The Lichenologist **37**(5): 409–423 (2005) © 2005 The British Lichen Society
doi:10.1017/S0024282905014933 *Printed in the United Kingdom*

Manganese as a site factor for epiphytic lichens

Markus HAUCK and Alexander PAUL

Abstract: Decreasing abundance of epiphytic lichens with increasing Mn supply from the substratum or from stemflow was found in several coniferous forests of Europe (Germany) as well as western (Montana, British Columbia) and eastern North America (New York State). Experiments carried out with *Hypogymnia physodes* and other species of chloro- and cyano-lichens suggest that these correlations are causal. High Mn concentrations e.g. reduce chlorophyll concentrations, chlorophyll fluorescence and degrade the chloroplast in lichen photobionts. Excess Mn inhibits the growth of soredia of *H. physodes* and causes damage in the fine- and ultra-structure of the soredia. Adult lichen thalli remain structurally unaffected by Mn. Manganese uptake does not result in membrane damage. Calcium, Mg, Fe and perhaps also Si alleviate Mn toxicity symptoms in *H. physodes*. *Lecanora conizaeoides* is not sensitive to Mn in laboratory experiments or in the field. The data suggest that high Mn concentrations are an important site factor for epiphytic lichens in coniferous forests that until recently has been overlooked. Manganese reaching the microhabitat of epiphytic lichens is primarily soil-borne and is usually not derived from pollution.

Key words: bark chemistry, heavy metal tolerance, *Hypogymnia physodes*, *Lecanora conizaeoides*, precipitation chemistry, transition metals

Introduction

The significance of cation concentrations for the physiology and distribution of vascular plants is widely accepted and has been the subject of extensive studies. Exhaustive knowledge is available, for example, for the effects of Ca, Mg, Na, Fe, Al, Mn, Zn, Cu and a range of non-essential heavy metals. Elemental analyses of the soil are routinely carried out with standardized extraction methods to describe the chemical environment of a vascular plant. The situation is quite different in lichen ecology. Here, scientists occasionally even debate which principal environmental sources contribute to the cation supply of lichens. Schade (1970) and Rasmussen *et al.* (1980), for instance, denied that there was any cation uptake from the substratum. Nevertheless, there is conclusive evidence from several field studies that lichens take up cations from air-borne particles, precipitation and the substratum (Nash 1989; Farmer *et al.* 1991; Gauslaa *et al.* 1998; Hauck 2003).

Detailed investigations into the pathways cations take into the lichen thallus or of the fate of cations within the thallus deal principally with heavy metals. Based on correlations between high Mn concentrations and low epiphytic lichen abundance repeatedly found in the field, we have conducted a case study of the ecophysiological role of this transition metal in lichens. Our research on the effects of Mn on lichens differs from previous heavy metal and lichen studies of, for example, Nieboer *et al.* (1972), Nash (1975), Puckett (1976), Brown & Beckett (1983), Beckett & Brown (1984), Purvis & Halls 1996, or Garty *et al.* (1992). This is because the studies cited generally deal with transition metals that either occur in extreme heavy metal-rich rock environments or are found in 'normal' ecosystems as a result of anthropogenic pollution. In the case of Mn toxicity, we are dealing with toxic

M. Hauck and A. Paul: Albrecht von Haller Institute of Plant Sciences, University of Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany.

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

concentrations that occur naturally in forest ecosystems.

Biological function of manganese in lichens

Any study dealing with toxicity effects of Mn should consider that Mn is also an essential micronutrient. Manganese participates in many basic vital processes, for example, in the water-splitting reaction of photosynthesis or in translation (Burnell 1988; Crowley *et al.* 2000). Furthermore, Mn is a constituent of many enzymes, for instance, of several dehydrogenases, transferases and hydrolases. The Mn-containing enzymes isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase take part in the citrate cycle (Burnell 1988; Crowley *et al.* 2000). Manganese-dependent superoxide dismutase occurs in every lichen species (Whittaker 2000; Stroupe *et al.* 2001). These examples show that lichens are not generally limited by Mn, but only by excess concentrations. However, the concentration of Mn required by a lichen in the field is apparently very low, since limitation of lichen abundance caused by Mn deficiency has never been found. Culture media used for the cultivation of lichen bionts, such as Bold's Basal Medium (BBM), contain only a few μM of Mn (Ahmadjian 1993).

Field data

Field observations on the correlation between epiphytic lichen abundance and Mn supply were made in several coniferous forests of central Europe as well as western and eastern North America. Decreasing cover values of the foliose chlorolichen *Hypogymnia physodes* and other lichens with increasing Mn concentration in bark or stemflow were found repeatedly in stands of *Picea abies* in the Harz Mountains, Germany (Hauck 2000, 2003; Hauck *et al.* 2001, 2002*a*). In one spruce stand, the cover of *H. physodes* was most closely correlated with the ratio of Mn to Ca concentrations in stemflow (Hauck *et al.* 2002*a*). In general, however, Mn concentrations in bark revealed more often an influence on lichen diversity than the Mn content of stemflow (Hauck 2003, 2005). Cover of the crustose chlorolichen *Lecanora conizaeoides*, which is known for its toxitolerance, did not respond to Mn concentrations. A wide range of chemical site factors was analyzed in these investigations so that intercorrelation with another ion concentration could be ruled out as the cause of the correlations between lichen abundance and Mn concentrations (Hauck *et al.* 2001, 2002*a*, *b*; Hauck & Runge 2002).

In a montane *Picea rubens–Abies balsamea* forest in the Adirondacks, upstate New York, chlorolichen species including *Hypogymnia physodes* decreased with increasing Mn/Fe ratio in *P. rubens* bark (Schmull & Hauck 2003*a*), while a few lichen species decreased with the Mn content of stemflow (Schmull *et al.* 2002). In *Picea engelmannii–Abies lasiocarpa* stands of the North American Intermountain West (in northwestern Montana) cover of several epiphytic chlorolichens decreased with increasing Mn concentration in the substratum or its ratio to Ca, Mg or Fe (Hauck & Spribille 2005). While epiphytic cyanolichens were absent from all these sites mentioned, so far, observations on *Pinus monticola* growing in the dripzone of *Populus balsamifera* in northwestern Montana indicated that cyanolichens could be more sensitive to high Mn supply than chlorolichens. Bipartite cyanolichens of the genera *Leptogium*, *Lobaria* and *Nephroma* were restricted to microhabitats with the lowest Mn content of bark, i.e. to pine branches within the dripzone of *Populus* (Hauck & Spribille 2002). The tripartite lichen *Lobaria pulmonaria*, which contains the green alga *Dictyochloropsis* and the cyanobacterium *Nostoc* (Rikkinen 2002), grew on bark of medium Mn content. The most Mn-rich microhabitats were inhabited only by chlorolichens. These observations on cyanolichens by Hauck & Spribille (2002) are based on a very small data set. However, similar findings have been reported previously by Goward & Arsenault (2000) on *Picea engelmannii* × *glauca* in British

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

Columbia, though these authors did not notice the correlation with Mn concentrations in their data.

Biogeochemical cycling of manganese in forest ecosystems

Manganese in tree bark is primarily soil-borne. Its solubility increases strongly with increasing acidity, which is also increased by water-logging, because insoluble Mn^{3+} and Mn^{4+} ions are converted into soluble Mn^{2+} in a reducing environment (Norvell 1988). Manganese^{2+} is readily taken up by the roots of vascular plants (Clarkson 1988) and, in the transpiration flux, reaches the stem and is allocated to all living tissues, the young foliage in particular (Lin *et al.* 1996). Although it has an essential metabolic role, excess concentrations of Mn are toxic to vascular plants (El-Jaoual & Cox 1998) including coniferous trees, where exposure to high Mn concentrations results in damage related to reduced uptake of Fe, Ca and Mg (Keil *et al.* 1986; Gartner *et al.* 1990; Kaus & Wild 1997). Translocation of Mn to the bark is apparently a widespread mechanism in trees to avoid toxic concentrations of free Mn^{2+} in physiologically more important tissues. Lövestam *et al.* (1990) and Sloof & Wolterbeek (1993) found evidence for the radial transport of Mn through the xylem parenchyma in *Picea abies* and *Populus* sp. Manganese is often deposited in the inner layers of the bark, which was taken as additional evidence for the mainly soil-borne (and not atmospheric) origin of the ions (Lövestam *et al.* 1990). *Abies balsamea* from upstate New York, however, was found to deposit Mn primarily in the outer bark, though comparison of element concentrations in bark, soil, stemflow and incident precipitation indicated that the bark Mn content was most significantly affected by the concentration available from the soil (Schmull *et al.* 2002; Schmull & Hauck 2003*a*). Fink (1999) provided detailed information on Mn deposition in the inner bark of *Malus domestica*, where cells with high Mn concentration become necrotic, while surrounding parenchyma cells undergo repeated cell divisions and, finally, form a periderm that seals the areas with Mn deposits. Other species deposit Mn in lignified tissues before their death. *Abies balsamea* immobilizes Mn in crystals in spongy cork and sclerotic phelloid cells of the outer bark (Schmull & Hauck 2003*a*). The chemical composition of these crystals is not known, but the absence of significant amounts of P, S, and Cl suggests that oxalate could be the corresponding anion. This is supported by Ca oxalate crystals, which are known to be formed in the bark of *Picea abies* under conditions of Ca profusion (Schmidt-Vogt 1986), and by the fact that Mn is deposited in oxalate crystals in the epiphytic lichen *Hypogymnia physodes* (Paul *et al.* 2003) and the saxicolous lichen *Pertusaria corallina* (Wilson & Jones 1984).

While the disposal of Mn in dying bark tissues is economic for the tree, it means that the part of a tree, which is inhabited by epiphytes, namely the bark surface, is particularly rich in Mn. Manganese^{2+} is a very mobile ion in plant tissues and, thus, together with K^+, is readily leached from the foliage and from bark (Fassbender 1977; Schmidt 1987). Leaching of Mn depends strongly on weather conditions. Levia & Herwitz (2000) found the most intense Mn leaching from *Carya glabra* during snowmelt at temperatures around 0 °C, when the viscosity of stemflow and the surface tension were particularly high. This agrees with observations of marked peaks in the Mn content of stemflow during the cold season in stands of *Picea abies* in the Harz Mountains, which could not be attributed to peak concentrations in incident precipitation (Hauck 2000). Thus, snowmelt water and stemflow from rainfall at low temperature contribute considerably to the Mn budget of bark surfaces, although the amount of such stemflow is negligible compared to stemflow that occurs in the warm season in temperate and boreal forests (Hauck 2000; Levia & Underwood 2004).

As a consequence of their high mobility, Mn^{2+} ions are also readily (re-)adsorbed by tree canopies. Most of the adsorbed Mn

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

remains on the canopy surface, i.e., in the foliage (especially in epicuticular wax layers) or in the bark (Lin *et al.* 1995; Lin & Schuepp 1996). Manganese adsorbed in the canopy is partly derived from precipitation and partly from aerosols (Schmidt 1987), whereas some ions are re-adsorbed after leaching. Quantification of the latter process has not been published. Atmospheric deposition of Mn is generally of less significance for the Mn content of bark than the amounts that are deposited in a tree after root uptake. This is supported by the close correlation of the Mn content of bark with the Mn content of the soil, but not of incident precipitation, which was found in five *Picea abies* stands of the Harz Mountains (Fig. 1A–D). Stemflow concentration is more difficult to predict (Fig. 1E), as it seems to depend on several variables, such as the Mn content of soil, incident precipitation and dry deposition and on the physiological condition of the trees. Since the bark Mn content has usually a more significant effect on lichen abundance than the Mn content of stemflow and moreover since the deposition of Mn from the atmosphere is primarily of natural origin in many areas, these data suggest that any effects of Mn on lichen vegetation are generally natural, but might be enhanced due to anthropogenic soil acidification. Canada is an exception to this rule, because the Mn-containing compound methylcyclopentadienyl manganese tricarbonyl (MMT) has been used since 1977 as an anti-knock additive in gasoline on a large scale (Lin *et al.* 1995). However, even there the quantitative role of anthropogenic Mn emissions on the total atmospheric Mn input is controversial (Forget *et al.* 1994; Zayed *et al.* 1999). In our own measurements of Mn concentrations in incident precipitation from northern New York State and north-western Montana, not far from the Canadian border (Schmull *et al.* 2002; Hauck & Spribille 2005), we did not find significantly higher concentrations than in Germany, where MMT is not used (Hauck & Runge 2002; Hauck *et al.* 2002*a*, *b*).

In vitro manganese uptake in lichens

Uptake of Mn from $MnCl_2$ solutions has been studied in the chlorolichens *Hypogymnia physodes* and *Lecanora conizaeoides* (Hauck *et al.* 2002*c*), *Alectoria sarmentosa* and *Bryoria fuscescens* (Paul 2005), and in the tripartite lichen *Lobaria pulmonaria* as well as in the bipartite cyanolichens *Leptogium saturninum* and *Nephroma helveticum* (Hauck *et al.* 2005). Both extracellular adsorption and intracellular uptake of Mn are commensurate with the concentration applied in the incubation medium (Table 1). At a given Mn concentration, extracellular adsorption and intracellular uptake reach a saturation level after 10–20 min. Rates of extracellular adsorption are generally much higher than of intracellular uptake (Hauck *et al.* 2002*c*). Similar temporal dynamics of cation uptake were found for Cu^{2+} in *Ramalina fastigiata* (Branquinho *et al.* 1997*a*). The spatial pattern of Mn in the thallus with significant concentrations at extracellular exchange sites and intracellularly is a result of the physico-chemical properties of Mn^{2+}, which has been classified as a borderline ion with strong class A character by Nieboer & Richardson (1980).

While the aquatic green alga *Scenedesmus subspicatus* is known to convert significant proportions of extracellular Mn^{2+} into Mn^{3+} and Mn^{4+} (Knauer *et al.* 1999), only a small percentage of the total extracellular Mn is Mn^{3+} or Mn^{4+}. This percentage involved less than 1% of the total extracellularly bound Mn in samples of *Hypogymnia physodes* and 3% of the total extracellularly bound Mn in samples of *Lecanora conizaeoides* exposed to millimolar concentrations of $MnCl_2$ for 1 h (Hauck *et al.* 2002*c*). Extracellular cation exchange sites that bind to Mn are not only found on the surface of cell walls (Richardson 1995), but also in extracellular polysaccharide matrices. In *H. physodes*, as much Mn was found in the interhyphal matrix as in the cell walls of cortical hyphae (Paul *et al.* 2003).

Photobiont cell walls in thallus pieces of *H. physodes* incubated with 5 mM $MnCl_2$ for 1 h contained about 20% of the

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

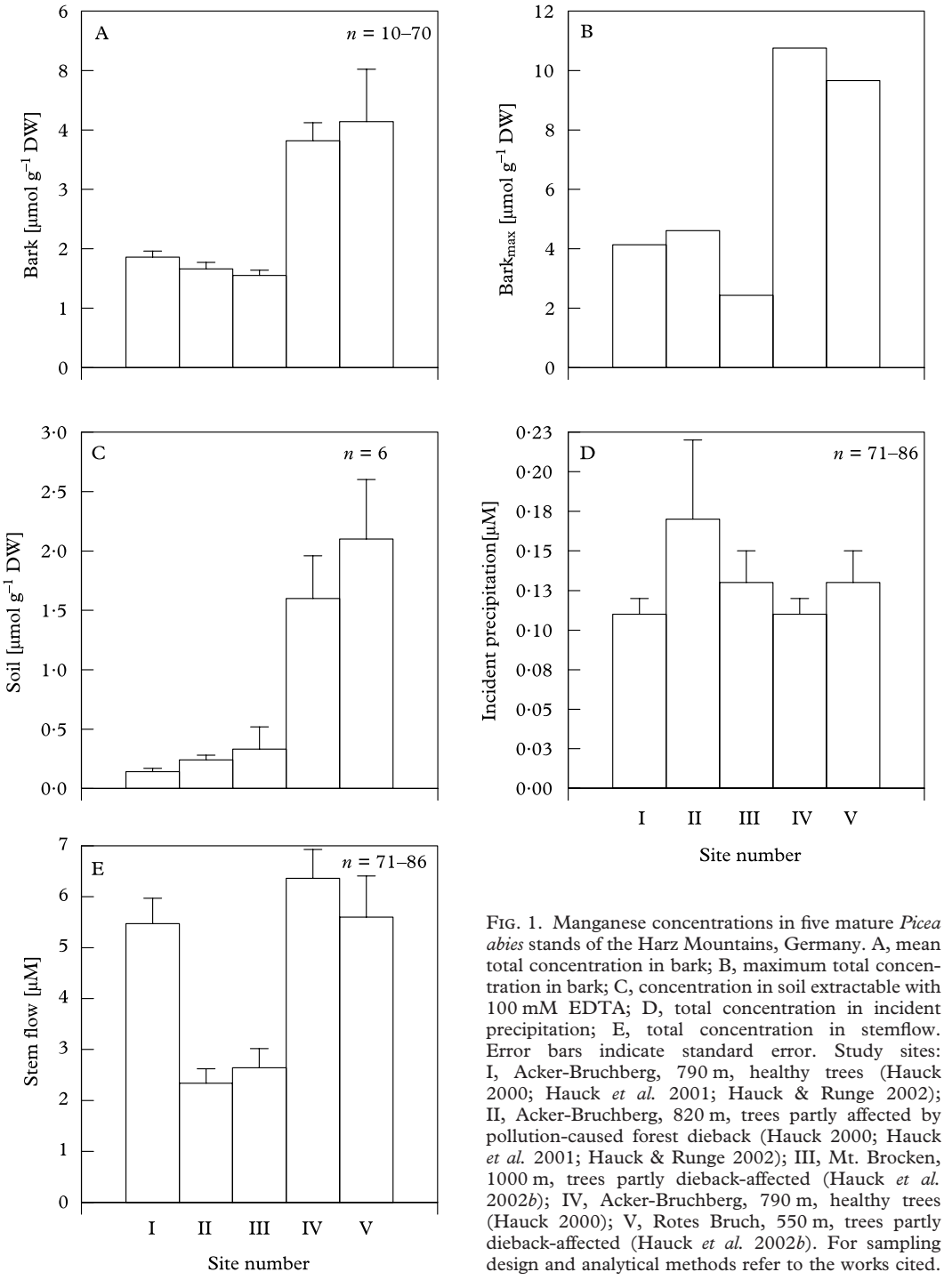


FIG. 1. Manganese concentrations in five mature *Picea abies* stands of the Harz Mountains, Germany. A, mean total concentration in bark; B, maximum total concentration in bark; C, concentration in soil extractable with 100 mM EDTA; D, total concentration in incident precipitation; E, total concentration in stemflow. Error bars indicate standard error. Study sites: I, Acker-Bruchberg, 790 m, healthy trees (Hauck 2000; Hauck *et al.* 2001; Hauck & Runge 2002); II, Acker-Bruchberg, 820 m, trees partly affected by pollution-caused forest dieback (Hauck 2000; Hauck *et al.* 2001; Hauck & Runge 2002); III, Mt. Brocken, 1000 m, trees partly dieback-affected (Hauck *et al.* 2002*b*); IV, Acker-Bruchberg, 790 m, healthy trees (Hauck 2000); V, Rotes Bruch, 550 m, trees partly dieback-affected (Hauck *et al.* 2002*b*). For sampling design and analytical methods refer to the works cited.

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

TABLE 1. *Concentrations of extracellularly bound and intracellular Mn (μmol g^{-1} DW*) in* Alectoria sarmentosa *and* Bryoria fuscescens *subsequent to 1 h-incubation with metal solutions containing* $MnCl_2$

Incubation (mM)	*Alectoria sarmentosa*		*Bryoria fuscescens*	
	Extracellularly bound	Intracellular	Extracellularly bound	Intracellular
Control	1·36 ± 0·13a†	0·35 ± 0·06a	2·12 ± 0·29a	0·63 ± 0·16a
Mn 0·1	2·60 ± 2·29a	0·53 ± 0·03a	2·51 ± 0·08a	0·90 ± 0·13a
Mn 1	13·0 ± 2·2b	1·38 ± 0·15b	16·5 ± 0·4b	1·84 ± 0·08a
Mn 100	49·6 ± 2·4c	3·88 ± 0·30c	80·1 ± 5·9c	9·68 ± 1·24b

*Mean values ± 1 SE (n=3).
†Within a column, means followed by the same letter do not differ significantly (Duncan's multiple range test, $P \leq 0{\cdot}05$, df=10).
Controls were incubated with deionized water.

concentration of Mn found in the fungal cell walls of the medulla (Paul *et al.* 2003). The Mn concentration in the algal cell lumina was less than 10% of that in the fungal cell interior. In soredia of *H. physodes* cultured on agar plates with BBM and varying $MnCl_2$ concentrations for 8 days, neither extracellular nor intracellular Mn concentrations differed between the fungus and the alga (Hauck *et al.* 2002*d*). Since the soredia lack a differentiated thallus with a cortex, these observations suggest that the heteromerous thallus structure of *H. physodes* enables the mycobiont of adult thalli to protect the photobiont from invasion by Mn to a certain extent, for example, by adsorption of ions at cortical ion exchange sites or in the interhyphal polysaccharide matrix (Paul *et al.* 2003). *Hypogymnia physodes* immobilizes excess amounts of Mn in intracellular polyphosphate bodies. This has been found in fungal and algal cells of soredia and in cortical hyphae of fully developed thalli (Paul *et al.* 2003). Such accumulation of Mn in polyphosphate bodies is widespread in fungi, algae and cyanobacteria (Baxter & Jensen 1980; Tillberg *et al.* 1980; Turnau *et al.* 1993). In soredia of *H. physodes*, extracellular encrustations of Mn phosphate (Fig. 2) are frequent (Hauck *et al.* 2002*d*). The immobilization of Mn in phosphate deposits does not lead to intracellular P deficiency as shown by analyzing adenine nucleotide concentrations (Hauck *et al.* 2003). This is probably because of a very efficient phosphate uptake mechanism in lichens including *H. physodes* (Farrar 1976; Hyvärinen & Crittenden 1998).

Manganese uptake in the field

Manganese uptake in the field is mainly from the substratum as correlation analyses show (Bosserman & Hagner 1981; De Bruin & Hackenitz 1986; Sloof & Wolterbeek 1993). In the Harz Mountains, Hauck (2000) found a close correlation between the total Mn content of *H. physodes* and the bark of *Picea abies* on which *H. physodes* grew (r=0.84, $P \leq 0.001$), but no significant correlation between the thallus Mn content and the mean Mn concentration in stemflow. In another spruce stand in the Harz Mountains with above-average content of plant-available Mn in the soil, Mn concentrations from stemflow were found to be more significant for the performance of *H. physodes* than for those in bark (Hauck *et al.* 2002*c*). The availability of Mn from bark differs between tree species. While it is obvious from correlations between Mn concentrations and the abundance of epiphytic lichen species that Mn is readily available from spruce bark as shown for *Picea abies*, *P. engelmannii* and *P. rubens* (Hauck *et al.* 2001; Schmull & Hauck 2003*a*; Hauck & Spribille 2005), Mn was found to be poorly available from the bark of *Abies balsamea* (Schmull & Hauck 2003*a*). This is because of the

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

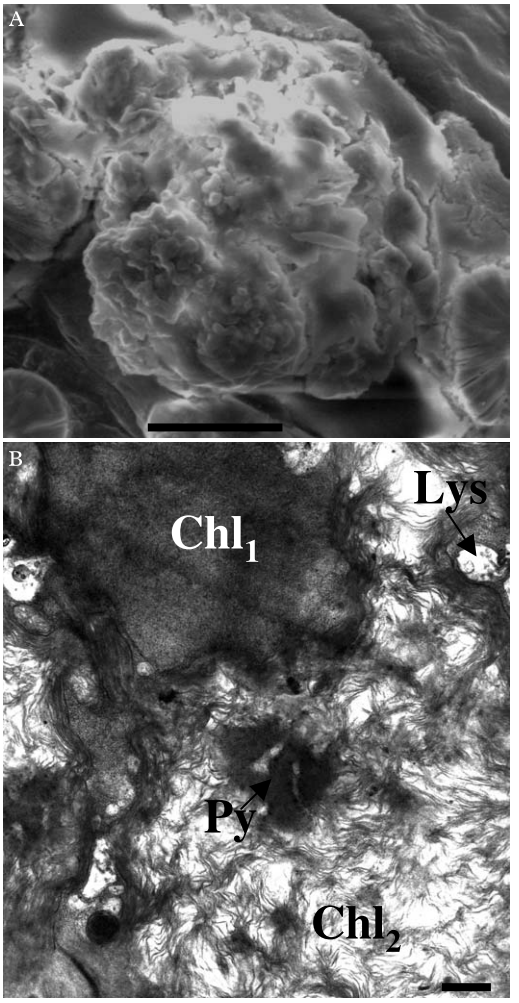


FIG. 2. Soredia of *Hypogymnia physodes*. A, manganese accumulation in extracellular phosphate encrustation in a soredium cultured on BBM with 7 mM $MnCl_2$ for 8 days; B, ultrathin section of *Trebouxia* photobiont in a soredium cultured on BBM with 500 μM $MnCl_2$ for 11 days showing areas, where the chloroplast is completely (Chl_1) or partly (Chl_2) degenerated, the central pyrenoid reduced in size and lysosome-like vesicles (Lys) involved in the break-down of organelles destroyed by Mn. Scales: A=5 μm; B=500 nm.

above-mentioned crystals (see Biogeochemical cycling) that *A. balsamea* forms in its bark. These crystals benefit not only the tree, but also its epiphytes. Though only studied in *A. balsamea*, so far, this result of Schmull & Hauck (2003*a*) could explain the general high abundance of epiphytic lichens on *Abies*. This hypothesis is supported by results of Hauck & Spribille (2005) from Montana, where lichen abundance was correlated to bark Mn concentrations on *Larix occidentalis* and *Picea engelmannii*, but not on *Abies lasiocarpa*. The availability of Mn from bark also differs between living and dead trees of the same tree species (Schmull & Hauck 2003*b*).

The maximum depth to which hyphae of the mycobiont penetrate the substratum is controversial (Ascaso *et al.* 1980; Estevez *et al.* 1980), but certainly depends on the lichen species. Fungal hyphae even of species that lack rhizinae, such as *H. physodes*, are in close contact with the bark (Fig. 3). Uptake of Mn from the substratum by these hyphae in the presence of a minimum amount of water is easily conceivable. The uptake of Mn from stemflow is also plausible, although the stemflow volume is rather small in coniferous forests and the contact time of lichens with stemflow is limited. This is because of the relatively high binding affinity of Mn to the extracellular exchange sites in lichen thalli (Nieboer & Richardson 1980). Manganese^{2+} ions efficiently replace Ca^{2+} and Mg^{2+} from these binding sites (Hauck *et al.* 2002*c*, *d*; Paul *et al.* 2003) so that short times of exposure to Mn are sufficient for the extracellular adsorption of significant amounts of ions. In this respect, Mn and other transition metals differ principally from ions with weak binding affinity to the extracellular binding sites. Hauck & Gross (2003) showed for K that concentrations found in *Picea abies* stemflow of the Harz Mountains were not high enough to cause significant extracellular adsorption (or intracellular uptake) in *H. physodes* despite an incubation time of 2 h, which is often not achieved by soaking with stemflow. In the case of Mn, experiments with cyanolichens showed that ions adsorbed at extracellular binding places are transferred into cells long after the actual exposure of the lichen thallus to the source of Mn^{2+} ions (Hauck *et al.* 2006). This agrees with a study of Brown & Beckett (1985) of Cd uptake in *Cladonia portentosa* and *Peltigera horizontalis*. These results suggest that even short exposures to

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

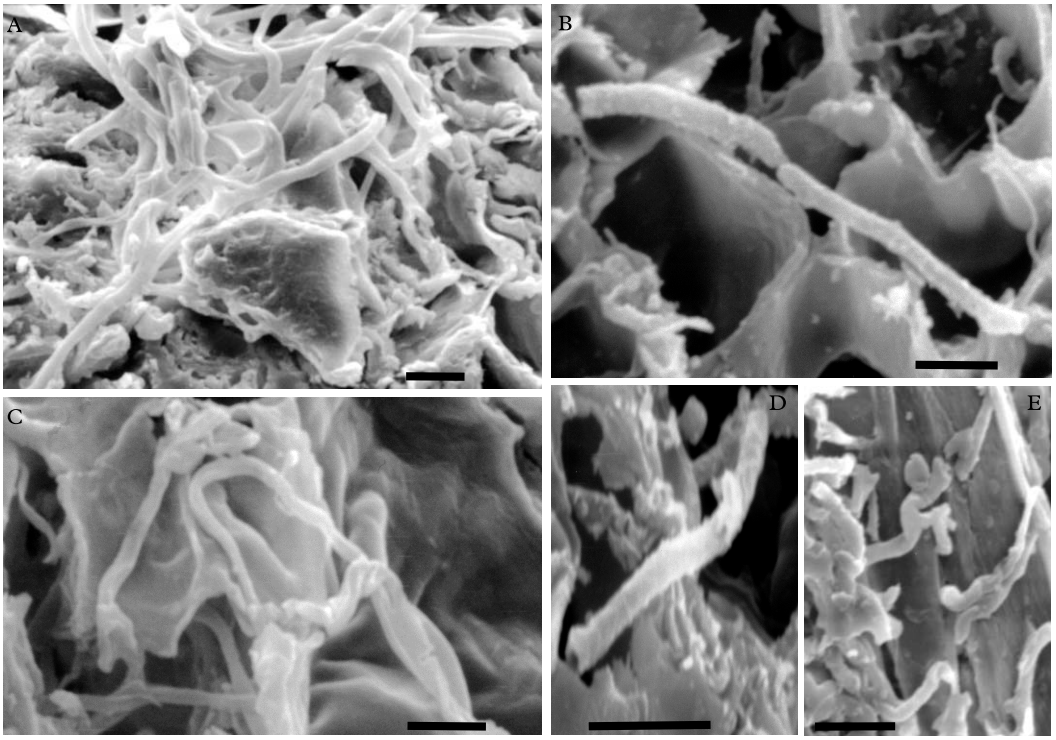


FIG. 3. Lichen/substratum interface of *Hypogymnia physodes* (A, B, D) and *Lecanora conizaeoides* (C, E) showing the close contact of mycobiont hyphae with bark and wood, which is the structural basis for cation uptake from the substratum. A & C, on *Picea abies* bark; B & D, on *Quercus robur* bark; E, on conifer wood (E). Scales: A–E = 10 µm.

Mn^{2+} in stemflow, for example, during a peak leaching event in the cold season, could be sufficient to affect epiphytic lichens. Unfortunately, our knowledge of the temporal and spatial dynamics of cation uptake in lichens under field conditions is still very limited. Exemplary measurements showed that epiphytic lichens growing on acidic bark contained between 0·3–1·6 µmol g^{-1} DW intracellularly and around 1–2 µmol g^{-1} DW at the extracellular exchange sites (Table 2). The bipartite cyanolichens *Leptogium saturninum* and *Nephroma helveticum* had a higher intracellular Mn content than the tripartite *Lobaria pulmonaria* or the bipartite chlorolichens (Table 2). Values shown in Table 2 refer to well-developed lichen thalli without visual damage and represent typical Mn concentrations of thalli from Mn-poor conifer bark. A study using soredia of *H. physodes* transplanted on to *Picea abies* bark with varying Mn content showed that intracellular uptake and extracellular adsorption of Mn is significantly increased with increasing Mn supply from the substratum (A. Paul, M. Hauck & E. Fritz unpublished). Bennett (1995) found total Mn concentrations of up to 3·9 µmol g^{-1} DW in *H. physodes*, which are above the sum of extracellular and intracellular Mn concentrations given for *H. physodes* in Table 2.

Manganese related damage in lichens

When we obtained our first field data that suggested a possible limitation of epiphytic lichen abundance in coniferous forests by high, ambient Mn concentration only a few studies of lichens and heavy metals, involving Mn had been published. Burton *et al.* (1981) used Mn as an example of a scarcely

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

TABLE 2. *Concentration of extracellularly absorbed and intracellular Mn (μmol g^{-1} DW*) in epiphytic lichens sampled from acidic bark in Europe and North America*

Species	Origin	Substratum	Intracellular†	Extracellular†
Alectoria sarmentosa	Sweden	*Picea abies*	0·4	1·4
Bryoria fuscescens	Sweden	*Picea abies*	0·6	2·1
Hypogymnia physodes	Germany	*Quercus robur*	0·6	1·5–1·6
Lecanora conizaeoides	Germany	*Picea abies*	0·7	1·1–1·3
Leptogium saturninum	Canada	*Picea engelmannii*	1·5	1·0
Lobaria pulmonaria	Canada	*Picea engelmannii*	0·3	0·9
Nephroma helveticum	Canada	*Abies lasiocarpa*	1·6	1·1

*Arithmetic means from 5–8 replicate samples.
†Extracellularly absorbed ions analyzed in 20 mM $NiCl_2$ extracts and intracellular ions measured in acid digests with 65% HNO_3 by means of atomic absorption spectrophotometry.

toxic transition metal and showed that significant K^+ losses in *Cladonia rangiferina* were induced by Mn only at a concentration as high as 1 M applied for 90 minutes. Goyal & Seaward (1982) found the total thallus content of K in the heteromerous cyanolichen *Peltigera canina* to be reduced by 50–70% after exposure to 2–16 mM $MnSO_4$ for 2 hours. Garty *et al.* (1992) reported a reduced chlorophyll content in *Ramalina lacera* after incubation with 20 mM $MnCl_2$ for 30 min, however, this experiment was conducted at pH 2·0 and the acidity alone reduced the chlorophyll content in an untreated control to the same extent as in the combined treatment of acidity and $MnCl_2$. Against the background of this scarce evidence for Mn toxicity in lichens, we initiated experiments on Mn toxicity in epiphytic lichens occurring in coniferous forests of Europe and North America to test whether the correlations between Mn concentration and epiphytic lichen abundance from our study plots were causal.

In most lichens investigated, Mn strongly reduced chlorophyll concentrations and chlorophyll fluorescence. In *H. physodes* chlorophyll concentrations were reduced both in adult thalli and in soredia exposed to excess amounts of Mn (Hauck *et al.* 2002*d*, 2003). A concentration of 1 mM $MnCl_2$ applied to thalli of *H. physodes* for 1 h reduced the chlorophyll content even more than an equimolar solution of $CuCl_2$ (Hauck *et al.* 2003). Ultrastructural studies of *H. physodes* soredia showed that excess Mn leads to the degradation of the chloroplast (Fig. 2) of the *Trebouxia* photobiont (Paul *et al.* 2004). Among the cyanolichens, Mn reduced chlorophyll fluorescence most in the homoiomerous, bipartite *Leptogium saturninum*, second most in the heteromerous, bipartite *Nephroma helveticum* and least in the tripartite *Lobaria pulmonaria*, which contains *Nostoc* in cephalodia (Hauck *et al.* 2006). Remarkably, chlorophyll fluorescence was affected more in *H. physodes* than in *L. pulmonaria*, though the latter is much more sensitive to SO_2 and related compounds than the former (Türk *et al.* 1974). This clearly shows that different mechanisms lead to the partly similar toxicity symptoms induced by Mn and SO_2.

While structural damage due to Mn uptake has not yet been observed in adult lichen thalli (Paul *et al.* 2003), various symptoms of damage have been detected in soredia of *H. physodes* with scanning and transmission electron microscopy. Scanning electron microscopy revealed strongly swollen and shortened mycobiont hyphae in the soredia (Hauck *et al.* 2002*d*). While intact soredia grown on agar medium with only 7 μM $MnCl_2$ had a fine, densely branched network of hyphae that closely surrounded the photobiont, many hyphae in soredia cultured with excess amounts of $MnCl_2$ lost their contact to the *Trebouxia* photobiont. Since the fungal cell walls are the only water source for the photobiont

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

(Honegger *et al.* 1996), this loss of contact must be fatal for the photobiont. This agrees with the observation that many algal cells were smaller or collapsed in Mn treated soredia (Hauck *et al.* 2002*d*). Ultrastructural studies showed that $MnCl_2$ further reduced the quantity of mesosome-like structures in the mycobiont (Paul *et al.* 2004). These organelles are supposed to be involved in the metabolite transfer from the photobiont to the fungus (Peveling 1972, 1976; Boissière 1982). Thus, surplus Mn apparently interferes with both the transfer of water and metabolites between the lichen bionts. Degradation of concentric bodies in soredia grown on a Mn-rich substratum suggests that high Mn concentrations are also capable of reducing the drought resistance of lichens (Paul *et al.* 2004), as concentric bodies are thought to be somehow involved in enabling the lichen fungus to withstand repeated drying and rewetting cycles (Peveling *et al.* 1985; Honegger 1995). Moreover, excess Mn inhibited the formation of *Trebouxia* autospores and, thereby, the propagation of the photobiont in *H. physodes* soredia (Paul *et al.* 2004).

A constant accompaniment of Mn uptake in lichens is the release of basic cations, such as Ca and Mg. Under natural conditions, the extracellular exchange sites in lichens are primarily occupied by Ca and Mg (Brown & Beckett 1984). Since the binding affinity of Mn to such sites is higher than that of Ca or Mg, extracellular Mn adsorption releases large amounts of Ca and Mg (Hauck *et al.* 2002*c*,*d*; Paul *et al.* 2003). This result parallels those obtained with other transition metals (Brown & Beckett 1984). Intracellular Ca concentrations were only slightly reduced (Hauck *et al.* 2002*d*; Paul *et al.* 2003), and intracellular Mg concentration never altered due to Mn uptake (Hauck *et al.* 2002*c*, *d*; Paul *et al.* 2003). The constant Mg content implies that Mn does not induce severe membrane damage (Branquinho *et al.* 1997*a*). In contrast to other transition metals, such as Cu (Branquinho *et al.* 1997*a*; Cabral 2003), Pb (Branquinho *et al.* 1997*b*) or Ni (Puckett 1976), Mn does not induce K efflux (Hauck *et al.* 2002*c*, *d*; Paul *et al.* 2003), which would indicate an increase of membrane permeability (Garty *et al.* 1998). The lack of membrane damage may be one reason why the toxicity even of ambient Mn concentrations to lichens has been overlooked for so long, as membrane permeability was considered as a principal criterion for heavy metal toxicity in early studies of this topic even more so than today (Burton *et al.* 1981). The significant K efflux induced by $MnSO_4$ in *Peltigera canina* (Goyal & Seaward 1982) was not confirmed in our studies with other species of cyanolichens so that there is probably no general trend for higher sensitivity of the membranes to Mn in cyanolichens than in chlorolichens (Hauck *et al.* 2006).

Interaction of calcium, magnesium, iron and silicon with manganese toxicity

There is experimental evidence from *H. physodes* to suggest that Ca, Mg, Fe and probably also Si reduce Mn uptake and alleviate Mn toxicity symptoms. Reduced extracellular adsorption and reduced intracellular uptake of Mn in the presence of Ca or Mg is a direct consequence of the competition of these ions with Mn for the extracellular exchange sites and perhaps also for transmembrane transport (Hauck *et al.* 2002*c*). This is consistent with an alleviation of Mn-induced growth inhibition in *H. physodes* soredia exerted by Ca and Mg singly or in combination (Hauck *et al.* 2002*d*). Reduced extracellular adsorption and intracellular uptake of Mn due to the addition of Ca or Mg was also observed in the cyanolichens *Lobaria pulmonaria* and *Nephroma helveticum*, but remarkably not in *Leptogium saturninum*, where Ca and Mg had no effect on intracellular Mn uptake (Hauck *et al.* 2006). The mitigation of Mn toxicity in *H. physodes* soredia by addition of $FeCl_3$ suggests that Mn causes Fe deficiency in lichens, as known from cyanobacteria (Csatorday *et al.* 1984) and vascular plants (Clairmont *et al.* 1986; El-Jaoual & Cox 1998). Part compensation of Mn-induced chlorophyll degradation by $FeCl_3$ supports

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

this view (Hauck *et al.* 2003). Lower Mn/Si ratios in the cell walls of collapsed versus intact *Trebouxia* cells in soredia of *H. physodes* may indicate an alleviating effect of Si on Mn toxicity in lichens. Such effects of Si are known from vascular plants, but the mechanisms are poorly understood (Horiguchi 1988; Iwasaki *et al.* 2002). Cases where correlations between the ratios of Mn to Ca, Mg or Fe and the cover of *H. physodes* and other epiphytic lichen species have been found in the field indicate together with the experimental results that the ratio of Mn to nutrient cations may sometimes be more significant than the Mn concentration itself. This agrees with results from organisms other than lichens (Goss & Carvalho 1992; Issa *et al.* 1995; Blackwell *et al.* 1998). Silicon concentrations were not analyzed during our field work.

Lecanora conizaeoides, a manganese tolerant lichen

Chlorophyll concentrations and chlorophyll fluorescence of *L. conizaeoides*, the cover of which was not found to be correlated with Mn concentrations in bark or stemflow, remained unaffected by treatment with excess concentrations of $MnCl_2$, albeit concentrations ten times higher than in *H. physodes* were applied (Hauck *et al.* 2003). In contrast to $MnCl_2$, $CuCl_2$ reduced the concentrations of chlorophylls *a* and *b* significantly in *L. conizaeoides* (Hauck *et al.* 2003). The decisive cause of the high Mn resistance of *L. conizaeoides* is probably its ability for effective intracellular immobilization of excess Mn (Paul *et al.* 2003). Paul *et al.* (2003) found that most Mn was accumulated in the apothecia, viz. in polyphosphate bodies and in S-containing deposits, which might be glutathione. While the Mn concentrations in the cell lumina outside such deposits were much lower in *L. conizaeoides* than in *H. physodes* (Paul *et al.* 2003), total intracellular Mn concentrations did not differ between the two species (Hauck *et al.* 2002*c*). Extracellular adsorption of Mn is significantly lower in *L. conizaeoides* than in *H. physodes* (Hauck *et al.* 2002*c*). Different sensitivities of the photobionts probably play no role in the different Mn sensitivities of *L. conizaeoides* and *H. physodes*. Material of both species sampled from the Harz Mountains constantly contained different ITS variants of *Trebouxia*, indeed, but the genotype found in *L. conizaeoides* is also known from some taxa thought to be much more sensitive to toxic chemicals than *L. conizaeoides*, such as *Pseudevernia cladonia* and *P. consocians* (G. Helms, M. Hauck & T. Friedl unpublished).

Linking field and experimental results

Relating laboratory results to field data is always problematic, especially in the present case, because little is known about the availability of ions from the substratum (Schmull & Hauck 2003*a*, *b*). Experimental work with soredia of *H. physodes* transplanted to *Picea abies* bark of known Mn content for two vegetation periods and one winter in a spruce forest of the Harz Mountains has been carried out to link the laboratory and field data (A. Paul, M. Hauck & E. Fritz unpublished). Before transplantation, the Mn concentration of bark pieces that supported the soredia was adjusted to the upper concentration range occurring naturally in bark of *P. abies* (11 μmol g^{-1} DW), *P. engelmannii* (13 μmol g^{-1} DW) or *P. rubens* (14 μmol g^{-1} DW). These concentrations were maximum values found during the studies of Hauck (2000), Hauck *et al.* (2001), Schmull & Hauck (2003) and Hauck & Spribille (2005). The experimental adjustment of the Mn content of bark in the experiment was done by incubation with $MnCl_2$. Stable bark Mn concentrations over the experimental period showed that soredia did not suffer from unnaturally high leaching rates. Soredia on Mn-rich bark were poorly developed and often disappeared from the bark pieces during the experiment. Soredia on Mn-poor bark showed better development including the beginning of thallus lobe formation. Manganese-rich bark was spontaneously colonized by *L. conizaeoides*, whereas this rarely happened on Mn-poor bark. The concentration of free, intracellular Mn in both fungal and algal

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

cells exceeded considerably that of soredia cultured for 11 days on agar plates in BBM at 500 μM $MnCl_2$. Paul *et al.* (2004) demonstrated in the latter type of soredia severe ultrastructural damage including the degradation of chloroplasts, mesosome-like structures and concentric bodies. These results thus provide strong support for the hypothesis that decreasing epiphytic lichen abundance with increasing Mn supply in coniferous forests is caused by toxic effects of excess concentrations of Mn. The results from the transplant experiment also substantiate the assumption that *L. conizaeoides* is not affected by high ambient Mn concentrations.

Outlook: what we know and what we don't know about manganese toxicity in epiphytes

Though never considered as a relevant site factor for epiphytic lichens until recently, there are now multiple lines of evidence from the field and from laboratory experiments that high Mn concentrations limit the abundance of many epiphytic chlorolichen species including *H. physodes*, whereas *L. conizaeoides* is tolerant to excess Mn. Studies with epiphytic cyanolichens suggest that at least some of them are particularly sensitive to high Mn concentrations (Hauck *et al.* 2005). While these results suggest a relevance of the naturally occurring variation of Mn concentrations in bark and stemflow for the performance of epiphytic lichens in coniferous forests of the Northern Hemisphere, some questions are still to be resolved. It is unclear, for instance, whether Mn is also a relevant site factor in deciduous forests, where pH values in the soil, bark and stemflow are usually higher and, thus, Mn is less soluble. Apart from lichens, it would be very interesting to include other epiphytic organisms in the studies of Mn toxicity. In this context it would be instructive, for example, to study whether the often poor epiphytic bryophyte vegetation of conifers is, as well as the result of factors such as microclimate, also the result of high Mn concentrations. Furthermore, it is unknown whether the distribution of non-epiphytic lichens is controlled by the supply of Mn. Lower Mn concentrations are extractable from gabbro rock with rich lichen vegetation than from gabbro with sparse lichen cover in Ireland (Boyle *et al.* 1987) suggesting the possible significance of ambient Mn levels for saxicolous lichens, but no studies have been carried out to investigate this special case or any possible interaction between the performance of rock-dwelling lichens and their Mn supply. In the case of the saxicolous communities investigated by Boyle *et al.* (1987), the lichen-rich rocks were simultaneously characterized by higher supply of P and K, which could also be responsible for the differences in lichen vegetation.

Our studies have been supported by grants from the Deutsche Forschungsgemeinschaft to M. Hauck (Ha 3152/1-1, 2-1, 2-2, 3-1) and of the Deutsche Bundesstiftung Umwelt to A. Paul.

References

Ahmadjian V. (1993) *The Lichen Symbiosis*. New York: Wiley.

Ascaso, C., Gonzales, C. & Vicente, C. (1980) Epiphytic *Evernia prunastri* (L.) Ach.: ultrastructural facts. *Cryptogamie, Bryologie et Lichénologie* **1:** 43–51.

Baxter M. & Jensen T. (1980) Uptake of magnesium, strontium, barium, and manganese by *Plectonema boryanum* (*Cyanophyceae*) with special reference to polyphosphate bodies. *Protoplasma* **104:** 81–89.

Beckett, R. P. & Brown, D. H. (1984) The relationship between cadmium uptake and heavy metal tolerance in the lichen genus *Peltigera*. *New Phytologist* **97:** 301–311.

Bennett, J. P. (1995) Abnormal chemical element concentrations in lichens of Isle Royale National Park. *Environmental and Experimental Botany* **35:** 259–277.

Blackwell, K. J., Tobin, J. M. & Avery, S. V. (1998) Manganese toxicity towards *Saccharomyces cerevisiae*: dependence on intracellular and extracellular magnesium concentrations. *Applied Microbiology and Biotechnology* **49:** 751–757.

Boissière, M.-C. (1982) Cytochemical ultrastructure of *Peltigera canina*: some features related to its symbiosis. *Lichenologist* **14:** 1–27.

Bosserman, R. W. & Hagner, J. E. (1981) Elemental composition of epiphytic lichens from Okefenokee Swamp. *Bryologist* **84:** 48–58.

Boyle, A. P., McCarthy, P. M. & Stewart, D. (1987) Geochemical control of saxicolous lichen communities on the Creggaun gabbro, Letterfrack, Co. Galway, western Ireland. *Lichenologist* **19:** 307–317.

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

Branquinho, C., Brown, D. H. & Catarino, F. (1997*a*) The cellular location of Cu in lichens and its effects on membrane integrity and chlorophyll fluorescence. *Environmental and Experimental Botany* **38:** 165–179.

Branquinho, C., Brown, D. H., Máguas, C. & Catarino, F. (1997*b*): Lead (Pb) uptake and its effects on membrane integrity and chlorophyll fluorescence in different lichen species. *Environmental and Experimental Botany* **37:** 95–105.

Brown, D. H. & Beckett, R. P. (1983) Differential sensitivity of lichens to heavy metals. *Annals of Botany* **52:** 51–57.

Brown, D. H. & Beckett, R. P. (1984) Uptake and effect of cations on lichen metabolism. *Lichenologist* **16:** 173–188.

Brown, D. H. & Beckett, R. P. (1985) The role of the cell wall in the intracellular uptake of cations by lichens. In *Lichen Physiology and Cell Biology* (D. H. Brown, ed.): 247–258. London: Plenum Press.

Burnell, J. N. (1988) The biochemistry of manganese in plants. In *Manganese in Soils and Plants* (R. D. Graham, R. J. Hannam & N. C. Uren, eds): 125–137. Dordrecht: Kluwer.

Burton, M. A. S., LeSueur, P. & Puckett, K. J. (1981) Copper, nickel, and thallium uptake by the lichen *Cladina rangiferina*. *Canadian Journal of Botany* **59:** 91–100.

Cabral, J. P. (2003) Copper toxicity to five *Parmelia* lichens in vitro. *Environmental and Experimental Botany* **49:** 237–250.

Clairmont, K. B., Hagar, W. G. & Davis, E. A. (1986) Manganese toxicity to chlorophyll synthesis in tobacco callus. *Plant Physiology* **80:** 291–293.

Clarkson, D. T. (1988) The uptake and translocation of manganese by plant roots. In *Manganese in Soils and Plants* (R. D. Graham, R. J. Hannam & N. C. Uren, eds): 101–111. Dordrecht: Kluwer.

Crowley, J. D., Traynor, D. A. & Weatherburn, D. C. (2000). Enzymes and proteins containing manganese: an overview. In *Metal Ions in Biological Systems. Vol. 37. Manganese and its Role in Biological Processes* (A. Sigel & H. Sigel, eds): 209–278. New York: Dekker.

Csatorday K., Gombos Z. & Szalontai B. (1984) Mn^{2+} and Co^{2+} toxicity in chlorophyll biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **81:** 476–478.

De Bruin, M. & Hackenitz, E. (1986) Trace element concentrations in epiphytic lichens and bark substrate. *Environmental Pollution B* **11:** 153–160.

El-Jaoual, T. & Cox, D. A. (1998) Manganese toxicity in plants. *Journal of Plant Nutrition* **21:** 353–386.

Estevez, M. P., Orus, M. I. & Vicente, C. (1980) Estudios morfologicus sobre *Evernia prunastri* de vida saprofitica. *Cryptogamie, Bryologie et Lichénologie* **1:** 33–41.

Farmer, A. M., Bates, J. W., Bell, J. N. B. (1991) Seasonal variations in acidic pollutant inputs and their effects on the chemistry of stemflow, bark and epiphyte tissues in three oak woodlands in N.W. Britain. *New Phytologist* **118:** 441–451.

Farrar, J. F. (1976) The uptake and metabolism of phosphate by the lichen *Hypogymnia physodes*. *New Phytologist* **77:** 127–134.

Fassbender, H. W. (1977) Modellversuch mit jungen Fichten zur Erfassung des internen Nährstoffumsatzes. *Oecologia Plantarum* **12:** 263–272.

Fink, S. (1999) *Pathological and Regenerative Plant Anatomy*. Berlin: Borntraeger.

Forget, E., Courchesne, F., Kennedy, G. & Zayed, J. (1994) Response of blue spruce (*Picea pungens*) to manganese pollution from MMT. *Water, Air and Soil Pollution* **73:** 319–324.

Gartner, E. J., Urfer, W., Eichhorn, J., Grabowski, H. & Huss, H. (1990): Mangan—ein Bioindikator für den derzeitigen Schadenszustand mittelalter Fichten in Hessen. *Forstarchiv* **61:** 229–233.

Garty, J., Karary, Y. & Harel, Y. (1992) Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. *Environmental and Experimental Botany* **32:** 229–241.

Garty, J., Kloog, N. & Cohen, Y. (1998) Integrity of lichen cell membranes in relation to concentration of airborne elements. *Archives of Environmental Contamination and Toxicology* **34:** 136–144.

Gauslaa, Y., Ohlson, M. & Rolstad, J. (1998) Fine-scale distribution of the epiphytic lichen *Usnea longissima* on two even-aged neighbouring *Picea abies* trees. *Journal of Vegetation Science* **9:** 95–102.

Goss, M. J. & Carvalho, M. J. G. P. R. (1992) Manganese toxicity: the significance of magnesium for the sensitivity of wheat plants. *Plant and Soil* **139:** 91–98.

Goward, T. & Arsenault, A. (2000) Cyanolichen distribution in young unmanaged forests: a dripzone effect? *Bryologist* **103:** 28–37.

Goyal, R. & Seaward, M. R. D. (1982) Metal uptake in terricolous lichens. III. Translocation in the thallus of *Peltigera canina*. *New Phytologist* **90:** 85–98.

Hauck, M. (2000) Ecology of epiphytic lichens in a montane spruce forest: influence of forest dieback and forest management on chemical habitat conditions. *Dissertationes Botanicae* **327:** 1–232.

Hauck, M. (2003) Epiphytic lichen diversity and forest dieback: the role of chemical site factors. *Bryologist* **106:** 257–269.

Hauck, M. (2005) Epiphytic lichen diversity on dead and dying conifers under different levels of atmospheric pollution. *Environmental Pollution* **135:** 111–119.

Hauck, M. & Gross, S. (2003) Potassium uptake in the epiphytic lichen *Hypogymnia physodes* at concentrations and pH conditions as found in stemflow. *Flora* **198:** 127–131.

Hauck, M. & Runge, M. (2002) Stemflow chemistry and epiphytic lichen diversity in dieback-affected spruce forest of the Harz Mountains, Germany. *Flora* **197:** 250–261.

Hauck, M. & Spribille, T. (2002) The Mn/Ca and Mn/Mg ratios in bark as possible causes for the occurrence of *Lobarion* lichens on conifers in the

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

dripzone of *Populus* in western North America. *Lichenologist* **34:** 527–532.

Hauck, M. & Spribille, T. (2005) The significance of precipitation and substrate chemistry for epiphytic lichen diversity in spruce-fir forests of the Salish Mountains, northwestern Montana. *Flora* **200:** (in press).

Hauck, M., Hesse, V. & Runge, M. (2002*a*) Correlations between the Mn/Ca ratio in stemflow and epiphytic lichen abundance in a dieback-affected spruce forest of the Harz Mountains. *Flora* **197:** 361–369.

Hauck, M., Hesse, V. & Runge, M. (2002*b*) The significance of stemflow chemistry for epiphytic lichen diversity in a dieback-affected spruce forest on Mt. Brocken, northern Germany. *Lichenologist* **34:** 415–427.

Hauck, M., Jung, R. & Runge, M. (2001) Relevance of element content of bark for the distribution of epiphytic lichens in a montane spruce forest affected by forest dieback. *Environmental Pollution* **112:** 221–227.

Hauck, M., Mulack, C. & Paul, A. (2002*c*) Manganese uptake in the epiphytic lichens *Hypogymnia physodes* and *Lecanora conizaeoides*. *Environmental and Experimental Botany* **48:** 107–116.

Hauck, M., Paul, A., Gross, S. & Raubuch, M. (2003) Manganese toxicity in epiphytic lichens: chlorophyll degradation and interaction with iron and phosphorus. *Environmental and Experimental Botany* **49:** 181–191.

Hauck, M., Paul, A., Mulack, C., Fritz, E. & Runge, M. (2002*d*) Effects of manganese on the viability of vegetative diaspores of the epiphytic lichen *Hypogymnia physodes*. *Environmental and Experimental Botany* **47:** 127–142.

Hauck, M., Paul, A. & Spribille, T. (2006) Uptake and toxicity of manganese in epiphytic cyanolichens. *Environmental and Experimental Botany* (in press).

Honegger, R. (1995) Experimental studies with foliose macrolichens: fungal responses to spatial disturbance at the organismic level and to spatial problems at the cellular level during drought stress events. *Canadian Journal of Botany* **73** (Suppl. 1)**:** 569–578.

Honegger, R., Peter, M. & Scherrer, S. (1996) Drought-induced structural alterations at the mycobiont-photobiont interface in a range of foliose macrolichens. *Protoplasma* **190:** 221–232.

Horiguchi, T. (1988) Mechanism of manganese toxicity and tolerance of plants. IV. Effects of silicon and alleviation of manganese toxicity of rice plants. *Soil Science and Plant Nutrition* **34:** 65–73.

Hyvärinen, M. & Crittenden, P. D. (1998) Phosphate uptake in *Cladonia portentosa*. *Lichenologist* **30:** 297–301.

Issa, A. A., Abdel-Basset, R. & Adams, M. S. (1995) Abolition of heavy metal toxicity on *Kirchneriella lunaris* (Chlorophyta) by calcium. *Annals of Botany* **75:** 189–192.

Iwasaki, K., Maier, P., Fecht, M. & Horst, W. J. (2002): Effects of silicon on apoplastic manganese concentrations in leaves and their relation to manganese tolerance in cowpea [*Vigna unguiculata* (L.) Walp.]. *Plant and Soil* **238:** 281–288.

Kaus, A. & Wild, A. (1997) Physiologische Aspekte der Erkrankung der Douglasie (*Pseudotsuga menziesii* var. *viridis*). *Mitteilungen der Forstlichen Versuchsanstalt Rheinland-Pfalz* **41:** 117–127.

Keil, P., Hecht-Buchholz, C. & Ortmann, U. (1986) Zum Einfluß von erhöhtem Mangan-Angebot auf Fichtensämlinge. *Allgemeine Forstzeitschrift* **41:** 855–858.

Knauer, K., Jabusch, T. & Sigg, L. (1999) Manganese uptake and Mn (II) oxidation by the alga *Scenedesmus subspicatus*. *Aquatic Sciences* **61:** 44–58.

Levia, D. F. & Herwitz, S. R. (2000) Physical properties of water in relation to stemflow leachate dynamics: implications for nutrient cycling. *Canadian Journal of Forest Research* **30:** 662–666.

Levia, D. F. & Underwood, S. J. (2004) Snowmelt induced stemflow in northern hardwood forests: a theoretical explanation on the causation of a neglected hydrological process. *Advances in Water Resources* **27:** 121–128.

Lin, Z.-Q. & Schuepp, P. H. (1996) Concentrations and distribution of metal elements at the surface of balsam fir foliage by micro-proton induced X-ray emission. *Environmental Science and Technology* **30:** 246–251.

Lin, Z.-Q., Barthakur, N. N., Schuepp, P. H. & Kennedy, G. G. (1995) Uptake and translocation of ^{54}Mn and ^{65}Zn applied on foliage and bark surfaces of balsam fir [*Abies balsamea* (L.) Mill.] seedlings. *Environmental and Experimental Botany* **35:** 475–483.

Lin, Z.-Q., Barthakur, N. N., Schuepp, P. H. & Kennedy, G. G. (1996) Uptake and translocation studies in balsam fir seedlings with ^{54}Mn and ^{65}Zn radioisotopes applied to soil surfaces. *Journal of Environmental Quality* **25:** 92–96.

Lövestam, G., Johansson, E.-M., Johansson, S. & Pallon, J. (1990) Elemental micro patterns in tree rings—a feasibility study using scanning proton microprobe analysis. *Ambio* **19:** 87–93.

Nash III, T. H. (1975) Influence of effluents from a zinc factory on lichens. *Ecological Monographs* **45:** 183–196.

Nash III, T. H. (1989) Metal tolerance in lichens. In *Heavy Metal Tolerance in Plants: Evolutionary Aspects* (A. J. Shaw, ed.): 119–131. Boca Raton: CRC Press.

Nieboer, E. & 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 B* **1:** 3–26.

Nieboer, E., Ahmed, H. M., Puckett, K. J. & Richardson, D. H. S. (1972) Heavy metal content of lichens in relations to distance from a nickel smelter in Sudbury, Ontario. *Lichenologist* **5:** 292–304.

Norvell, W. A. (1988) Inorganic reactions of manganese in soils. In *Manganese in Soils and Plants*

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press

(R. D. Graham, R. J. Hannam & N. C. Uren, eds): 37–58. Dordrecht: Kluwer.

Paul, A. (2005) Manganese as a site factor for epiphytic lichens. Dissertation: University of Göttingen.

Paul, A., Hauck, M. & Fritz, E. (2003) Effects of manganese on element distribution and structure in thalli of the epiphytic lichens *Hypogymnia physodes* and *Lecanora conizaeoides*. *Environmental and Experimental Botany* **50:** 113–124.

Paul, A., Hauck, M. & Langenfeld-Heyser, R. (2004) Ultrastructural changes in soredia of the epiphytic lichen *Hypogymnia physodes* cultivated with manganese. *Environmental and Experimental Botany* **52:** 139–147.

Peveling, E. (1972) Mesomen-ähnliche Strukturen im Mycobionten von *Lichina pygmaea*. *Zeitschrift für Naturforschung* **27b:** 1388–1392.

Peveling, E. (1976) Investigations into the ultrastructure of lichens. In *Lichenology: Progress and Problems* (D. H. Brown, D. L. Hawksworth & R. H. Bailey, eds): 17–26. London: Academic Press.

Peveling, E., Robenek, H. & Berns, B. (1985) The architecture of the concentric bodies in the mycobiont of *Peltigera praetextata*. In *Lichen Physiology and Cell Biology* (D. H. Brown, ed.): 275–285. London: Plenum Press.

Puckett, K. J. (1976) The effect of heavy metals on some aspects of lichen physiology. *Canadian Journal of Botany* **54:** 2695–2703.

Purvis, O. W. & Halls, C. (1996) A review of lichens in metal-enriched environments. *Lichenologist* **28:** 571–601.

Rasmussen, L., Pilegaard, K. & Gydessen, H. (1980) The application of cryptogams as monitoring organisms of metal air pollution in Denmark. *Botanisk Tidskrift* **75:** 93–99.

Richardson, D. H. S. (1995) Metal uptake in lichens. *Symbiosis* **18:** 119–127.

Rikkinen, J. (2002) Cyanolichens: an evolutionary overview. In *Cyanobacteria in Symbiosis* (A. N. Rain, B. Bergman & U. Rasmussen, eds): 175–188. Dordrecht: Kluwer.

Schade, A. (1970) Über Herkunft und Vorkommen der Caliciumoxalat-Exkrete in kortizikolen Parmeliaceen. *Nova Hedwigia* **19:** 159–187.

Schmidt, M. (1987) Atmosphärischer Eintrag und interner Umsatz von Schwermetallen in Waldökosystemen. *Berichte des Forschungszentrums Waldökosysteme/Waldsterben Reihe A* **40:** 1–174.

Schmidt-Vogt, H. (1986) *Die Fichte. Band II/1: Wachstum, Züchtung, Boden, Umwelt, Holz*. Hamburg: Parey.

Schmull, M. & Hauck, M. (2003*a*) Element microdistribution in the bark of *Abies balsamea* and *Picea rubens* and its impact on epiphytic lichen abundance on Whiteface Mountain, New York. *Flora* **198:** 293–303.

Schmull, M. & Hauck, M. (2003*b*) Extraction methods for assessing the availability of cations for epiphytic lichens from bark. *Environmental and Experimental Botany* **49:** 273–283.

Schmull, M., Hauck, M., Vann, D. R., Johnson, A. H. & Runge, M. (2002) Site factors determining epiphytic lichen distribution in a dieback-affected spruce-fir forest on Whiteface Mountain, New York: stemflow chemistry. *Canadian Journal of Botany* **80:** 1131–1140.

Sloof, J. E. & Wolterbeek, B. T. (1993) Substrate influence on epiphytic lichens. *Environmental Monitoring and Assessment* **25:** 225–234.

Stroupe, M. E., DiDonato, M. & Tainer, J. A. (2001) Manganese superoxide dismutase. In *Handbook of Metalloproteins. Vol. 2* (A. Messerschmidt, R. Huber, T. Poulos & K. Wieghardt, eds): 941–951. Wiley, Chichester.

Tillberg, J.-E., Rowley, J. R. & Barnard, T. (1980) X-ray microanalysis of leakage from polyphosphate granules in *Scenedesmus*. *Journal of Ultrastructure Research* **72:** 316–324.

Türk, R., Wirth, V. & Lange, O. L. (1974) CO_2-Gaswechsel-Untersuchungen zur SO_2-Resistenz von Flechten. *Oecologia* **15:** 33–64.

Turnau, K., Kottke. I. & Oberwinkler, F. (1993) *Paxillus involutus – Pinus sylvestris* mycorrhizae from heavily polluted forest. I. Element localization using electron energy loss spectroscopy and imaging. *Botanica Acta* **106:** 213–219.

Whittaker, J. W. (2000) Manganese superoxide dismutase. In *Metal Ions in Biological Systems. Vol. 37. Manganese and its Role in Biological Processes* (A. Sigel & H. Sigel, eds): 587–611. Dekker, New York.

Wilson, M. J. & Jones, D. (1984): The occurrence and significance of manganese oxalate in *Pertusaria corallina* (Lichenes). *Pedobiologia* **26:** 373–379.

Zayed J., Thibault C., Gareau L. & Kennedy G. (1999) Airborne manganese particulates and methylcyclopentadienyl manganese tricarbonyl (MMT) at selected outdoor sites in Montreal. *Neurotoxicology* **20:** 151–157.

Accepted for publication 15 *April* 2005

https://doi.org/10.1017/S0024282905014933 Published online by Cambridge University Press