Chemosphere 199 (2018) 655–660

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

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

Luca Paoli*, Andrea Vannini, Fabrizio Monaci, Stefano Loppi

Department of Life Sciences, Via Mattioli 4, I-53100 Siena, University of Siena, Italy

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.

ARTICLE INFO

Article history:
Received 20 October 2017
Received in revised form
8 February 2018
Accepted 9 February 2018
Available online 20 February 2018

Handling Editor: R. Ebinghaus

Keywords:
Bioaccumulation
Heavy metals
Ionic uptake
Xanthoria parietina

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 μM of $CdCl_2$, $CuCl_2$, and $ZnCl_2$ as well as 5 and 50 μM of $Pb(NO_3)_2$, tested individually and in combination (Cd^{2+}+Cu^{2+}+Pb^{2+}+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.

© 2018 Elsevier Ltd. All rights reserved.

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).

* Corresponding author.
E-mail address: paoli4@unisi.it (L. Paoli).

https://doi.org/10.1016/j.chemosphere.2018.02.066
0045-6535/© 2018 Elsevier Ltd. All rights reserved.

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 Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}, either individually or in combination (Cd^{2+}+Cu^{2+}+Pb^{2+}+Zn^{2+}).

2. Materials and methods

2.1. Lichen material

Thalli of the foliose lichen *Xanthoria parietina* (L.) Th·Fr. (Teloschistaceae) were collected in a rural area of Tuscany (43°14′07″ N, 11°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 ± 2 °C, RH 55 ± 5%, photoperiod of 12 h at 40 μmol m^{-2} s^{-1} photons PAR and then used for the treatments.

2.2. Experimental design

Solutions 10 and 100 μM of $CdCl_2$, $CuCl_2$, and $ZnCl_2$ as well as 5 and 50 μM of $Pb(NO_3)_2$ were tested individually and in combination (Cd^{2+}+Cu^{2+}+Pb^{2+}+Zn^{2+}). Hereafter, metal ions Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}, will be indicated as Cd, Cu, Pb and Zn. Metals have been provided as chloride salts, being highly soluble in water: $CdCl_2$ (1400 g/L), $CuCl_2$ (757 g/L), $ZnCl_2$ (4320 g/L). It was not possible to use $PbCl_2$ owing to the low water solubility of this salt (10.8 g/L), therefore we selected $Pb(NO_3)_2$ (525 g/L). In the mixed solutions, the total metal concentrations were 35 and 350 μM, 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 (Cd+Cu+Pb+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 Na_2EDTA 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 °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% HNO_3, 0.2 mL of 60% HF and 0.5 mL of 30% H_2O_2 in a microwave digestion system (Milestone Ethos 900, Milestone Srl, Sorisole (BG), Italy) at 280 °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 μmol/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 μmol/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

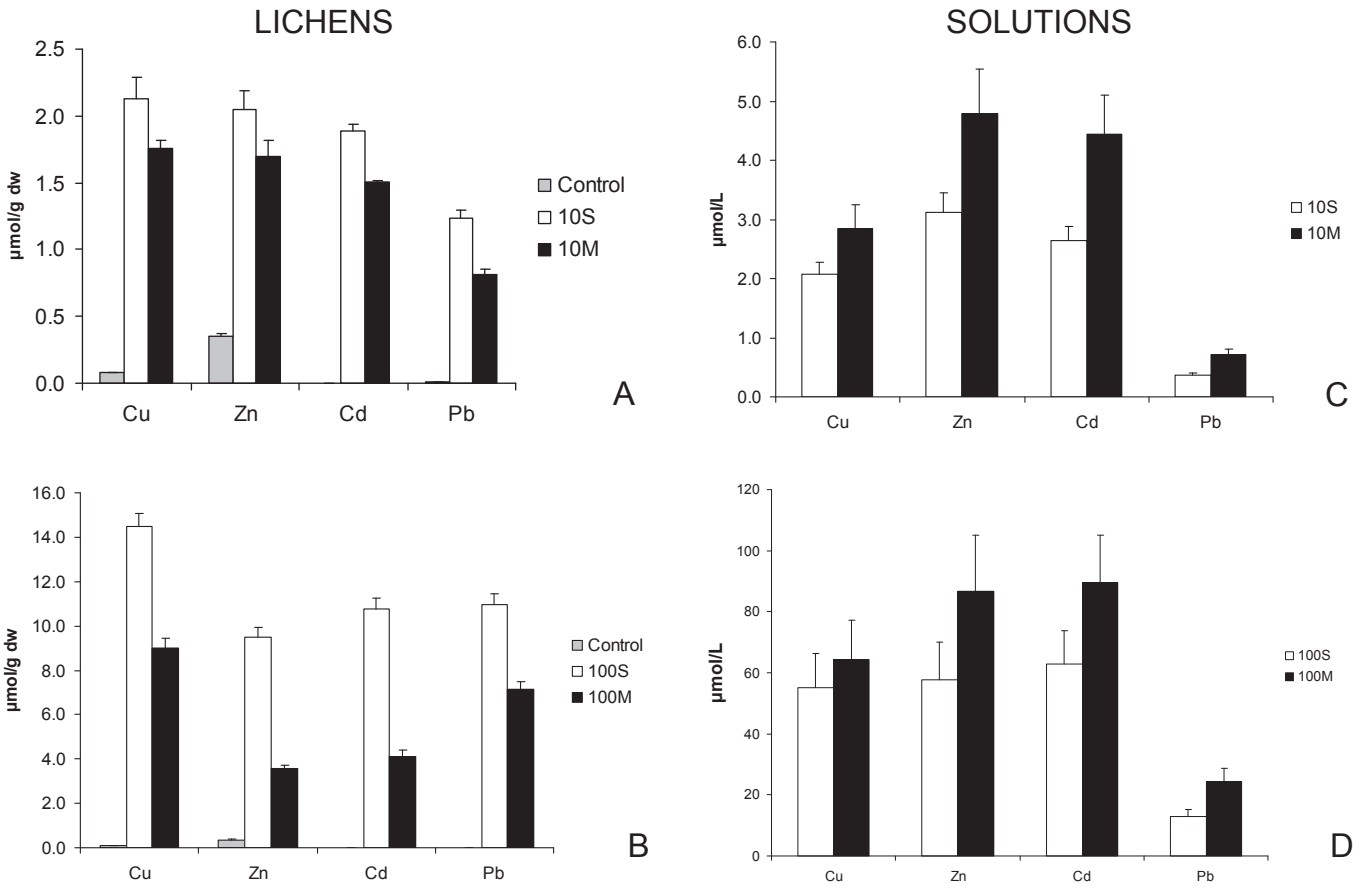

Fig. 1. Total concentrations of Cd, Cu, Pb and Zn (µmol/g dw) in the lichen *X. parietina* (A, B) and within the treating solutions (µmol/L) (C, D) after incubation with single (S) and mixed (M) salt solutions 10 and 100 µM. Pb was provided at a 50% concentration compared with the other metal ions. For each condition, differences between S and M treatments are statistically significant (Mann-Whitney *U* test, $P < 0.05$, $N = 9$).

of the same element compared with the single solutions. The difference in net uptake (mean values) between single and mixed solutions, accounting also the pre-exposure concentration, ranged 14–29% at the lowest concentration and 38–68% at the highest concentration.

The analysis of metal concentrations in the treatment solutions after lichen incubation allowed the calculation of rough mass balances (Table 1), which were in good agreement with nominal values. The percent net uptake in lichen samples showed a constant trend of decreasing values (for Cd, Cu, Zn) in the order 10 µM single > 10 µM mixed > 100 µM single > 100 µM mixed (Table 2). The same order applied to Pb (5 and 50 µM).

Fig. 2 shows the extracellular fraction for each metal. Interestingly, in control samples the extracellular fraction of Zn and Cu (micro-nutrients) was very low (1–5%), while that of Cd and Pb (toxic elements) was ca. 37% for both metals. After metal incubation, the extracellular fraction greatly increased, reaching up to 97% for Pb. While Cu and Zn fluctuated, it was constantly around 90% for Cd and Pb, irrespective of concentration and treatment (S or M).

Table 1
Rough mass balances: sum of metal concentrations (µmol) measured in lichen samples and treatment solutions, after lichen (200 mg) incubation with single (S) and mixed (M) salt solutions (50 mL). *Pb was provided at a 50% concentration compared with the other metal ions.

	Cu	Zn	Cd	Pb*
S10	0.51	0.50	0.51	0.26
S100	0.48	0.51	0.52	0.20
M10	5.64	4.72	5.29	2.84
M100	5.00	4.97	5.29	2.65

Table 2
Percent (%) net uptake in *X. parietina* incubated with single (S) and mixed (M) salt solutions (10 and 100 µM). *Pb was provided at a 50% concentration compared with the other metal ions.

	Cu	Zn	Cd	Pb*
S10	79.8%	68.5%	74.1%	93.0%
S100	70.3%	52.9%	57.6%	81.9%
M10	51.1%	38.7%	40.7%	77.2%
M100	35.7%	12.9%	15.5%	53.9%

4. Discussion

The results indicated that under the experimental conditions a ionic competition between Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} for binding sites of lichen thalli does exist. The uptake was proportionally lower for richer solutions. The contemporary supply of Cd, Cu, Pb, Zn determined a decreased uptake of each metal compared with the uptake measured when each metal was supplied alone. Hauck et al. (2002) found that Ca and Mg in combination may reduce the uptake of Mn in the lichen *Hypogymnia physodes* under laboratory conditions. Similarly, Chettri et al. (1997) reported that the uptake of a metal from a mixed solution with the same amount of each

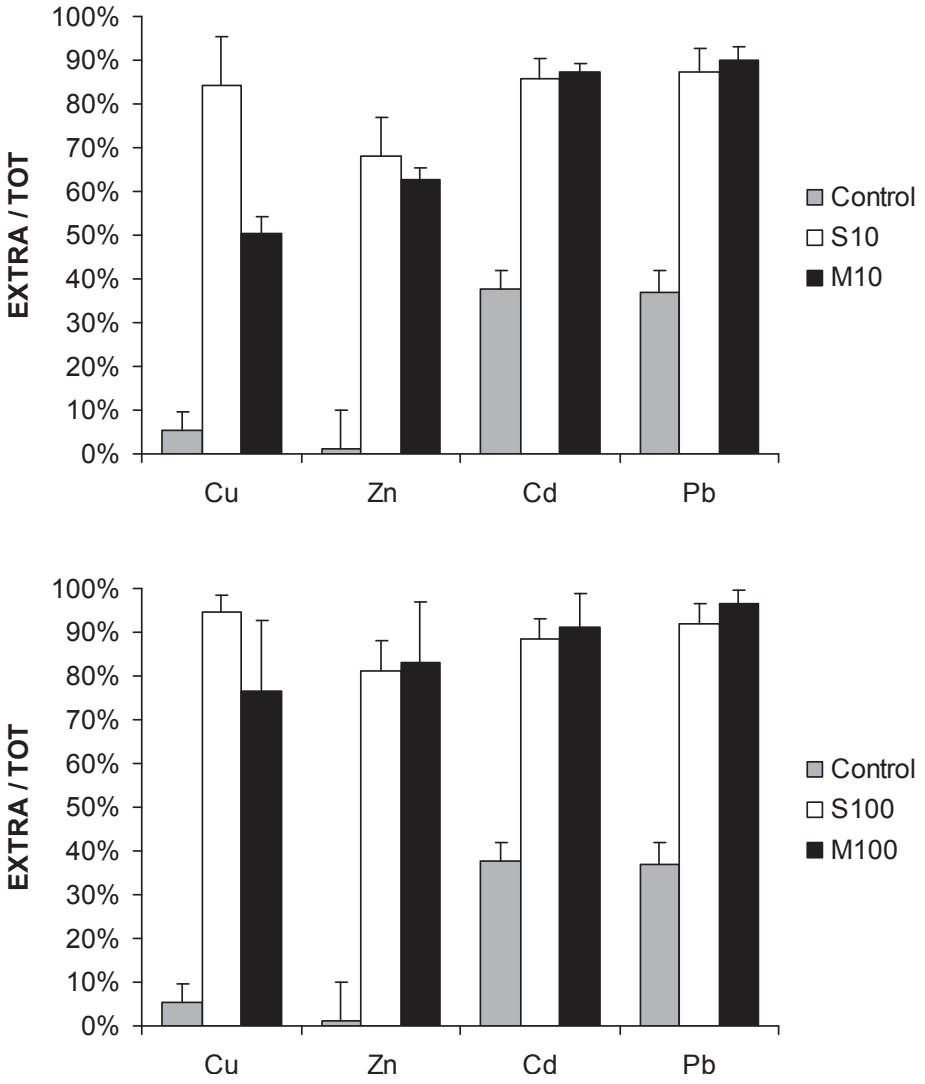

Fig. 2. Extracellular concentrations (as percentage) of Cd, Cu, Pb and Zn in control samples of *X. parietina* and after the treatments with single (S) and mixed (M) salt solutions. For each element, differences between S and M solutions are all statistically significant (Mann-Whitney *U* test, $P < 0.05$, $N = 9$).

metal is affected by the presence of the others and that the competitive uptake followed the sequence Pb > Cu > Zn. The authors assessed the uptake on a mass basis, i.e. results are expressed as μg/g dw. Our results, based on % net molar uptake compared to control values, reflect the real ionic competition and indicate that Pb is always taken up in higher proportions. The uptake of different metals can change according to the pH of the solution (and also to the lichen species), being overall stable for Cu, Pb and Zn in the range 4–7 (our experimental range), with peaks of absorption in the range 5.0–6.5 (Chettri et al., 1997).

Our results are consistent with the findings of Renaudin et al. (2018), that the competition between Pb^{2+} and Na^{+} for binding sites in the moss *Hypnum cupressiforme* may affect their reciprocal uptake. The authors demonstrated that high concentrations of Pb^{2+} prevented Na^{+} from binding to the cell wall, since both elements compete for the same sites in the extracellular compartment. Furthermore, specimens from a coastal environment (acclimated to high salt concentrations), accumulated more Na^{+} and less Pb^{2+} than specimens from continental populations (Renaudin et al., 2018). Consequently, biomonitoring programs that use mosses to investigate heavy metals pollution in coastal environments should account for a possible risk of underestimating real environmental levels due to the "saturation" of binding sites by marine cations.

Previous experiments suggested that a mechanism of ionic exchange is involved in cation uptake also in lichens (Brown and Slingsby, 1972). Puckett et al. (1973) suggested the formation of metal-complexes on binding sites located in the cell wall (represented by carboxylic and hydrocarboxylic acids and chitin) and argued that the stability of these complexes reduces the possibility for a cation of being exchanged by other cations. In the case of elements forming weaker complexes on the cell wall, lichen thalli may not reflect the actual proportion of the same elements in the environment (Chettri et al., 1997). Hence, ionic metal uptake in lichens consists of a ion exchange process modified by metal-complex formation, which is parallel to the process of labile extracellular cation bonding and can be followed by cations accumulated at intracellular level (Klos et al., 2007).

Field studies on the proportion of extra- and intracellular amounts of elements accumulated in lichens are still limited. Cations may enter inside mycobiont and photobiont cells through energy-dependent and plasma membrane controlled systems and this fraction represents the intracellular component originating

from trace element depositions. If the aim of the research does not deal with the assessment of particulate air pollution, it could be suggested to quickly wash the samples before the analysis to remove particles simply deposited over the thallus surface; in addition, in the case of lichen transplants, relatively short exposure times (e.g. 1–3 months) are recommended. As an example, Vannini et al. (2017) reported a marked intracellular accumulation of Cd and Sb (evinced by means of elution techniques) in the lichen *Evernia prunastri* exposed (3 months) to traffic pollution.

As a rule of thumb, it is known that Pb has a strong affinity for cell wall ligands, whereas Zn and Cu, being essential micronutrients, may easily penetrate the plasma membrane; Cd has an intermediate behaviour (Bačkor and Loppi, 2009). The results of our study indicated that the proportion between extracellular and total contents varied for those elements accumulated also intracellularly (Cu, Zn), while it did not change so much for those elements mainly accumulated in the cell wall (Cd and Pb). In fact, Cu and Zn are components of several enzymes (Van Assche and Clijsters, 1990), hence they may easily penetrate the plasmalemma, assuming an intermediate distribution and accumulation inside the cell. This is confirmed also in control samples, which showed that (as a normal condition), Cu and Zn are almost all distributed intracellularly, while ca. 1/3 of Cd and Pb was extracellular. The 90% extracellular uptake of Cd and Pb explains how lichens cope with the toxicity of these metals. However, this is valid in general, since the extracellular accumulation was 68–95% for all metals when provided in single solutions.

Passive uptake is a physico-chemical process influenced by environmental concentrations and chemical affinities, and in lichens it is the main process of extracellular accumulation (Goyal and Seaward, 1982), while active uptake of cations is linked to lichen metabolism and correlates with the respiration rate (Kershaw, 1985). However, at high concentrations also those elements with metabolic value may become toxic, especially Cu (Bačkor and Loppi, 2009), and passive uptake may prevail as well. In general, the fraction of elements accumulated extracellularly can be more easily displaced by wetting and drying cycles and by the competition with other elements, hence having a shorter turnover time (Richardson and Nieboer, 1980), while the fraction accumulated at intracellular level has a longer residence time (Nieboer et al., 1979). This is relevant especially in bioaccumulation studies, since lichens are successfully applied in environmental monitoring given their ability to take up metals from the surrounding environment to levels far exceeding their requirements (Bačkor and Loppi, 2009).

Lichens tend to the reach an equilibrium with their surrounding environment, reflecting the chemical composition of bulk atmospheric deposition even when field-exposed (transplanted) for periods as short as one month (Loppi and Paoli, 2015). On the other hand, specimens exposed from polluted to clean sites may require periods as long as one year to reflect the improvement of environmental conditions (Paoli et al., 2018). Extracellular element accumulation depends both on environmental levels and physico-chemical characteristics of these elements, as well as environmental features of the site (e.g. rain), and a variable equilibrium with the surrounding environment is reached. However, our results suggest that when element uptake occurs mainly via wet (water-soluble) deposition, the element content of lichens may not reflect the actual proportions of elements in the environment, but also under- or overestimate the real amount of some elements. As an example, assuming that the single-metal solution is representative of the environmental pollution at a certain site and air pollution increases by the release of other additional air pollutants along with the original one (the mixed-metal solution): by analyzing lichen samples one should conclude that air pollution by the original metal has decreased, which is not actually the case. The same holds true for the reverse case.

5. Conclusions

The experiment demonstrated that a competition between cations (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}) for binding sites in *X. parietina* does exist. Treating lichen thalli with single salt solutions caused a relevant accumulation for all elements. When the elements were provided in a mixed solution, lichens accumulated a lower amount of the same element. The uptake occurred chiefly at extracellular binding sites. Furthermore, the uptake was proportionally lower for richer solutions. These evidences suggest that bioaccumulation data might result in an underestimation of some element levels measured in biomonitoring studies. Since it is known that a mechanism of ionic exchange is involved in cations uptake in mosses (Renaudin et al., 2018) and lichens, in order to limit the possible risk of underestimating the real environmental pollution by heavy metals due to this competition, research is needed i.e. to clarify the saturation of cell wall ligands under the influence of multiple sources of pollution and later on, to improve data interpretation.

Acknowledgements

We acknowledge three anonymous reviewers for their comments on an earlier version of the manuscript. We are grateful to Gianni Henson (University of Siena) for kindly reviewing the English of the manuscript.

References

Bačkor, M., Loppi, S., 2009. Interactions of lichens with heavy metals. Biol. Plant. 53, 214–222.
Bargagli, R., Nimis, P.L., 2002. Guidelines for the use of epiphytic lichens as biomonitors of atmospheric deposition of trace elements. In: Nimis, P.L., Scheidegger, C., Wolseley, P.A. (Eds.), Monitoring with Lichens – Monitoring Lichens. Kluwer Academic Publishers, pp. 295–299.
Branquinho, C., Brown, D.H., 1994. A method for studying the cellular location of lead in lichens. Lichenologist 26, 83–90.
Brown, D.H., Brown, R.M., 1991. Mineral cycling and lichens: the physiological basis. Lichenologist 23, 293–307.
Brown, D.H., Slingsby, D.R., 1972. The cellular location of lead and potassium in the lichen *Cladonia rangiformis* (L.) Hoffm. New Phytol. 71, 297–305.
Chettri, M.K., Sawidis, T., Zachariadis, G.A., Stratis, J.A., 1997. Uptake of heavy metals by living and dead *Cladonia* thalli. Environ. Exp. Bot. 37, 39–52.
Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S.M., Siegel, B.Z., 1983. Removal of uranium (VI) from solution by fungal biomass and fungal wall-related biopolymers. Science 219, 285–286.
Goyal, R., Seaward, M.R.D., 1982. Metal uptake in terricolous lichens. New Phytol. 90, 85–98.
Hauck, M., Mulack, C., Paul, A., 2002. Manganese uptake in the epiphytic lichens *Hypogymnia physodes* and *Lecanora conizaeoides*. Environ. Exp. Bot. 48, 107–117.
Kershaw, K.A., 1985. Physiological Ecology of Lichens. Cambridge University Press, p. 293.
Klos, A., Rajfur, M., Waclawek, M., Waclawek, W., 2007. Heavy metal sorption in the lichen cationactive layer. Bioelectrochemistry 71, 60–65.
Loppi, S., Paoli, L., 2015. Comparison of the trace element content in transplants of the lichen *Evernia prunastri* and in bulk atmospheric deposition: a case study from a low polluted environment (C Italy). Biologia 70, 460–466.
Loppi, S., Paoli, L., Gaggi, C., 2006. Diversity of epiphytic lichens and Hg contents of *Xanthoria parietina* thalli as monitors of geothermal air pollution in the Mt. Amiata area (central Italy). J. Atmos. Chem. 53, 93–105.
Nieboer, E., Richardson, D.H.S., Lavoie, P., Padovan, D., 1979. The role of metal ion binding in modifying the toxic effects of sulphur dioxide on the lichen *Umbilicaria muhlenbergii*. I. Potassium efflux studies. New Phytol. 82, 621–632.
Nieboer, E., Richardson, D.H.S., Tomassini, F.D., 1978. Mineral uptake and release by lichens: an overview. Bryologist 81, 226–246.
Paoli, L., Fiorini, E., Munzi, S., Sorbo, S., Basile, A., Loppi, S., 2013. Antimony toxicity in the lichen *Xanthoria parietina* (L.) Th. Fr. Chemosphere 93, 2269–2275.
Paoli, L., Vannini, A., Fačkovcová, Z., Guarnieri, M., Bačkor, M., Loppi, S., 2018. One year of transplant: is it enough for lichens to reflect the new atmospheric conditions? Ecol. Indic. https://doi.org/10.1016/j.ecolind.2018.01.043.
Puckett, K.J., Nieboer, E., Gorzynski, M.J., Richardson, D.H.S., 1973. The uptake of metal ions by lichens: a modified ion-exchange process. New Phytol. 72,

329–342.
Renaudin, M., Leblond, S., Meyer, C., Rose, C., Lequy, E., 2018. The coastal environment affects lead and sodium uptake by the moss *Hypnum cupressiforme* used as an air pollution biomonitor. Chemosphere 193, 506–513.
Richardson, D.H.S., Nieboer, E., 1980. Surface binding and accumulation of metals in lichens. In: Cook, C.B., Pappas, P.W., Rudolph, E.D. (Eds.), Cellular Interactions in Symbiosis and Parasitism. OH. Ohio State University Press, Columbus, pp. 75–94.
Van Assche, F., Clijsters, H., 1990. Effects of metals on enzyme activity in plants. Plant Cell & Environ 13, 195–206.
Vannini, A., Paoli, L., Nicolardi, V., Di Lella, L.A., Loppi, S., 2017. Seasonal variations in intracellular trace element content and physiological parameters in the lichen *Evernia prunastri* transplanted to an urban environment. Acta Bot. Croat. 76, 171–176.