

REVIEW

## Interactions of lichens with heavy metals

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### Abstract

Recent developments in knowledge about the interactions between lichens and heavy metals at different levels, from populations to cells and from ecology to molecular biology are reviewed. Sources of heavy metals, mechanisms of heavy metal accumulation and detoxification by lichens are discussed. Special emphasis is placed on ultrastructural changes as well as physiological parameters such as membrane integrity, pigment composition, chlorophyll *a* fluorescence, photosynthesis, respiration, contents of ATP, amino acids, ergosterol, ethylene, non-protein thiols, activity of antioxidant enzymes and expression of stress proteins.

*Additional key words:* biomonitoring, cadmium, copper, detoxification, exclusion, lead, manganese, mercury, zinc.

### Introduction

Lichens are the dominant organisms of *ca.* 8 % terrestrial ecosystems (Larson 1987) and are typically found in environments subject to extremes of temperature, desiccation and nutrient status. As lichens have no root apparatus, their mineral nutrition depends mainly on atmospheric inputs and owing to the lack of protective cuticle and stomata, thalli can accumulate mineral elements, including heavy metals, at levels exceeding their metabolic requirements. Moreover, lichens are long-living, ectohydric organisms with a limited control on water and gas exchange. These characteristics make them

very good bioindicators of air pollution (Garty 2001).

Lichens are widely used through the world for biomonitoring atmospheric heavy metals and progress in this field has been already reviewed (*e.g.* Garty 2001, Bargagli and Mikhailova 2002). However, in the last few years research has chiefly focused on the physiological and biochemical effects of heavy metal accumulation in lichens. This paper aims at reviewing recent developments in the study of interactions of lichens with heavy metals at different levels, from populations to cells and from ecology to molecular biology.

### Lichens and excess heavy metals

The effectiveness of lichens in intercepting particles not only from the atmosphere but also from substrate has been documented in many studies (Loppi *et al.* 1999, Pirintsos *et al.* 2006). These particles may be deposited onto the lichen surface or trapped in the intercellular

spaces of the medulla (Garty *et al.* 1979) and can remain unaltered for a long time. Lichens thus accumulate and retain many heavy metals in quantities that vastly exceed their physiological requirements and tolerate these high concentrations by sequestering metals extracellularly as

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*Abbreviations:* APX - ascorbate peroxidase; CAT - catalase; d.m. - dry mass; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; HPCD - hydroperoxi-conjugated dienes; HSP - heat shock protein; MDA - malodialdehyde; PCs - phytochelatins; SOD - superoxide dismutase; TPF - triphenyl formazan; TTC - triphenyl tetrazolium chloride.

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oxalate crystals or complexing them with lichen acids (details below). The toxicity of metals in deposited or entrapped particles is determined by chemical and physical factors, including their abundance, chemical form, differing solubilities in water, pH and temperature.

Owing to the rapid expansion of industry and agriculture during the last few centuries, lichens are frequently exposed to excess metals which they may tolerate as a result of detoxification mechanisms. Considerable amounts of heavy metals are immobilized by cell wall components, and the main processes for maintaining metal homeostasis in lichens, including transport of heavy metals across membranes, are chelation and sequestration.

As lichens are differentially sensitive to heavy metals, presence of these elements in the environment may produce changes at community level. Recently, Mikhailova (2007) studied survival strategies of epiphytic lichens growing close to a copper smelter in the Middle Urals. She investigated the responses of *Hypogymnia physodes* and *Tuckermanopsis sepincola*, as an asexually reproducing strong competitor, and a sexually repro-

ducing weak competitor, respectively, and showed that *T. sepincola* benefits from the Cu-sensitivity of *H. physodes*. Under Cu stress, *H. physodes* produced only esorediate or low sorediate thalli and sharply decreased in abundance, while abundance of *T. sepincola* on birch trunks increased.

Specific lichen communities on rock and soil occurring in heavy metal-polluted areas worldwide, mainly related to mining of metals, have been investigated by several authors (e.g. Nash 1989, Purvis and Halls 1996, Bačkor and Fahselt 2004a, Banášová *et al.* 2006). Some lichens associated with heavy metal-rich substrates are common species that tolerate metals and occur in both polluted and unpolluted areas. Other species, however, are restricted to heavy metal-rich substrates and thus have a disjunctive distribution reflecting the availability of suitable sites. Most lichens requiring metal-rich substrates belong to the genera *Acarospora*, *Aspicilia*, *Lecanora*, *Lecidea*, *Porpidia*, *Rhizocarpon* or *Tremolecia* (Nash 1989, Purvis and Halls 1996, Bačkor and Fahselt 2004a).

### Accumulation of heavy metals by lichens

Accumulation of heavy metals by lichens is one of the most widely-studied aspects of modern lichenology, because of its importance to biomonitoring. Processes are very complex and many factors affect heavy metal accumulation by lichens and their individual bionts.

Some lichens accumulate considerable amounts of heavy metals. *Acarospora rugulosa* Körb. accumulated up to 16 % of Cu on a dry mass basis (Chisholm *et al.* 1987). *Lecidea lactea* and *Acarospora rugulosa* from cupriferous pyritic rocks in Central Scandinavia contained Cu in concentrations as high as 5 % (d.m.) (Purvis 1984). Pawlik-Skowrońska *et al.* (2006) found that apothecia of *Lecanora polytropa* accumulated Cu up to 1.3 % (d.m.), approximately 50 % of which was in an exchangeable form. However, there are considerable differences between total and intracellular contents. Sanità di Toppi *et al.* (2005a) found in short-term experiments that more than half of total accumulated Cd was immobilized by the cell wall. However, owing to the biochemical and physiological effects of heavy metals, intracellular concentrations of metals must necessarily be considered.

Heavy metal accumulation by lichens is a dynamic process. Short-term investigations on the effects of excess metals showed that lichens soaked into metal solutions accumulated metals quickly, in most cases within a few hours. In the case of Cu, maximum accumulation was observed after 3 - 6 h (Monnet *et al.* 2006). Transplantation studies showed that most lichens respond to changes in atmospheric heavy metals within a few months. The residence time of many elements in lichen thalli is 2 - 5 years (Walther *et al.* 1990).

Although one might expect that the heavy metal

content of lichens would increase as a function of time, the situation is really much more complicated. In fact contents of several elements in transplanted lichens go up and down during the study period. One possible explanation is that contents of these elements are, at least partly, controlled by physiological processes and turnover mechanisms (Bergamaschi *et al.* 2007). Moreover, metals can be removed by rainwater which dislodges contaminating particles on the thallus surface (Brown and Brown 1991), resulting in lower content during periods of rain and higher content in the dry season. The water-leachable fraction (deposited plus intercellular) is generally assumed to represent mainly metals originated from dry deposition, and the elements present in leachates show distinct temporal patterns with concentrations being usually higher in summer than in winter (Boonpragob and Nash 1990). However, because lichens are more active metabolically when wet, winter months are suitable to growth and mineral uptake (Nash and Gries 1995).

In contrast to "washing", rainfall also richly contributes to the total element content of lichens (Knops *et al.* 1991). Although the balance between these two processes could thus produce temporal differences in metal contents in spite of constant pollution loads, the concentrations in lichen thalli mainly varies according to amounts of incident pollutants. These may produce a high degree of biological stress, and in turn, alter element uptake (Bergamaschi *et al.* 2007).

The time of exposure of lichens transplanted into polluted areas may influence the vitality of thalli and consequently the active processes of element uptake. In the absence of time-studies designed to determine the minimal exposure required to produce significant change,

the critical period of exposure remains unknown (Garty 2001). Relatively short exposure times of 1 - 3 months are generally sufficient to affect transplanted lichens. After longer exposures they become saturated with elements, lose biomass, and alter surface structures and physiological performance (Bargagli and Mikhailova 2002).

The accumulation of trace elements by lichens is both passive over the long term or dynamic and immediate involving uptake and release to the point of equilibrium. Reis *et al.* (1999) introduced the concept of "remembrance time", which is the time over which lichen functioning reflects recently-experienced environmental availability. If the "remembrance time" of lichens transplanted from a remote area to a polluted site is longer than the period over which transplant performance is recorded, assessment of the current levels of pollutants, which is the ultimate aim of pollution biomonitoring, would be seriously hampered.

Only a few studies investigated the heterogeneity in distribution of heavy metals in a lichen thallus. Goyal and Seaward (1982a) found that rhizinae and medulla play an important role in metal accumulation and translocation through thalli of terri-colous lichens. Goyal and Seaward (1982b) found that the terricolous lichens *Peltigera canina* and *P. rufescens* respond to metal pollution by reducing thallus size and rhizine length, associated with darkening of their colour caused by the increased heavy metal content. On the other hand, thalli from metal-polluted sites showed denser rhizinae, more profusely branched, network-forming veins, hypertrophic medulla and increased bleaching of thalli. Also reproductive structures can be affected by heavy metals. Field studies demonstrated decreased production of apothecia, which are involved in sexual reproduction, and increased asexual reproduction by conidia (Nash and Gries 1995). Mikhailova (2007) found that Cu pollution caused growth abnormalities, distortion of apothecia and colour changes in thalli of *Tuckermanopsis sepincola* and decreased production of soredia in *Hypogymnia physodes*. Excess Mn caused ultrastructural damage and inhibited soredial growth in *Hypogymnia physodes* (Hauck and Paul 2005).

The relationship between morphology and acquisition of elements is still poorly understood. Lichens with crustose morphology are capable of accumulating considerable amounts of heavy metals, sometimes reaching several percents of their dry mass (see above) but crustose species are seldom considered by experimental lichenologist, mainly because insufficient biomass is available for physiological and biochemical tests. Surface structure, adhesiveness and water-holding capacity of thalli also affect metal accumulation in lichens (Brown and Beckett 1985). Pores and holes on the cortex, which could trap particles or allow them to enter inside the thallus, in some species may be sealed by polymers, probably including lichenins and isolichenins (Richardson 1995).

In polluted areas, the central part of the thallus of

foliose lichens may have higher concentrations of certain metals than the peripheral parts, owing to the greater exposure times experienced (Bargagli *et al.* 1987). However, a regular zonation pattern is not always evident and, furthermore, several elements seem to be rather mobile within the thallus (Goyal and Seaward 1982a). The trace element content of the peripheral (younger) and central (older) parts of *Flavoparmelia caperata* thalli from an unpolluted area of central Italy was highly variable (Loppi *et al.* 1997), and contents of several elements (As, Cr, Fe, Hg, Mn, Ni, Sb) were similar. The contents of elements of limited metabolic significance (Al, Cd, Pb) were higher in the central thallus parts, suggesting that these are trapped in the medulla. Elements essential for metabolism (Co, Cu, Mo, Zn) were concentrated in peripheral thallus regions that are most active metabolically. These observations suggest that metals can move from one part of the thallus to another.

Nieboer *et al.* (1978) observed that dead thalli accumulate slightly more heavy metals than living thalli. Adamo *et al.* (2007) exposed living and dead (oven-treated) thalli of *Pseudevernia furfuracea* for 6 weeks in two Italian cities, Trieste and Naples, and found that living thalli did not generally accumulate more trace elements than dead ones and concluded that in these urban environments, surface interception of atmospheric particles is more important to accumulation than vitality.

Lichen bionts are differentially involved in heavy metal accumulation. The mycobiont, which represents more than 90 % of total lichen biomass, accumulates most of the heavy metals from the environment. Consistently to this expectation, Sanità di Toppi *et al.* (2005b) found that mycobiont hyphae, especially those forming the upper cortex of lichen thalli, were the main site of Cd accumulation.

Because heavy metal uptake is probably mainly physicochemical (Nieboer *et al.* 1976), pH is one of the most important factors affecting it. Decrease in pH can increase the solubility of some metals, releasing them from particles acquired either from the atmosphere or substrate. Decreased pH can thus increase the bioavailability of metals, *e.g.* for lichens growing in mining areas.

Temperature is another important factor influencing metal uptake, with metal accumulation increasing as temperature rises (Nieboer *et al.* 1976). Metal uptake is also dependent upon the location of lichens in relation to availability of heavy metals. For example, elevation plays an important role in metal accumulation, probably owing to the different amount of precipitation, and deposition of more soluble elements (*e.g.* Cd, Zn) thus increases with altitude (Král *et al.* 1989). On a smaller scale, lichens growing on isolated trees contain higher amounts of heavy metal-rich particles than those in dense tree stands. Even in individual trees, heavy metal accumulation depends on the extent of stemflow on a particular side and is usually higher near ground level (Bargagli and Mikhailova 2002).

## Exclusion of heavy metals by lichens

Exclusion of heavy metals is a relatively well known detoxification mechanism and is one of the most studied in lichens. Cell walls of both bionts are effective in metal exclusion, however, intact lichens possess other mechanisms potentially involving organic acids and lichen secondary metabolites.

Oxalates, considered as one of the most effective extracellular mechanisms of heavy metal detoxification, are widely distributed in lichens. Copper oxalate has been reported within the medulla of some Cu-tolerant lichen species (Purvis 1984, Chisholm *et al.* 1987). The role of extracellular oxalates in the immobilization of Zn and Pb in the lichen *Diploschistes muscorum* has been investigated by Sarret *et al.* (1998), while oxalic, citric and malic acids have been demonstrated in *Lecanora polytropa* (Pawlik-Skowrońska *et al.* 2006).

Secondary metabolites of lichens include extracellular phenols secreted by the fungal partner, which are deposited mainly on the surface of mycobiont hyphae. Usually these substances, which include dibenzofuran derivatives, depsides, and depsidones, occur in considerable amounts, typically constituting 0.1 - 5.0 % of thallus dry mass (Fahselt 1994), but in some cases reach 20 % (d.m.) (Barnes *et al.* 1992). So far, more than 800 lichen secondary metabolites have been identified (Hauck and Huneck 2007). These substances have many ecological roles, confirmed experimentally, including allelopathy and antimicrobial and antiherbivory activity (Fahselt 1994). They are also implicated in rock mineralization and pedogenesis, although some organic acids, such as oxalic acid, are probably more important compounds in weathering of rocks. Lichen substances are known to function *in vitro* as chelators of cations, including heavy metals, but the chelating ability of these compounds in lichen thalli requires further study. Purvis *et al.* (1987) reported complexing of copper with norstictic acid in the cortex of the lichen *Acarospora smaragdula* and *Lecidea lactea* grown on cupriferous substrates. Bačkor and Fahselt (2004b) found that usnic acid, one of the most widely distributed secondary metabolite of lichens, may be associated with Cu, Ni, Fe

and Al in *Cladonia pleurota* growing on metal-rich soils near Sudbury (Ontario, Canada). Although metal complexes with secondary metabolites of lichens have been reported several times, knowledge of their impact on metabolic processes is limited. Buďová *et al.* (2006) found that usnic acid does not protect the cells of the lichen photobiont *Trebouxia erici* against the toxic effect of Cu in culture medium; both usnic acid and Cu were phytotoxic and inhibited photobiont growth, viability and chlorophyll *a* fluorescence. Although there is no doubt about ecological roles of secondary metabolites of lichens, these substances may be phytotoxic and adversely affect the growth of photobiont cells (Bačkor *et al.* 1998).

Cations, including heavy metals, can bind to extracellular sites of the mycobiont and photobiont cell walls, and cation binding is one of the most important mechanisms of defense against metal excess. The cell wall is a significant site for metal accumulation owing to the presence of negatively charged anionic sites (Collins and Farrar 1978). These probably include carboxyl and phosphate groups, although amine and hydroxyl groups are also involved. Metal accumulation is usually accompanied by a rapid release of protons (Nieboer *et al.* 1976). Sanità di Toppi *et al.* (2005a) showed that for some metals (*e.g.* Cd and Cr) mycobiont hyphae are the main sites of accumulation.

Cations differ in binding capacities and as a consequence they may displace each other. Competition experiments have established a sequence of ion affinities for exchange sites: monovalent class A < divalent class A < borderline divalent < divalent class B (Nash 1996).

Conventional methods which involve the use of NiCl<sub>2</sub> or Na<sub>2</sub>-EDTA serve to distinguish cell wall accumulation of heavy metals from total metal accumulation by lichen thalli (Brown and Beckett 1985, Monnet *et al.* 2005). Sanità di Toppi *et al.* (2005a) demonstrated that thalli of the lichen *Xanthoria parietina* may bind more than half the total accumulated Cd in short term (24 - 48 h) experiments.

## Heavy metals and plasmalemma

The plasmalemma is the first site of biological interaction of heavy metals with lichens. Frequently used standard methods to determine the degree of cell membrane damage in experimental lichenology involve assessment of electrolyte (mainly potassium) leakage, contents of malondialdehyde (MDA) and hydroperoxy-conjugated dienes (HPCD) and ultrastructural changes.

Metal transport across the plasma membrane in lichens is probably similar to that in model plants (*Arabidopsis*, *Thlaspi*) with protein transport and intracellular high-affinity binding sites mediating the uptake of heavy metals across membranes. Recently,

genes for heavy metal transporters of plasma membranes have been identified (Yang *et al.* 2005). Lipids are a key component of biological membranes and it has been recently demonstrated that lipid metabolism of lichen photobionts may be affected by Cu and Pb (Guschina and Harwood 2006).

Heavy metals which pass through the plasmalemma are accumulated intracellularly. Although in lichens the intracellular fraction of metals may be as low as 5 % of the total, this component is essential for normal physiological processes and vitality (Branquinho *et al.* 1999).

Sanità di Toppi *et al.* (2004) showed that in the

mycobiont hyphae, intracellular Cd is mostly localized in concentric bodies, organelles which are typically present in lichenized ascomycetes. Although their role is still far from being understood, they are probably important for metal detoxification. The vacuole is another important

site for intracellular metal accumulation, resulting in compartmentalization that prevents free circulation of metal ions in the cytosol. Chloroplasts and mitochondria may also be involved in heavy metal sequestration, as in the alga *Euglena gracilis* (Perales-Vela *et al.* 2006).

### Lichen metabolism and heavy metals

Several physiological parameters are used in higher plants to assess the degree of heavy metal stress (Shah and Mongkynrih 2007), and these may be applied as well to lichens.

A simple test to check the integrity of the plasma membrane of lichen cells is to place a piece of lichen thallus in deionized water and record electric conductivity (Marques *et al.* 2005). Determination of leakage of ions from lichen thalli, especially K, is one of the most useful characteristics of stress occurrence. Puckett (1976) found gradual K loss induced by increased concentrations of Cu, Hg and Ag in *Umbilicaria muhlenbergii*.

Products indicating peroxidation of membrane lipids such as malondialdehyde (MDA) and hydroperoxy-conjugated dienes (HPCD) have also been used as indicators of heavy metal damage to lichens, similarly as to higher plants (González and Pignata 1997, Panda 2008). For example, Carreras *et al.* (2005) found that airborne metal pollutants increased the MDA content in lichen thalli. Rodríguez *et al.* (2007) showed that Ni treatment increased the content of HPCD significantly in two lichen species, making them more sensitive to damage. Cuny *et al.* (2004) found parallel increases in the MDA content and electrical conductivity of water in which thalli were immersed. Monnet *et al.* (2005, 2006) measured the MDA and HPCD content in the lichen *Dermatocarpon luridum* to demonstrate responses to Cu stress.

Lichen algal cells respond to Cu and Ni excess by strong plasmolysis. At the ultrastructural level, mitochondrial cristae and chloroplast thylakoids swell in young photobiont cells (Tarhanen 1998). Paul *et al.* (2004) found that Mn excess in the *Trebouxia jamesii* photobiont reduced the size of cells and swelled or contracted thylakoids.

Mycobiont ultrastructure is usually less affected than photobionts by excess heavy metals. Although concentric bodies were sites of intracellular Cd accumulation in mycobiont hyphae (Sanità di Toppi *et al.* 2004), concentric bodies were almost completely absent in Mn-treated mycobiont cells (Paul *et al.* 2004).

The composition of photosynthetic pigments is one of the most sensitive and widely used parameters for the assessment of heavy metal stress in lichens (Boonpragob 2002). Decreased chlorophyll *a* content is a sensitive marker for excess Cu in both lichens as well as axenic photobiont cultures (Bačkor and Zetíková 2003, Bačkor and Dzubaj 2004). Cu stress can promote conversion of chlorophyll *a* to chlorophyll *b* by oxidation of the methyl group on the ring II (Chettri *et al.* 1998), so total

chlorophyll (*a+b*) content remains stable, while the chlorophyll *a/b* ratio is decreased. Hg excess, however, decreases both chlorophyll *a* and chlorophyll *b* content (Bačkor and Zetíková 2003, Bačkor and Dzubaj 2004). The total content of carotenoids is also sensitive to the presence of some heavy metals *e.g.* Cu (Bačkor *et al.* 2003).

One of the most frequently used parameters in lichen stress physiology is chlorophyll degradation, expressed as the phaeophytinization quotient, which reflects the ratio of chlorophyll *a* to phaeophytin *a* (Garty 2001). This is defined as the ratio of absorbances at 435 and 415 nm (Ronen and Galun 1984). In healthy lichens and photobionts the  $A_{435}/A_{415}$  ratio is about 1.4 and the presence of heavy metals (*e.g.* Cu and Hg) may cause a marked decrease of this value.

The potential quantum yield of electron transfer through photosystem 2 (PS 2), reflecting photosynthetic efficiency, is usually expressed by the variable to maximum fluorescence ratio ( $F_v/F_m$ ). Healthy lichens have  $F_v/F_m$  in the range 0.6 - 0.7 (Dzubaj *et al.* 2008) and lower values indicate damage to PS 2 and hence a decreased photosynthetic efficiency. This parameter is used routinely to establish the physiological status of thalli in experimental and environmental lichenology (Garty 2001). Branquinho *et al.* (1997) showed that intracellular Cu > 4.0  $\mu\text{mol g}^{-1}$  decreased chlorophyll *a* fluorescence in the lichen *Ramalina fastigiata*. Garty *et al.* (2007) observed a decrease of  $F_v/F_m$  in *R. lacera* thalli following short-term exposure to  $\text{CuSO}_4$ , and Bačkor *et al.* (2007a) in short term laboratory experiments found a decrease in chlorophyll *a* fluorescence in axenic cultures of the lichen photobiont *Trebouxia erici* due to Cd and Cu excess.

Changes in respiratory and photosynthetic rates caused by air pollution have been determined by measuring  $\text{CO}_2$  gas exchange (Tretiach and Baruffo 2001). Dzubaj *et al.* (2008) measured  $\text{CO}_2$  gas exchange in the lichen *Xanthoria parietina* collected close to a steel factory in Košice (Slovakia) and in a control locality. Lichen thalli near the steel factory had higher heavy metal (Al, Cd, Fe, Mn, Pb, Sb and Zn) contents, while effects on respiration and photosynthesis were only weakly negative. Reduction of a colorless water-soluble compound, triphenyl tetrazolium chloride (TTC) to a red colored water-insoluble product, the triphenyl formazan (TPF) is driven by respiratory dehydrogenases (Towill and Mazur 1975), thus measurement of TPF is a rapid and inexpensive way to evaluate the response of whole lichens, as well as their cultured bionts to a wide range of

environmental stressors, including heavy metals (Bačkor and Fahselt 2005).

Several studies have shown that the ATP content in lichens may be used as stress marker in air pollution studies (Silberstein *et al.* 1996). Garty *et al.* (1988) showed that the ATP content in transplanted thalli of the lichen *Ramalina duriaei* decreases with increasing Cu and Pb accumulation.

Accumulation of free proline has been observed in response to a wide range of abiotic and biotic stresses in plants, including heavy metals. Proline can detoxify free radicals by forming a stable complex with these, maintaining NAD(P)<sup>+</sup>/NAD(P)H ratios during metal stress at values similar to those of normal cells (Hare and Cress 1997). The role of proline in heavy metal tolerance of lichens is essentially unknown, however, free proline increased in the lichen photobiont *Trebouxia erici* in response to Cu treatment (Bačkor *et al.* 2004).

Cysteine is unique among amino acids owing to the presence of thiol (-SH) groups, which are responsible for binding of heavy metals. Cysteine is a limiting substrate for glutathione (GSH) synthesis. GSH is an important thiol compound involved in protection of cells against active oxygen species, including toxic metals (May *et al.* 1998). The ratio of reduced to oxidized glutathione within lichen cells may thus be used as an indicator of toxicity (Pawlik-Skowrońska *et al.* 2002). Bačkor *et al.* (2007b) demonstrated that the Cu-tolerant strain of the lichen photobiont *Trebouxia erici* synthesized more cysteine than the wild strain, even at nutritional levels of heavy metals in the culture medium. Cysteine pool may play a key role in defense against heavy metals in lichen photobionts, similar to that reported for free-living algae. Bačkor *et al.* (2006a) found that GSH increased in aposymbiotically grown lichen mycobiont cultures of *Cladonia cristatella* at 10  $\mu$ M Cu. However, the same Cu concentration was very toxic for cultures of the lichen photobiont *Trebouxia erici* and decreased GSH was observed (Bačkor *et al.* 2007b).

In the presence of some heavy metals, phytochelatin synthase (EC 2.3.2.15) catalyzes the synthesis of phytochelatin (PCs) from glutathione (Grill *et al.* 1985). Cysteine sulfhydryl is the primary site of metal binding in phytochelatin (PCs), as well as in metallothioneins (Sanità di Toppi *et al.* 2003). All these groups of chelators are responsible for metal sequestration in living cells. Pawlik-Skowrońska *et al.* (2002) found that lichen thalli are able to synthesize phytochelatin in response to Cd, Pb and Zn, but phytochelatin has not been observed in aposymbiotically grown lichen mycobionts. The cultured *Cladonia cristatella* mycobiont also did not produce phytochelatin (Bačkor *et al.* 2006a), although photobionts produce phytochelatin under heavy metal stress. Cd and Cu are strong activators of PC biosynthesis in the lichen photobiont *Trebouxia erici* (Bačkor *et al.* 2007a).

The content of ergosterol, the main sterol of fungal

plasma membranes, is correlated with the basal respiration rates of lichens (Sundberg *et al.* 1999). The typical ergosterol content in lichens is in the range 0.1 - 1.8 mg g<sup>-1</sup>(d.m.) (Dahlman *et al.* 2002), and Bačkor *et al.* (2006a) found similar values in the *Cladonia cristatella* mycobiont. Tarhanen *et al.* (1999) found that the ergosterol content in the lichen *Bryoria fuscescens* was decreased by the presence of Cu and Ni, and suggested that the toxicity of these heavy metals is due to reduced membrane integrity in mycobiont hyphae. Decreased content of ergosterol caused by Cu stress has also been found in axenic cultures of the lichen mycobiont *Cladonia cristatella* (Bačkor *et al.* 2006a).

Monnet *et al.* (2006) investigated the activities of antioxidant enzymes in response to Cu excess and found different reactions depending upon Cu concentration; at 0.25 mM the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were unchanged from controls, but they increased at 0.5 mM Cu. The activity of glutathione reductase (GR) decreased at both Cu concentrations. In *Xanthoria parietina* metal excess caused increased activity of guaiacol peroxidase but no change, or only a moderate decrease in the activity of SOD, APX, catalase (CAT) and some other enzymes (Cuny *et al.* 2004, Sanità di Toppi *et al.* 2005a).

Although soluble proteins in lichens are relatively easy to determine (Bradford 1976), they are still not frequently used in the assessment of heavy metal stress, possibly because their contents in lichens are typically low, *e.g.* 1 mg g<sup>-1</sup>(f.m.) in thalli of *Xanthoria parietina* (Sanità di Toppi *et al.* 2005b). Bačkor *et al.* (2006a) found mean soluble protein content of *ca.* 5 mg g<sup>-1</sup>(d.m.). Monnet *et al.* (2006) reported decreased soluble protein content due to increased Cu accumulation in thalli of the lichen *Dermatocarpon luridum*, and a similar decrease was observed in cultures of lichen photobionts exposed to Cu excess (Bačkor *et al.* 2006b). However, the soluble protein content in lichen photobionts was not affected by Cd excess in short-term experiments (Sanità di Toppi *et al.* 2005b).

Extreme environmental conditions, including pollution with heavy metals, may activate change in the transcript levels of numerous genes encoding proteins with protective functions against stress-related damage (Mittra *et al.* 2008). Highly conserved heat shock proteins (HSP) expressed in response to stress, such as low and high temperature, heavy metal exposure and oxygen depletion (Bierkens 2000) are involved in the maintenance of protein homeostasis in untreated cells. These are generally grouped into families according to their molecular mass, forming multiple isoforms. The expression of stress protein HSP70 in the lichen photobiont *Trebouxia erici* was relatively stable over all tested Cu concentrations, while Cd caused a strong increase in the expression even at concentrations as low as 1  $\mu$ M during a short-term exposure (Bačkor *et al.* 2006b).

## Conclusions

Notwithstanding the high number of papers dealing with interactions of lichens with heavy metals, there is still much to be learned about their ecophysiological aspects and cellular location. It is thus important to consider that total concentrations have little relevance for ecophysiological investigations because only metals inside cell directly affect physiological and metabolic processes related to toxicity. Careful study of the mechanisms regulating bioavailability, accumulation, toxicity and detoxification of heavy metals in lichens, as well as of

parameters reflecting lichen vitality is needed. Analysis of intraspecific genetic diversity will also contribute to a better understanding of the variability in the metal content in thalli of one species, as well as responses under different environmental and experimental conditions. Lastly, the use of genotoxicity tests, along with the study of mechanisms of bioavailability and biotoxicity of metals, could further enhance the utility of lichens as biomonitors of air pollutants.

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