieboer, 1980). Potassium may be particularly abundant in the alga zones (Asta and Garrec, 1980).

Reports of uronic acid content of lichens need to be treated with caution, due

to inadequacies of the techniques employed, according to Brown (1976). Aspartic and glutamic acid residues might be involved. Lichen compounds ('acids') seem not to be responsible for detectable cation binding according to Brown (op.c.). Nieboer *et al.* (1978) concluded that carboxylic and hydrocarboxylic groups were probably involved in the cation exchange but considered the phenolic (-OH), carboxylic acid (-COOH) and other functional groups (e.g., -CH=O) of lichen compounds appropriate for metal-complex formation.

2.1. SOURCES AND LOCALIZATION OF MINERAL NUTRIENTS AND HEAVY METALS

Precipitation and throughfall is a main or major source of mineral nutrition and heavy metals in many lichens (cf., e.g., de Bruin, 1985; Puckett, 1985). Pilegaard (1979) found concentrations of several metals linearly correlated with the atmospheric deposition, measured as bulk precipitation. However, soil particulates ('dust') and substrate seem to be of great importance in certain forms, of less or little importance in others. Lounamaa (1956) in his early work on baseline concentrations of metals in Finnish plants concluded that the nature of the substrate is accountable for differences in the metal content of lichens. The concentrations of Cr, Mn, and Ni were consistently higher in lichens from ultrabasic rocks, Pb and Zn higher from siliceous rocks, thus related to substrate concentrations. According to Nieboer *et al.* (1978) there is evidence that lichen compounds play an important role in rock weathering. Being present extracellularly on the hyphae they provide conditions favorable to the chemical decomposition of trapped particulates, as well.

The occurrence of extracellular heavy-metal containing particles in the medullar region of the thallus, entangled amongst the mycobiont hyphae, was demonstrated by Garty *et al.* (1979), Galun *et al.* (1984). Evidence of a widespread occurrence of trapped particulates in lichens was given by, e.g. Nieboer *et al.* (1978). Distinction has to be made between real 'uptake' and 'accumulation' as all chemical species are certainly not instantaneously available to the lichen (cf. Kershaw, 1985). In a longer perspective, however, elements contained in particulate matter may become soluble and incorporated in the ecosystem circulation.

That a transport of metals from the substrate to the thallus may occur in certain species is indicated from several studies. De Bruin (1985) and De Bruin and Hackenits (1986) suggested that epiphytic lichens may obtain Ca, Mn, Zn and Cd from the bark substrate, whereas bark was probably not a significant source for several other metal elements (e.g., V, Fe, Co, and As). A close relationship between the heavy metal content of the rhizinae of *Peltigera canina* and of the soil substrate was reported by Goyal and Seaward (1981), though it is not wholly evident that a further transport occurred to the main parts of the thallus. However, Goyal and Seaward (1982b) concluded that heavy metal cations were able to move freely from the rhizinae to the upper thallial surface and *vice versa*. It was suggested that the rhizinae are able to absorb, accumulate, translocate and regulate metals, thus preventing toxic amounts from reaching or penetrating the sensitive phycobiont.

It seems unquestionable that many lichens, capable of growing on metalliferous

rocks (*vide infra*), absorb and accumulate heavy metals from the substrate. Noeske *et al.* (1970) found Fe and Cu to accumulate in the form of a crust in and on the upper surface and sides of the areoles of *Acarospora sinoptica*, and (to a lesser extent) along the juncture between the rocks surface and the attachment of the thallus. According to Nieboer *et al.* (1978) all that is required for uptake is a film of moisture bridging the interface between rock surface and thallus.

Translocation of (mainly deposited) metals are also known to occur within different parts of the thallus. The diffusion coefficient for Pb in the top layer of a *Cladonia alpestris* carpet was estimated to be ca. $0.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Persson *et al.*, 1974) being one order of magnitude lower than the results obtained by Tuominen (1968) for ^{90}Sr ions in dead thalli of the same species. In a study of *Cladonia alpestris* by Mattsson (1975) indications were obtained of a shorttime internal cycling of ^{137}Cs in the lichen carpet. In dry periods certain amounts of the ion were forced to the crown region of the lichen and in periods of high rainfall relocated downwards again. Only a small proportion, however, did leave the carpet due to leaching.

2.2. INTRACELLULAR UPTAKE

Heavy metals immobilized in cell wall constituents or otherwise extracellularly located are usually considered non-toxic to the plant. The knowledge of intracellular uptake of heavy metals in lichens is scanty, but information on uptake mechanisms is also obtainable from studies using alkali and alkaline metal ions.

Handley and Overstreet (1968) studying the uptake of ^{137}Cs by *Ramalina reticulata*, concluded that uptake did not appear to be directly linked to metabolism. Results indicated the presence of a barrier, stabilized by Ca, to the entry of Cs. The barrier membranes involved could be the plasmalemmae of the algal or the fungal cells, or both. In *Cladonia rangiformis* most of the K was present intracellularly (Brown and Slingsby, 1972) whereas no intracellular Pb could be detected in spite of excessive quantities of Pb present in the thallus. Cesium ions are probably incorporated by an active process into the cytoplasm, whereas the divalent Sr ion is reversibly bound in the cell wall (Brown, 1976).

Heavy metal ions with lower affinity values for cell wall ligands than, e.g., Pb may to some extent penetrate the cytoplasm. Differences between taxa and populations of the same species seem to occur. Populations of lichens with enhanced photosynthetic resistance to Zn and Cd showed a lower intracellular Cd uptake than non-resistant populations (Beckett and Brown, 1983b). Light enhanced the intracellular Cd uptake indicating an active mechanism.

However, only a small share (2.4 to 7%) of the Zn supplied in short time experiments with ZnSO_4 on *Peltigera horisontalis* and *Cladonia rangiferina* was recovered intracellularly. In non-treated thalli the intracellular Zn fraction amounted to 29 to 47% of the total. Though not perfect, the presence of a barrier against excessive intracellular uptake was evident (Brown and Beckett, 1983). *Peltigera membranacea* previously shown to be tolerant to Zn-induced inhibition of photosynthesis, displayed a reduced rate of Cd uptake into the cells, particularly of the cyanobacterial

phycobiont. However, it seems that a severe metal stress is required before metal tolerance by exclusion occurs. As light stimulated the intracellular uptake of Cd this was considered to represent an active entry into the algal cells. It was also postulated that intracellular Cd uptake occurs by a system which normally transports Mg. With uptake in the dark it was not clear which symbiont was involved and whether energy was required (Beckett and Brown, 1984a, b). *Cladonia rangiformis* from Pb-rich habitats contained K mostly in an intracellular, soluble form, Ca and Pb in an extracellular, exchangeable form, with Mg and Zn showing an intermediate pattern, partly dependent on total element concentration (Brown and Buck, 1985).

Further information on evidence for intracellular uptake in lichens is given in Kershaw (1985). The role of the cell wall in the intracellular uptake of cations was recently reviewed by Brown and Beckett (1985).

It must be concluded that the uptake of heavy metal ions is primarily a passive extracellular process of ion exchange and complex formation. Ligands are present in the cell walls of both symbionts, those of the mycobiont being quantitatively most important. Depending on the degree of affinity for the ligands and the total metal load certain heavy metal ions, at least Cd and Zn, may penetrate the plasmalemmae and occur in the cytoplasm. In metals with a high affinity for the cell wall ligands (e.g., Pb) only very small amounts are supposed to be present intracellularly. Copper and Ni, though practically no experimental evidence exists, must be postulated to take an intermediate position.

3. Toxicity and Tolerance

As is the case with bryophytes (see Tyler, 1989) the tolerance of lichens to elevated tissue concentrations of heavy metals varies greatly between species, populations and elements. Extreme tolerance to high tissue concentrations has been reported in several cases (Table I). Few studies have been carried out where several metals have been considered simultaneously, which makes comparisons between elements difficult. Generally, however, Hg, Ag and Cu seem to be more toxic than Pb, Zn, and Ni, as concluded by Nieboer *et al.* (1975).

3.1. EXPERIMENTAL EVIDENCE

A depression of gas exchange rates at elevated Pb concentrations in lichen thalli has been reported by, e.g., James (1973), Puckett (1976), Punz (1979) and Déruelle and Petit (1983). Lower Pb concentrations (80 to 300 $\mu\text{g g}^{-1}$) were found to enhance gas exchange rates in *Parmelia caperata* and *Cladonia portentosa* (Lemaistre, 1985). The relative toxicity of several elements tested to the photosynthetic activity of non-tolerant *Umbilicaria muhlenbergii* and *Stereocaulon paschale* was, on average, Ag, Hg > Cu, Cd > Pb, Ni (Puckett, 1976). Increasing concentrations (>0.1 mM) of Cu, Hg or Ag salts produced a gradual loss of K from the thallus, interpreted as an increased cell permeability, as most K is located intracellularly.

TABLE I

High total concentrations of heavy metals recorded in lichens (mg kg⁻¹ dry weight) according to literature evidence. Unless otherwise stated a metalliferous substrate was the main source of the metal

| Species | Concentration in thallus | Reference |
|---|---|---|
| <i>Acarospora sinoptica</i> | Cu = 1100 | Lange and Ziegler (1963) |
| <i>Alectoria pubescens</i> | Cu = 1000 | Alstrup and Hansen (1977) |
| <i>Cladonia</i> sp. | Cu = 2100 | Dykeman and de Sousa (1966) |
| <i>Cornicularia muricata</i> | Pb = 12000 Zn = 5600 | Rao <i>et al.</i> (1977) |
| <i>Lasallia populosa</i> ^a | Zn = 2500 Cd = 335 | Nash (1971, 1975) |
| <i>Lecanora muralis</i> | Pb = 3120 Zn = 2470 | Seaward (1973, 1974) |
| <i>Lecanora polytropia</i> | Cu = 4900 | Alstrup and Hansen (1977) |
| <i>Lecanora vinetorum</i> ^b | Cu = 5000 | Poelt and Huneck (1968) |
| <i>Parmelia</i> sp. | Pb = 1620 Zn = 3270 | Rao <i>et al.</i> (1977) |
| <i>Peltigera canina</i> | Zn = 880 Pb = 9900 Pb = 7700 Zn = 3500 | Goyal and Seaward (1982a) Seaward (1974) Rao <i>et al.</i> (1977) |
| <i>Peltigera rufescens</i> | Pb = 1560 | Goyal and Seaward (1982a) |
| <i>Stereocaulon nanodes</i> | Zn = 3300 | Maquinay <i>et al.</i> (1961) |
| <i>Stereocaulon paschale</i> | Cu = 3000 | Lounamaa (1956) |
| <i>Umbilicaria lyngei</i> | Cu = 2100 | Alstrup and Hansen (1977) |
| <i>Verrucaria nigrescens</i> ^a | Zn = > 23000 | Nash (1975) |

^a Zinc smelter emission.

^b Fungicide spray.

Lichen photosynthesis seems to be more sensitive than respiration to heavy metals. Dark respiration was not adversely affected in lichens incubated in solutions containing 0.1 to 10 mg L⁻¹ of Pb, Cd, or V (Johnsen, 1976). Respiration exceeded photosynthetic rate (net photosynthesis < 0) in non-tolerant *Lasallia populosa* containing 480 to 3300 mg kg⁻¹ Zn and 860 to 7600 mg kg⁻¹ Cd. Corresponding concentration values for *Cladonia uncialis* were 450 mg kg⁻¹ Zn and 320 mg kg⁻¹ Cd (Nash, 1975).

Lichens containing cyanobacterial phycobionts seem to be more sensitive to heavy metals (Zn, Cd, Cu, Pb) than those containing green algae. A 50% reduction of photosynthesis was measured in *Peltigera horisontalis* (containing cyanobacteria) exposed to 0.05 to 0.10 mM solutions of Zn, Cd and Cu (as sulphate), whereas > 1M solutions of Zn and Cd were required to produce the same reduction in *Cladonia rangiformis* (containing green algae) (Brown and Beckett, 1983). Ten species of lichens containing cyanobacteria, exposed to 1 mM ZnSO₄, decreased their photosynthetic activity to 5 to 40% of the controls, whereas nine other species

containing green algae were almost unaffected.

In other studies 0.3 mM Zn or Cu was shown to be toxic to the photosynthesis of the cyanobacteria-containing genus *Peltigera* (Beckett and Brown, 1983b). A non-tolerant population of *Peltigera membranacea* lost 90% of its photosynthetic activity on exposure to 0.1 mM ZnSO₄ (Beckett and Brown, 1984a). The same species from a polluted site proved to be much less sensitive.

The nitrogen fixation of two species of Nostoc, one of them a lichen phycobiont, was inhibited by 0.025 to 0.125 mg L⁻¹ of Cd, Pb, and Zn. Lower concentrations had a stimulating effect (Henriksson and DaSilva, 1978). The phosphatase activity of *Cladonia rangiferina*, probably extracellularly located on the cell wall or plasmalemma, was inhibited by vanadate, vanadyl, uranyl and molybdate ions, whereas a slight enhancement was observed with Cu and Ni ions (Lane and Puckett, 1979; Le Sueur and Puckett, 1980). Urea-induced urease in *Evernia prunastri* was competitively inhibited by Ni at >40 μM, though this metal is essential for producing the active protein (Perezurria *et al.*, 1986).

3.2. TOLERANCE MECHANISMS

Based on metal-induced reduction in photosynthesis *Peltigera* spp., sampled from a range of sites with contrasting metal status, were demonstrated to possess Zn tolerance directly proportional to the Zn content of the thallus. Differences in Cu tolerance were not related to the Cu content of the thallus but rather to the Zn content (Beckett and Brown, 1983a). *P. membranacea* from an uncontaminated site, pretreated with 0.005 mM ZnSO₄ (30 min) one week before was much more resistant to 0.1 mM ZnSO₄ than not pretreated material. There was also some increase in Cd but not in Cu resistance. This indicates that at least part of the Zn resistance found in lichens from metal polluted localities may be phenotypically acquired.

Peltigera membranacea, shown to be tolerant to Zn-induced inhibition of photosynthesis, displayed a reduced rate of Cd intracellular uptake, suggesting that metal exclusion was a tolerance mechanism. However, a severe metal stress was required before metal tolerance by exclusion occurred (Beckett and Brown, 1984a).

Already Lange and Ziegler (1963) proposed that inherent cytoplasmatic tolerance, cytoplasmatic immobilization of ions and transport of ions to regions external to the plasma membrane on the cell wall were the mechanisms of heavy metal tolerance in lichens. However, the high affinity of Pb for cell wall ligands makes it unnecessary to postulate any cytoplasmatically located tolerance mechanism for Pb in lichens, according to Brown and Slingsby (1972).

Lichens able to endure extremely metalliferous sites may possess various mechanisms of metal detoxification, as suggested by Purvis *et al.* (1985, 1987) and Purvis and James (1985). *Acrospora smaragdula* and closely related taxa develop a greenish hue (normally pale reddish brown) when growing on Cu-rich rocks, probably due to precipitation of a Cu-complex with a lichen compound (norstictic acid) on the thallus surface and within the upper cortex. Copper oxalate has also been detected in the medulla of several lichens from cupriferous rocks and *Lecanora*

cascadensis can accumulate a dark green Cu-salt of unknown composition.

A special form of detoxification seems to be accomplished by the rhizinae of certain lichen species. It was suggested by Goyal and Seaward (1982b) that the capability of rhizinae to accumulate and regulate metals may prevent toxic amounts from reaching the sensitive parts of the thallus.

However since so few lichen culture experiments have been performed or reported it is difficult to conclude in more detail the nature of mechanisms involved in metal tolerance. As being the case with bryophytes, extreme resistance to Cu (possibly other heavy metals as well) is often, though not always, accompanied by a high resistance to acidity.

3.3. FIELD EVIDENCE OF METAL TOXICITY

Morphological changes of lichen thalli related to high levels of heavy metals have occasionally been reported (e.g., Lambinon *et al.*, 1964). The morphology of *Peltigera canina* and *Peltigera rufescens* was changed in a metal polluted environment: the rhizinae became shorter, the thallus smaller with profusely branched veins, and hypertrophy of the medulla occurred (Goyal and Seaward, 1982a).

Otherwise, toxic effects on lichens under field conditions have been recorded as a sudden or gradual decrease in species abundance or species numbers/diversity along a known gradient of heavy metal exposure. Very few such studies do exist, the main ones being those of Le Blanc *et al.* (1974), Folkeson (1981) and Folkeson and Andersson-Bringmark (1988).

Thresholds for survival measured as metal concentration in thalli, were reported by Rao *et al.* (1977). It was suggested that the following maximum combined concentrations (mg kg^{-1}) could be endured under field conditions when accumulation occurs over a long period of time:

| | Pb | Cu | Zn | Cd |
|----------------------------|---------|---------|---------|---------|
| <i>Hypogymnia physodes</i> | 718-918 | 210-405 | 168-233 | 5.0 |
| <i>Parmelia squarrosa</i> | 175-575 | 255-283 | 200-260 | 3.5-4.7 |

The values should only be interpreted as the highest concentrations of these four elements measured in the thalli in a heavy metal gradient before total disappearance of the species. Which of the elements were really limiting to the distribution towards the metal source is not known, but Cd is quite unlikely in this case. Emissions of SO_2 may further complicate the picture. That several lichens under certain conditions may tolerate much higher concentrations is evident from Table I.

The highest concentrations in lichens recorded in the heavily SO_2 and metal polluted Sudbury district, Canada, were 250 mg kg^{-1} for Cu and 310 mg kg^{-1} for Ni in the most tolerant species, *Stereocaulon paschale* (Nieboer *et al.*, 1975; Hutchinson, 1979). These limits might have been influenced by SO_2 or acidification as well.

LeBlanc *et al.* (1974) studied the response of lichens to SO₂ and heavy metal (i.a. Pb, Cu) pollution around a primary copper smelter in Quebec, Canada. From their results the degree of tolerance in several widespread lichen species may be concluded (Table II), but no metal concentration data were reported. The lichen flora within an area greatly influenced by SO₂ and metal (Zn, Cd) pollution from a Zn smelter at Palmerton, Pennsylvania, was studied by Nash (1971, 1972). Only 9 species were recorded in the polluted area, compared to 84 species in an otherwise similar control area. The tolerant species were mainly crust-forming saxicolous forms; only one epiphyte could survive the pollution. The highest Zn concentration probably ever recorded in lichens, > 23 000 mg kg⁻¹, was measured in *Verrucaria nigrescens* from this area (Nash, 1975).

Only one field study of this kind where SO₂ was of definitely minor importance to the results, seems to have been reported. Folkesson (1981) and Folkesson and Andersson-Bringmark (1988) made an extensive mapping of the decline of the plant cover in a coniferous forest area of southeast Sweden (Gusum) polluted by Cu

TABLE II

Degree of tolerance in widespread lichen species to SO₂ and heavy metal pollution in the surroundings of a primary copper smelter in Quebec (LeBlanc *et al.*, 1974)

| Most tolerant | Rather tolerant | Less tolerant or sensitive |
|-------------------------|-----------------------------|-------------------------------|
| <i>Mycoblastus</i> | <i>Alectoria capillaris</i> | <i>Lecidea cinnabarina</i> |
| <i>Sanguinarius</i> | <i>Alectoria sarmentosa</i> | <i>Parmelia saxatilis</i> |
| | <i>Centraria pinastri</i> | |
| <i>Parmelia sulcata</i> | <i>Cetraria sepincola</i> | <i>Parmelia trabeculata</i> |
| | <i>Hypogymnia physodes</i> | <i>Pertusaria multipuncta</i> |
| | <i>Parmeliopsis ambigua</i> | <i>Pertusaria pertusa</i> |
| | <i>Platismatia glauca</i> | |
| | <i>Cladonia coniocraea</i> | |
| | <i>Ramalina minuscula</i> | |

TABLE III

Critical concentrations (mg kg⁻¹ dry weight) of Cu and Zn in two quantitatively important lichens of coniferous forest surrounding the Gusum brass foundry in Sweden (from: Folkesson and Andersson-Bringmark, 1988)

| | Conc. in remote sites | Conc. at first sign of cover decrease | Conc. at obvious sign of cover decrease | Apparent threshold conc. for survival |
|-----------------------------|-----------------------|---------------------------------------|---|---------------------------------------|
| <i>Cladonia rangiferina</i> | | | | |
| Cu | 5-7 | 300 | 320 | 350 |
| Zn | 55-75 | 500 | 550 | 600 |
| <i>Hypogymnia physodes</i> | | | | |
| Cu | 11-15 | 300 | 600 | 700 |
| Zn | 95-140 | 1300 | 1800 | 1900 |

TABLE IV

Levels of tolerance of Cu+Zn pollution in widespread epigeic or epilithic *Cladonia* lichens in the Gusum brass mill area, S Sweden (from: Folkeson, 1981; Folkeson and Andersson-Bringmark, 1988)

| Most tolerant | Tolerant | Rather sensitive | Most sensitive |
|---|-----------------------|-----------------------|---------------------|
| <i>Cladonia chlorophaea</i> agg. | <i>C. carneola</i> | <i>C. arbuscula</i> | <i>C. stellaris</i> |
| <i>C. coniocraea</i> et <i>ochrochlora</i> | <i>C. cenotea</i> | <i>C. rangiferina</i> | |
| | <i>C. cornuta</i> | <i>C. uncialis</i> | |
| | <i>C. fimbriata</i> | | |
| | <i>C. furcata</i> | | |
| | <i>C. gracilis</i> | | |
| | <i>C. macrophylla</i> | | |
| | <i>C. squamosa</i> | | |
| | <i>C. subulata</i> | | |

and Zn from a brass foundry. For the most frequent taxa the authors were able to determine the concentrations of Cu and Zn at which the first signs of a cover reduction was discernible, as well as the apparant threshold concentration for survival (Table III). The concentration difference between these critical levels is definitely small and it is probable that a sudden increase of the cytoplasmatic penetration (plasmalemma permeability) occurs in one or both of these elements.

However, several lichen taxa were more sensitive than those of Table III. A detailed study of the relative sensitivity of the many *Cladonia* species in the *Gusum* area is also reported by Folkeson and Andersson-Bringmark (1988) (Table IV). The highest number of *Cladonia* taxa was found at intermediate degrees of pollution, probably favored by the decline of otherwise dominating forest mosses. *Cladonia stellaris*, however, proved very sensitive to Cu + Zn pollution and *C. uncialis* was also absent in the more polluted sites mainly colonized by *C. chlorophaea*, *C. coniocraea*, and *C. ochrochlora*.

The first effects on the occurrence of epiphytes on spruce in the *Gusum* study were observed when the Cu and Zn concentrations of the comparatively tolerant *Hypogymnia physodes* were ca. 80 and ca. 500 mg kg⁻¹, respectively (Folkeson and Andersson-Bringmark, 1988). There was a continuous and marked decline of the epiphytic lichen flora with increasing metal load.

4. Conclusions

(1) Lichens are supplied with mineral nutrients and heavy metals from precipitation, throughfall and dust but also from the substrate, the proportion taken by the various sources being dependent on species characteristics, substrate properties and amounts contained in the deposition.

(2) The extracellular uptake of metal ions is essentially a passive process of ion exchange and complex formation with cell wall ligands, chiefly those of the fungal

constituent (mycobiont).

(3) Intracellular uptake is limited by the nature of the metal ion, the permeability properties of the cell membrane and the amount of extracellular ligands. It seems membrane permeability may increase considerably on exposure to elevated concentrations of toxic metal ions. Cations with a very high affinity for the extracellular ligands, e.g., Pb^{2+} , are almost exclusively encountered extracellularly in the thallus, whereas those with a low affinity for ligands, e.g., K^+ , are mainly found intracellularly. Most heavy metal ions are supposed to take an intermediate position between these two extremes, but there is a lack of detailed knowledge in this field.

(4) The sensitivity of lichens to elevated tissue concentrations of heavy metals varies greatly between species, population and elements. The relative toxicity seems to decrease in the order $Hg, Ag > Cu, Cd (?) > Zn, Ni \geq Pb$. However, the degree of sensitivity varies considerably within the same population or even individual, according to previous exposure to the metal. This indicates that at least part of the tolerance found in lichens from metal polluted localities may be phenotypically acquired.

(5) Tolerance mechanisms mainly include immobilization of the toxic metal ions in biologically non-active forms: as extracellular complexes in metabolically less active thallus fractions and as precipitated organic salts (for Cu as oxalate, Cu-lichen compounds, etc.).

(6) Limited information is available from the field concerning threshold concentrations of metals in lichen thalli. Quantitatively important woodland species may endure ca. 300 mg kg^{-1} Cu and 500 to 1300 mg kg^{-1} Zn without visible damage. However, only slightly higher tissue concentrations may be lethal. There is evidence that the most sensitive lichens are affected already at a concentration of ca. 80 mg kg^{-1} (Cu), i.e. 5 to 10 times the current regional base-line level of south Sweden. In combination with SO_2 or strong acidity the critical metal concentrations would be lower, though it is usually not apparent which factor is limiting the distribution under such conditions.

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