



# Competition between heavy metal ions for binding sites in lichens: Implications for biomonitoring studies



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## HIGHLIGHTS

- *Xanthoria parietina* was treated with ionic single/mixed solutions of Cd, Cu, Pb, Zn.
- A mixed supply decreased the uptake, compared to each cation supplied alone.
- A competition between divalent cations for binding sites on lichen thalli does exist.
- The real environmental levels of such elements can be underestimated in field studies.

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## ABSTRACT

The competitive behavior of divalent heavy metals (Cd, Cr, Pb, Zn) during cation uptake was investigated in the foliose lichen *Xanthoria parietina*. Lichen thalli were incubated with solutions containing 10 and 100  $\mu\text{M}$  of  $\text{CdCl}_2$ ,  $\text{CuCl}_2$ , and  $\text{ZnCl}_2$  as well as 5 and 50  $\mu\text{M}$  of  $\text{Pb}(\text{NO}_3)_2$ , tested individually and in combination ( $\text{Cd}^{2+} + \text{Cu}^{2+} + \text{Pb}^{2+} + \text{Zn}^{2+}$ ). The analysis of molar concentrations suggests that a competition between cations for binding sites in *X. parietina* does exist. The decrease in net uptake between single and mixed solutions ranged between 14 and 29% at the lowest concentration and between 38 and 68% at the highest concentration. Furthermore, the uptake was proportionally lower for richer solutions. Each metal may behave differently when uptook: some (toxic elements) are preferentially stored at extracellular level (Cd, Pb), while others (micro-nutrients) are also present at intracellular level (Cu and Zn). The proportion between extracellular and total content changed for those elements accumulated also at intracellular level (Cu and Zn), while for Cd and Pb almost all the uptake occurred by passive mechanisms mainly at extracellular binding sites. The competition between metals for binding sites in the lichen surface entails that bioaccumulation data might result in an underestimation of some element levels measured in biomonitoring studies.

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## 1. Introduction

Lichens have a notable ability to take up atmospheric pollutants, especially heavy metals, from the surrounding environment, and it is widely acknowledged that the concentrations of trace elements in lichen thalli are correlated with their environmental levels (Loppi and Paoli, 2015). Lichens take up elements from the atmosphere through three mechanisms: particulate trapping, extracellular ion exchange and intracellular accumulation; the first two mechanisms explain the ability of these organisms to accumulate and tolerate toxic elements to levels far above their physiological

requirements (Bačkor and Loppi, 2009).

The lichen thallus is characterized by cation exchange properties: the uptake of soluble cations occurs according to chemical affinities for anionic sites in the cell wall and the concentration of the supplied elements; in this way, cations are bound to the cell walls forming metal-complexes, in particular with carboxylic and hydrocarboxylic groups and chitin (Galun et al., 1983). Cations may enter and accumulate intracellularly through energy-dependent and plasma membrane controlled systems (Bačkor and Loppi, 2009). However, the uptake and release of trace elements are reversible processes influenced by several parameters, such as thallus morphology and age, physiological status, pH, duration of exposure, microclimatic conditions, presence and type of pollutants in the environment and in the lichen thallus (Bačkor and Loppi, 2009).

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Notably, the contemporary supply of positively charged elements may displace the original cations from their extracellular exchange sites, depending on pH, chemical affinities and available concentrations (Nieboer et al., 1978; Hauck et al., 2002). Therefore, the accumulation of elements which form weaker complexes with the lichen thallus may be influenced by increases in concentrations of other heavy metals able to form stronger complexes (Chettri et al., 1997). As a consequence, it is possible that in lichen biomonitoring studies the nature of the elements and their different competitive capacities for exchange binding sites might mask the real environmental level of these elements. Bioaccumulation studies using lichens as biomonitors normally provide the total content of the investigated elements on the lichen thallus, without distinguishing between trapped particulate matter and ionic (extracellular and/or intracellular) fractions.

The present manuscript deals with cations uptake. Our working hypothesis was that a competition between divalent cations for binding sites on lichen thalli does exist. To test this hypothesis, the concentrations of selected heavy metals, namely Cd, Cu, Pb and Zn, were compared in lichen samples (*Xanthoria parietina*) incubated with solutions containing  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$ , either individually or in combination ( $\text{Cd}^{2+} + \text{Cu}^{2+} + \text{Pb}^{2+} + \text{Zn}^{2+}$ ).

## 2. Materials and methods

### 2.1. Lichen material

Thalli of the foliose lichen *Xanthoria parietina* (L.) Th·Fr. (Telo-schistaceae) were collected in a rural area of Tuscany ( $43^{\circ}14'07''$  N,  $11^{\circ}20'26''$  E, Ville di Corsano, Siena, Italy). The species, often forming extensive yellow patches ranging from flat to wrinkled rosettes, has been selected being extremely common in Tuscany and widely used in biomonitoring studies (e.g., Loppi et al., 2006) as well as in laboratory experiments of element accumulation and toxicity (e.g., Paoli et al., 2013). After collection, samples were brought to the laboratory and cleaned from impurities under a stereoscopic microscope by means of plastic tweezers. Then, the lichens were washed in deionized water, air dried (water content <10%) and divided into batches (each one of about 200 mg). The lichen material was left to acclimate for three days in a climatic chamber at  $15 \pm 2^{\circ}\text{C}$ , RH  $55 \pm 5\%$ , photoperiod of 12 h at  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  photons PAR and then used for the treatments.

### 2.2. Experimental design

Solutions 10 and 100  $\mu\text{M}$  of  $\text{CdCl}_2$ ,  $\text{CuCl}_2$ , and  $\text{ZnCl}_2$  as well as 5 and 50  $\mu\text{M}$  of  $\text{Pb}(\text{NO}_3)_2$  were tested individually and in combination ( $\text{Cd}^{2+} + \text{Cu}^{2+} + \text{Pb}^{2+} + \text{Zn}^{2+}$ ). Hereafter, metal ions  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$ , will be indicated as Cd, Cu, Pb and Zn. Metals have been provided as chloride salts, being highly soluble in water:  $\text{CdCl}_2$  (1400 g/L),  $\text{CuCl}_2$  (757 g/L),  $\text{ZnCl}_2$  (4320 g/L). It was not possible to use  $\text{PbCl}_2$  owing to the low water solubility of this salt (10.8 g/L), therefore we selected  $\text{Pb}(\text{NO}_3)_2$  (525 g/L). In the mixed solutions, the total metal concentrations were 35 and 350  $\mu\text{M}$ , so that each metal was at the same concentration of the single solution. Pb was provided at a 50% concentration compared with the other metal ions owing to its known characteristic of remaining largely extracellular without entering the protoplast (Branquinho and Brown, 1994). The metal concentrations supplied during our experiment are within the range used to induce heavy metals uptake in lichen samples in laboratory experiments, as well as within the ranges of ecologically relevant levels found in polluted environments, such as urban and industrial areas (Chettri et al., 1997; Hauck et al., 2002).

For each treatment, 200 mg of dry (living) lichen material were

incubated and gently shaken for 1 h in 50 mL of individual Cd, Cu, Pb and Zn, as well as combined ( $\text{Cd} + \text{Cu} + \text{Pb} + \text{Zn}$ ) solutions. It is known that the uptake of these metals is not influenced over a solution pH in the range 4–7 (Chettri et al., 1997) and our solutions were within this interval. Samples were removed from the solutions, briefly washed with deionized water to get rid of free apoplastic and unbound ions, and let air-dry on absorbing paper for 24 h in a climatic-chamber, as described above, in order to allow also a later uptake (Hauck et al., 2002). Control samples were treated in the same way, but incubated only in deionized water. To verify the effective adsorption and calculate mass balances, after the treatments, once the samples were removed, Cd, Cu, Pb and Zn were analyzed also in the treatment solutions. Due to a procedural mistake, nominal concentrations were not verified experimentally. The experiment was replicated independently 3 times.

### 2.3. Total and extracellular amounts

In order to distinguish between total and extracellular contents of Cd, Cu, Pb, Zn, an elution technique (Brown and Brown, 1991) was run. Samples were divided into two batches: the total content was determined in one batch, while the other batch was soaked by shaking for 1 h in 10 mL of a 20 mM  $\text{Na}_2\text{EDTA}$  solution and then rinsed in deionized water to remove the extracellular fraction of the elements bound to the cell wall (Branquinho and Brown, 1994). The difference between the total content and the concentration after EDTA washing was taken as the extracellular amount.

### 2.4. Chemical analysis

Prior to mineralization, samples were desiccated in oven at  $40^{\circ}\text{C}$  for 24 h to determine their dry weight. Then, they were pulverized with a ceramic mortar and pestle and mineralized with a mixture of 3 mL of 70%  $\text{HNO}_3$ , 0.2 mL of 60% HF and 0.5 mL of 30%  $\text{H}_2\text{O}_2$  in a microwave digestion system (Milestone Ethos 900, Milestone Srl, Sorisole (BG), Italy) at  $280^{\circ}\text{C}$  and 55 bars. The concentrations of Cd, Cu, Pb, Zn were determined by ICP–MS (Perkin Elmer – Sciex, Elan 6100, Waltham, MA). Since our aim was to check for ionic competition, results were expressed as  $\mu\text{mol/g}$  dry weight. Analytical quality was checked with the Standard Reference Material IAEA–336 ‘lichen’; recoveries were 98% for Cu, 110% for Zn, 108% for Cd and 96% for Pb. The precision of the analysis was estimated by the coefficient of variation of 5 replicates and was 5% for Cu, 3% for Zn, 10% for Cd and 3% for Pb. For comparison, the treating solutions used in the metal incubation were also analyzed, with the results expressed as  $\mu\text{mol/L}$ .

### 2.5. Statistics

Owing to the small data-set, for each element, the non-parametric Mann-Whitney U statistical test ( $P < 0.05$ ) was used to check for differences between single and mixed solutions.

## 3. Results

The concentrations of Cd, Cu, Pb and Zn in *X. parietina* after the treatments with single (S) and mixed (M) salt solutions are summarized in Fig. 1, where also the concentrations in the solutions after the treatments are represented. The element content of control samples corresponded to that of lichens from unpolluted environments (Bargagli and Nimis, 2002). Compared with control samples, the treatments caused a great accumulation for all elements, but the proportion 1:10 (as supplied) was roughly maintained only for Pb. When the elements were provided in a mixed solution, lichens always accumulated a significantly lower amount

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