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Uptake and toxicity of manganese in epiphytic cyanolichens

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Abstract

Mn uptake from $MnCl_2$ solution and chlorophyll fluorescence (as a selected vitality parameter) were studied in the epiphytic lichens *Lobaria pulmonaria* (tripartite, heteromerous lichen with the green alga *Dictyochloropsis* as primary photobiont and *Nostoc* in cephalodia), *Nephroma helveticum* (bipartite, heteromerous lichen with *Nostoc* photobiont) and *Leptogium saturninum* (bipartite, homoiomerous lichen with *Nostoc* photobiont). Extracellular adsorption and intracellular uptake of Mn increased in the order *L. pulmonaria* < *N. helveticum* < *L. saturninum*. Mn increasingly reduced the effective quantum yield of photosystem 2 (Φ_2) in the same order. $CaCl_2$ and $MgCl_2$ alleviated the Mn-induced reduction of Φ_2. Moist thalli of all species transferred significant amounts of extracellular Mn into the cells during a recovery day subsequent to incubation with metal solution. This suggests that even short exposures to Mn in the field, e.g. via stemflow, can affect the physiology of the lichen species studied. The experimental results support the hypothesis that cyanolichens are sensitive to excess Mn. Data also suggest that the tripartite *L. pulmonaria* is less Mn-sensitive than the bipartite cyanolichens. This agrees with published field observations from Montana, where bipartite cyanolichens (including *L. saturninum* and *N. helveticum*) occurred on conifer bark with the lowest Mn concentration, while *L. pulmonaria* was also found on bark with higher Mn concentrations.

Keywords: Chlorophyll fluorescence; Heavy metal tolerance; Intrathalline cation transfer; *Leptogium saturninum*; *Lobaria pulmonaria*; *Nephroma helveticum*

1. Introduction

Experimental evidence from the foliose epiphytic lichen *Hypogymnia physodes* shows that this lichen species is sensitive to excess Mn, e.g., in terms of chlorophyll content, chloroplast integrity, soredia growth, photobiont reproduction or in respect of the Ca and Mg budgets (Hauck et al., 2002b,c, 2003; Paul et al., 2003, 2004). Correlations between cover of *H. physodes* and Mn concentration in bark or stemflow suggest that the experimentally proven Mn sensitivity limits the abundance of *H. physodes* in the field. So far, such evidence is limited to coniferous forests of Europe and North America (Hauck et al., 2001, 2002a; Schmull and Hauck, 2003). Mn affecting epiphytes on the trunk surfaces is primarily soil-borne. It reaches the bark surface after root uptake, xylem transport and subsequent leaching from bark and foliage (Lövestam et al., 1990; Sloof and Wolterbeek, 1993; Levia and Herwitz, 2000). In the sites studied, so far, in Germany, New York and Montana, Mn was, therefore, a natural site factor and did not derive primarily from atmospheric deposition (Hauck, 2003, 2005). Whether Mn is effective only in conifer stands, with acidic soils, bark, stemflow and throughfall, where the availability of Mn is higher than at less acidic sites, or whether Mn toxicity is a widespread site factor for epiphytic lichens even in deciduous forest ecosystems is not known yet. Further, it is not known whether high ambient Mn concentrations limit the abundance of epiphytes other than lichens. In contrast to *H. physodes*, the crustose lichen *Lecanora conizaeoides* is not sensitive to Mn as shown by field data (Hauck et al., 2001, 2002a) and experimental evidence (Hauck et al., 2002b, 2003). This is due to its effective intracellular immobilization in polyphosphate granules and in S-containing deposits, which may be phytochelatines (Paul et al., 2003).

As yet, investigations on Mn toxicity have been limited to chlorolichens with the most common green-algal photobiont *Trebouxia*. Particularly with regard to SO_2 or heavy metals, cyanolichens are often supposed to be less toxitolerant than chlorolichens (Garty, 2001; Nash and Gries, 2002).

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In northwestern Montana, cyanolichens such as *Leptogium cellulosum*, *L. saturninum*, *Lobaria hallii*, *Nephroma helveticum*, *N. parile*, and *N. resupinatum* were found to be restricted to coniferous tree bark with low Mn content (Hauck and Spribille, 2002). Bark with high Mn content was only inhabited by chlorolichens. The tripartite lichen *Lobaria pulmonaria*, which has a green alga as the primary photobiont and the cyanobacterium *Nostoc* in internal cephalodia, had an intermediate position between bipartite cyano- and chlorolichens with respect to the Mn concentration of the substrate.

These field observations from Montana led to the hypothesis that cyanolichens are particularly sensitive to Mn. Furthermore, the observation with *L. pulmonaria* resulted in the hypothesis that bipartite cyanolichens are more sensitive to Mn than tripartite lichens with cephalodia. To test these hypotheses, three species were selected that occur on conifers of northwestern Montana (Hauck and Spribille, 2002) and represent three different types of cyanolichens. *Leptogium saturninum* is an homoiomerous, gelatinous, bipartite cyanolichen, *N. helveticum* is an heteromerous, bipartite cyanolichen lichen, and *L. pulmonaria* is an example of an heteromerous, tripartite lichen. All species contain the cyanobacterium *Nostoc*; the green-algal photobiont of *L. pulmonaria* is *Dictyochloropsis* (Rikkinen, 2002). To test the effect of Mn of these lichen species, we studied intra- and extra-cellular uptake as well as chlorophyll fluorescence. Since, firstly, field observations in Montana indicated that the ratios of Mn to Ca and Mg could be more significant for cyanolichens distribution than the Mn concentration itself (Hauck and Spribille, 2002), and secondly, experimental and field evidence for interaction of Mn with Ca and Mg is available from the chlorolichen *Hypogymnia physodes* (Hauck et al., 2002a,b,c, 2003), Mn uptake and chlorophyll fluorescence were also studied in assays where Mn was combined with Ca or Mg.

2. Materials and methods

2.1. In vitro Mn uptake

Thalli of *L. saturninum* (Dickson) Nyl., *L. pulmonaria* (L.) Hoffm. and *N. helveticum* Ach. were sampled from conifer bark in British Columbia, Canada. Samples of *L. saturninum* and *N. helveticum* were taken in Wells-Gray Provincial Park, ca. 25 km N Clearwater, 51°49′ N, 120°07′ W, 820 m, those of *L. pulmonaria* in Spahats Provincial Park, N Clearwater, 51°45′ N, 120°00′ W, 760 m. Air-dry thalli were stored in the dark at room temperature for a few days subsequent to collection and during transport by aircraft and were then frozen at −30 °C. For uptake experiments, thalli were cut into pieces of about 1 cm^2 at room temperature. These pieces were mixed (separately for each species) to avoid effects due to variation of vitality or element content between different thalli of the same species. Prior to the experiment, samples were stored in Petri dishes for 1 day at 80% relative humidity, a day temperature (for 13 h daily) of 13 °C during a photon flux of 30 μmol m^{-2} s^{-1}, and a night temperature of 10 °C. Experiments were carried out in five replicates, while each replicate sample consisted of ten thallus pieces. *L. pulmonaria* and *N. helveticum* were incubated in 20 ml of 5 mM $MnCl_2$ for 0, 2.5, 5, 10, 20, 40, or 80 min, respectively, in order to study time-dependent Mn uptake. The effect of Ca and Mg on Mn uptake was studied in *L. pulmonaria*, *N. helveticum* and *L. saturninum*. Samples were incubated in 20 ml either of 10 mM $MnCl_2$, 10 mM $MnCl_2$ and 2.5 mM $CaCl_2$, or 10 mM $MnCl_2$ and 2.5 mM $MgCl_2$ for 40 min. *L. saturninum* was only considered in this part of the experiment, because it was not possible to collect enough material of this species at one site for the entire experiment. All solutions were adjusted to pH 5 with HCl and NaOH. Incubation was stopped by removing the incubation solution by decantation, immediately shaking the samples with 20 ml deionized water for 2 min and subsequent removal of the water. After incubation, one half of the samples was stored in the growth chamber under the climatic conditions as described above for one day. By this recovery day, lagged uptake of Mn, i.e., translocation from extracellular binding sites into the cell was studied. The other half of samples was prepared for analysis immediately after incubation.

For this purpose, samples were shaken twice with 20 ml of deionized water to remove free apoplastic ions. These water samples were not analyzed, because Mn is primarily allocated at extracellular exchange sites and intracellularly. Extracellularly bound cations were exchanged by shaking samples twice with 20 ml $NiCl_2$. The two $NiCl_2$ solutions per sample were filtered with ash-free filters (Blue Ribbon Filters, Schleicher & Schuell, Dassel, Germany) and pooled. According to Vázquez et al. (1999) two washing procedures with 20 mM $NiCl_2$ are sufficient to release extracellularly bound ions of class A metals or borderline ions with class A character (Nieboer and Richardson, 1980), whereas $NiCl_2$ incubation with higher concentrations or for prolonged periods results in membrane damage. Then samples were dried at 105 °C, homogenized, and digested with 65% HNO_3 in order to determine the intracellular ions (Brown and Brown, 1991). Concentrations of Mn, Ca and Mg in $NiCl_2$ solutions and of Mn, Ca, Mg and K in acid digests were determined with AAS (AAS Vario 6, Analytik Jena, Germany); 0.1% $CsCl_2$ and 0.1% $La(NO_3)_3$ were added prior to analysis to suppress ionization of K or the to release Ca and Mg from refractory, insoluble salts, respectively.

2.2. Chlorophyll fluorescence

Thallus lobes of *L. pulmonaria*, *N. helveticum* and *L. saturninum* were preincubated in the growth chamber, incubated with either (1) deionized water (control), (2) 5 mM $MnCl_2$, (3) 10 mM $MnCl_2$, (4) 5 mM $MnCl_2$ and 2.5 mM $CaCl_2$, (5) 5 mM $MnCl_2$ and 2.5 mM $MgCl_2$, or (4) 5 mM $MnCl_2$, 1.25 mM $CaCl_2$ and 1.25 mM $MgCl_2$ at pH 5 for 40 min as described above. Afterwards the incuba-