Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Research 97 (2005) 50–57
Environmental Research
www.elsevier.com/locate/envres

# The role of urban air pollutants on the performance of heavy metal accumulation in *Usnea amblyoclada*

Hebe A. Carreras,[a,*] Eduardo D. Wannaz,[a] Carlos A. Perez,[b] and María L. Pignata[a]

[a]*Departamento de Química, Instituto Multidisciplinario de Biología Vegetal-IMBIV/CONICET-UNC, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Avda. Vélez Sársfield 1611, X5016 GCA Córdoba, Argentina*
[b]*Laboratorio Nacional de Luz Síncrotron-LNLS/CNPq, Caixa Postal 6192, 13038-970 Campinas, Brazil*

Received 12 November 2003; received in revised form 30 April 2004; accepted 17 May 2004
Available online 28 July 2004

**Abstract**

Lichens incorporate heavy metals according to a selectivity sequence; therefore, their uptake rate can be affected when elements with a high affinity for cell wall exchange sites or that provoke harmful alterations to the metabolism of lichen thalli are present in the environment. The aim of this study was to examine the effect of urban pollutants on the accumulation of some heavy metals in *Usnea amblyoclada*. Lichen samples were transplanted for 1 month to both a polluted and a nonpolluted area in Córdoba, Argentina. They were then collected and soaked in tridistilled water or in solutions containing different concentrations of Cu, Ni, Pb, and Zn salts. The uptake of $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$, and $Pb^{2+}$, and other parameters indicative of lichen damage were measured in all the lichen samples. The thalli retrieved from the polluted area showed significant increases in both the malonaldehyde content and the electrical conductivity of the water in which they had been immersed. These results indicate that the atmospheric pollutants could be responsible for the significant damage to the lichen's cellular membranes, thus altering several mechanisms related to the uptake of heavy metals. Both the area of transplantation and the concentration of the metallic solutions had significant effects on the levels of Cu, Ni, and Pb measured in lichen thalli; however, no significant differences were observed in Zn concentrations. The highest uptakes corresponded to Pb and Cu, suggesting that they probably have a higher affinity with the lichen cell wall exchange sites. This study confirms the fact that, although lichens can be useful biological indicators, the physiological mechanisms involved in metal uptake should be carefully analyzed. Therefore, when estimating the heavy metal content of an environment, the competitive mechanism for cation uptake should be considered especially in areas where the presence of high levels of metals with a strong binding affinity is suspected. The presence of secondary products in the lichens could be responsible for the selective uptake of cations and for a possible tolerance to their presence.


*Keywords:* Air pollution; Biomonitors; Chemical response; Lichen; Selective cation uptake

## 1. Introduction

Lichens have been extensively used for biomonitoring air quality because of their great sensitivity to environmental conditions that originate measurable changes in some of their components or specific parameters. The degradation of chlorophyll is one of the most obvious signs that damage has occurred in lichens (Manrique et al., 1993; Riga-Karandinos and Karandinos, 1998). However, many other parameters have also been employed to assess the physiological damage in lichens caused by atmospheric pollutants such as the malonaldehyde (MDA) content (González et al., 1996), the level of auxins or ethylene (Garty et al., 1997; Garty, 2000), the variations in respiratory rate (Conti and Cecchetti, 2001), and the biosynthesis of lipids and proteins (Silberstein et al., 1996). These changes have been measured even before the appearance of the first visible signs of damage.

*Corresponding author. Fax: +54-51-4334139.
*E-mail address:* hcarreras@com.uncor.edu (H.A. Carreras).

0013-9351/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2004.05.009

Several studies have been performed on the accumulation of heavy metals in many different species (Sawidis et al., 1995; Monaci et al., 1997; Scerbo et al., 1999; Loppi et al., 2002). There are also many other studies focused on the uptake, retention, localization, release, tolerance, and toxicity of different elements, all undertaken in controlled laboratory conditions (Branquinho et al., 1997; Chettri et al., 1998; Kauppi et al., 1998). However, when lichens are used as biomonitors of a specific area, their responses could well be different from those observed in the laboratory. This is due to the fact that the lichens are exposed not only to a single pollutant but to complex mixtures of them which are also affected by different meteorological conditions. Furthermore, lichen damage caused by the presence of other gaseous or particulate pollutants in the environment can modify or interrupt the physiological processes involved in the accumulation of heavy metals.

It has been assumed that the concentration of heavy metals in lichens reflects the proportion of these elements in the environment. However, competitive studies in lichens have revealed that heavy metals are incorporated according to a selectivity sequence by a cation uptake mechanism which involves ion exchange and is modified by the formation of metal complexes (Chettri et al., 1997). Thus, the uptake and/or accumulation rate of any element in the lichen thalli can be affected by an increase in the concentration of other elements present in the environment which form relatively stronger complexes and are therefore incorporated more efficiently. The main aim of this investigation was to examine the influence of real urban contamination conditions on the accumulation of some heavy metals in the lichen *Usnea amblyoclada*, a species commonly found in our country that has been successfully employed to monitor heavy metals in previous studies. Additionally, we studied the relationship between urban pollutants and some metallic cations, comparing their different uptake rates.

## 2. Materials and methods

### 2.1. Study area

The present study was undertaken in Córdoba city (31°24′S, 64°11′W), capital of the province of Córdoba, at an altitude of 440 m above sea level. The climate is subhumid, with an average annual precipitation of 790 mm, concentrated mainly in summer. The mean annual temperature is 17.4 °C and the prevailing winds are from the NE and SE. Two different sampling zones were selected in the urban area of Córdoba city, according to their degree of contamination. The polluted zone was located downtown, a densely populated area with great traffic congestion during most of the day, high buildings that limit wind circulation, and very scarce vegetation. During winter, the temperature inversion hinders the vertical circulation of air and the intense solar radiation favors the photochemical reactions that originate smog. The nonpolluted zone was located in a neighborhood topographically elevated with respect to the downtown area, with very little traffic, one or two flat buildings, and abundant natural and exotic vegetation.

### 2.2. Lichen material and sampling procedure

Thalli of *U. amblyoclada* (Müll. Arg.) Zahlbr. were collected from Los Gigantes, 70 km west of Córdoba city, which has a very low level of pollution. The area was chosen because the species is very abundant and therefore the collection of samples had very low impact on the population density. The basal parts of lichen thalli were detached with the adhering pieces of rock substrate and were stored in the laboratory at room temperature until exposure. These thalli corresponded to the baseline material.

Lichen bags were prepared with 6.0 g fresh material packed loosely in a fine nylon net; so each bag included several thalli. Three of these bags were tied on a nylon rope on different posts 3 m above the ground at each sampling zone and exposed for 1 month, from May 16 to June 17, 1999, and then collected.

The exposed samples and the baseline material were air-dried, homogenized in a mortar with a pestle, and then freeze-dried in polyethylene bags. From each lichen bag, three subsamples of the homogenized material were taken to obtain a mean arithmetic value ± standard deviation for each chemical determination.

### 2.3. Sample treatment

To evaluate the ability of the exposed lichens to incorporate $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$ and $Pb^{2+}$ from metallic solutions, lichens retrieved from the polluted and the nonpolluted areas were soaked for 30 min either in tridistilled water (control treatment) or in the following salt solutions: $CuSO_4$ (0.5, 1, 2.5, 5, and 10 mM), $NiSO_4$ (0.5, 1, 5, and 10 mM), $ZnSO_4$ (0.5, 1, 5, and 10 mM), and $Pb(NO_3)_2$ (0.5, 1, 5, and 10 mM). These concentrations correspond to the environmental concentrations of the metals in urban or industrial areas (McKinney, 1993). The lichen thalli were then rinsed twice in tridistilled water, shaken gently to remove any excess water, and left to dry at room temperature.

Previous studies showed that the incorporation of heavy metals can be affected by the acidity of the environment (Kauppi et al., 1998). For that reason the acidity of all solutions was adjusted to pH 3.5 by adding $H_2SO_4$ or $HNO_3$.

### 2.4. Physiological determinations

The procedures followed for the quantification of chlorophyll-*a* (Chl-*a*), chlorophyll-*b* (Chl-*b*), phaeophytin-*a* (Phaeo-*a*), phaeophytin-*b* (Phaeo-*b*), carotenoids (Carot), hydroperoxy conjugated dienes (HPCD), malondialdehyde (MDA), dry weight/fresh weight ratio (DW/FW), and other ratios measured are explained in detail in Carreras et al. (1998) and Carreras and Pignata (2001). All determinations were expressed on a fresh-weight basis.

The electrical conductivity (EC) parameter is a relative measure of membrane integrity (Garty et al., 2002).

Conductivity measurements were performed in lichen thalli that were previously incubated in a humidity chamber (80%) for 1 h. After that, entire thalli were divided into samples of 2 g each and immersed in 50 mL tridistilled water for 2 h at room temperature. The EC of the water was measured by an EC meter with a glass electrode (Oakton WD-35610) and expressed as $mS\,m^{-1}$.

### 2.5. Metal analysis

Lichen samples (0.5 g) were dried at 60°C until constant weight and then ashed at 650°C for 60 min. The ashes were digested using a 5:1 mixture of HCl (18%) and $HNO_3$ (conc.) at a mild temperature and the solid residue was separated by centrifugation. Finally, 10 ppm of Ge (internal standard) was added and the volume was adjusted to 50 mL with tridistilled water. Aliquots of 5 μL were taken from this solution and dried on an acrylic support. As a quality control, blank samples and samples of the standard reference material Hay IAEA-V-10 were prepared in the same way as described above. The system was calibrated with known concentrations of standard solutions of metals and Ge as an internal standard. Samples were irradiated for 200 s using the total-reflection technique, at an X-ray fluorescence beamline of the National Synchrotron Light Laboratory, Campinas, Brazil. For the excitation, a polychromatic beam of approximately 2 mm wide and 1 mm high was used. A Si detector with a resolution of 140 eV at 5.9 keV was used for the X-ray detection.

### 2.6. Statistical analysis

Results are expressed as the mean values of three independent determinations corresponding to each of the three sampling sites in each experimental area. Physiological parameters and metal concentrations were submitted to an analysis of variance (ANOVA). Post hoc comparisons were conducted using the least significant difference test. A $P$-value $<0.05$ was considered a significant difference. The assumptions of the ANOVA were previously verified by graphic methods (residuals vs. fitted values, box plots, and steam leaf plots).

## 3. Results and discussion

### 3.1. Effects of urban atmospheric pollutants on lichen thalli

Table 1 shows the physiological parameters quantified in thalli of *U. amblyoclada* transplanted to the polluted and the nonpolluted areas.

Pigment degradation in lichens is related to atmospheric pollutants because they can originate many free radicals that have important effects on different physiological processes such as photosynthesis (Gries, 1996). In the present experiment, the thalli exposed to the polluted area had higher concentrations of the photosynthetic pigments, although only Chl-*a* showed significant differences. This has already been found in previous studies of this species (Carreras et al., 1998). Von Arb and Brunold (1989) observed the same in *Parmelia sulcata* that had been transplanted in different areas in Biel, Switzerland and attributed the difference to urban air pollutants, mainly nitrogen oxides, which have a fertilizing effect on lichens. Furthermore, the Chl-*b*/Chl-*a* and Phaeo-*a*/Chl-*a* ratios were also higher

Table 1
Psiological parameters quantified in baseline *U. amblyoclada* and in thalli transplanted to a polluted and a nonpolluted area

| | $SP_1$ | Chl-*a* (mg/g FW) | Carot (mg/g FW) | Chl-*b*/Chl-*a* | Phaeo-*a*/Chl-*a* | EC (mS/m/g FW) | DW/FW | MDA (μmol/g FW) | HPCD (mmol/g FW) |
|---|---|---|---|---|---|---|---|---|---|
| Baseline | | | | | | | | | |
| | | 0.124 | 0.079 | 1.211 | 1.752 | 61.28 | 0.905 | 0.108 | 0.124 |
| | | 0.100 | 0.082 | 0.924 | 1.716 | 88.45 | 0.904 | 0.118 | 0.124 |
| | | 0.204 | 0.065 | 0.598 | 2.709 | 47.18 | 0.904 | 0.074 | 0.179 |
| Mean | | 0.142b | 0.075 | 0.911b | 2.059 | 65.64b | 0.904a | 0.100b | 0.142 |
| SD | | 0.054 | 0.009 | 0.307 | 0.593 | 20.98 | 0.001 | 0.023 | 0.032 |
| Nonpolluted area | | | | | | | | | |
| | 1 | 0.126 | 0.052 | 1.093 | 2.806 | 74.14 | 0.884 | 0.109 | 0.182 |
| | 2 | 0.115 | 0.087 | 0.732 | 2.401 | 77.39 | 0.883 | 0.106 | 0.158 |
| | 3 | 0.206 | 0.136 | 0.459 | 1.908 | 91.52 | 0.882 | 0.115 | 0.149 |
| Mean | | 0.139b | 0.092 | 0.670 c | 2.339 | 81.02b | 0.883b | 0.110b | 0.163 |
| SD | | 0.052 | 0.038 | 0.258 | 0.753 | 25.36 | 0.002 | 0.007 | 0.044 |
| Polluted area | | | | | | | | | |
| | 1 | 0.181 | 0.058 | 1.129 | 2.455 | 177.96 | 0.902 | 0.145 | 0.154 |
| | 2 | 0.140 | 0.064 | 1.056 | 2.210 | 84.42 | 0.927 | 0.116 | 0.138 |
| | 3 | 0.205 | 0.071 | 0.830 | 2.523 | 122.11 | 0.912 | 0.123 | 0.150 |
| Mean | | 0.179a | 0.064 | 1.005a | 2.369 | 139.27a | 0.914a | 0.128a | 0.148 |
| SD | | 0.033 | 0.029 | 0.156 | 0.358 | 59.37 | 0.011 | 0.015 | 0.009 |
| ANOVA (*P* value) | | 0.012 | ns | 0.004 | ns | 0.023 | 0.000 | 0.016 | ns |

Mean values on each vertical column followed by the same letter do not differ significantly ($P < 0.05$). $SP_1$, sampling point.

in the polluted area, indicating that atmospheric pollutants can also promote the degradation of Chl-*a*. Therefore, it can be concluded that atmospheric pollutants are possibly responsible for both the pigment degradation and the increase of its synthesis.

Atmospheric pollutants are potent catalyzers of the peroxidation of membrane lipids (Tarhanen et al., 1997). On the other hand, the presence of MDA in biological systems can be related to the peroxidation of unsaturated fatty acids that constitute cellular membranes (Turton et al., 1997). In this study, the concentration of MDA was significantly higher in lichens transplanted to the polluted area, suggesting that air pollutants may have caused some damage to the cellular membranes of lichen thalli. These results are in agreement with those of Egger et al. (1994) who documented an increase of the MDA content in thalli of *Hypogymnia physodes* transplanted to a highly polluted area.

The integrity of cell membranes in *U. amblyoclada* was also verified by changes in the EC of the water in which intact thalli had been immersed. The lichens transplanted to the polluted area had significantly higher ED values than those of thalli transplanted to the nonpolluted area and the baseline material, which is in accordance with the MDA concentration data. Similarly, Garty et al. (1992) found significant differences in the EC of lichens retrieved from industrial areas with respect to other thalli retrieved from rural areas.

The DW/FW ratio was significantly higher in lichens transplanted to the polluted area. The lowest value corresponded to the lichens transplanted to the nonpolluted area but did not significantly differ from the ratio of the baseline material. Previous investigations using other lichen species have revealed that the content of water can be altered in thalli transplanted to heavily polluted environments (Levin and Pignata, 1995; Cañas et al., 1997).

### 3.2. Uptake of some metallic cations in transplanted thalli

Mean values ± standard deviation of Cu, Ni, Zn, and Pb measured in baseline lichen material and in transplanted thalli are presented in Table 2. In general, the levels of all of these elements were higher in the thalli transplanted to the polluted area, reflecting the concentration of heavy metals characteristic of this area. The levels of Cu, Pb, and Zn were significantly higher in the thalli transplanted to the polluted area. However, in the thalli from the nonpolluted area only the levels of Cu were significantly higher than the baseline material, as Pb and Zn levels were not significantly different. The Ni content did not vary significantly in any of the transplanted thalli compared to the baseline samples.

When the concentrations of metals measured in lichen thalli were compared, the values of Zn were two and three times higher than the values of the other metals studied. This result could be related to the higher content of Zn in the granite rocks which are the characteristic substrate of this species. Saxicolous lichens are known to encrust onto rocks by exuding compounds that weather the rock and form minerals by complexing their cations. Among these compounds, lichen acids are suspected to play a prominent role as a result of their strong metal binding ability (Sarret et al., 1998). An elevated Zn content was also observed in the thalli of *Xanthoria parietina* collected from different areas of the Livorno province in Italy (Scerbo et al., 1999). Our findings indicate that the levels of Zn in *U. amblyoclada* are naturally high; therefore most of their binding sites could be already occupied.

Table 2
Concentration of Cu, Ni, Pb, and Zn in baseline *U. amblyoclada* and in thalli transplanted to a polluted and a nonpolluted area

| | Sampling point | Cu (μg/g FW) | Ni (μg/g FW) | Pb (μg/g FW) | Zn (μg/g FW) |
|---|---|---|---|---|---|
| Baseline | | | | | |
| | 1 | 3.20 | 3.49 | 3.86 | 21.37 |
| | 2 | 3.33 | 2.86 | 3.88 | 19.38 |
| Mean | | 3.18c | 3.27 | 3.87b | 20.38b |
| SD | | 0.44 | 0.09 | 0.01 | 1.41 |
| Polluted area | | | | | |
| | 1 | 5.32 | 5.16 | 7.74 | 33.07 |
| | 2 | 4.98 | 4.37 | 8.69 | 30.68 |
| Mean | | 5.15a | 5.13 | 8.21a | 31.87a |
| SD | | 0.24 | 0.27 | 0.67 | 1.69 |
| Nonpolluted area | | | | | |
| | 1 | 3.79 | 3.21 | 4.99 | 22.57 |
| | 2 | 5.10 | 4.63 | 4.31 | 24.95 |
| Mean | | 4.45b | 4.44 | 4.65b | 23.76b |
| SD | | 0.92 | 0.93 | 0.48 | 1.68 |
| ANOVA (*P* value) | | 0.022 | ns | 0.005 | 0.012 |

Mean values on each vertical column followed by the same letter do not differ significantly ($P < 0.05$).

### 3.3. Dependence of metal uptake on the physiological status of thalli

Table 3 shows the levels of Cu, Ni, Pb, and Zn measured in lichen thalli exposed to the polluted or nonpolluted areas and then immersed in different metallic solutions. Taking into account that no significant difference was observed between the baseline thalli and the samples immersed in tridistilled water, only the latter treatment was used as a control sample in the statistical analysis.

Both the transplanting area and the different metallic solutions had significant effects on the levels of Cu accumulated on and in the cortical cells of *U.*

Table 3
Ccentration of Cu, Ni, Pb, and Zn in *U. amblyoclada* thalli exposed to a polluted and a nonpolluted area and then immersed in different metallic solutions

| | Cu (μg/g FW) (mean ± SD) | Ni (μg/g FW) (mean ± SD) | Pb (μg/g FW) (mean ± SD) | Zn (μg/g FW) (mean ± SD) |
|---|---|---|---|---|
| *Treatment* | | | | |
| $H_2O$ | 5.535 ± 3.314 | 4.009 ± 0.722 | 5.349 ± 1.031 | 23.32 ± 3.237 |
| 0.5 mM | 26.91 ± 4.247 | 25.07 ± 8.110 | 181.8 ± 14.57 | 29.26 ± 8.888 |
| 1 mM | 61.30 ± 10.33 | 41.63 ± 8.625 | 321.5 ± 34.00 | 37.64 ± 2.291 |
| 5 mM | 134.0 ± 97.69 | 76.31 ± 15.10 | 387.6 ± 49.29 | 31.31 ± 1.663 |
| 10 mM | 300.0 ± 92.22 | 217.6 ± 44.76 | 610.8 ± 129.6 | 25.00 ± 2.519 |
| *Area (A)* | | | | |
| Polluted | 29.18 ± 56.82 | 30.55 ± 61.98 | 104.8 ± 201.7 | 28.46 ± 6.935 |
| Nonpolluted | 44.92 ± 100.6 | 24.12 ± 46.14 | 90.50 ± 161.2 | 31.18 ± 13.09 |
| ANOVA (*P* value) | | | | |
| Treatment (*T*) | 0.001 | 0.000 | 0.000 | 0.023 |
| Area (*A*) | 0.048 | 0.036 | 0.30 | ns |
| $T * A$ | ns | 0.014 | ns | ns |

*amblyoclada*. In both transplanting areas, the levels of Cu in lichen thalli increased simultaneously with its concentration in the solution and the samples treated with the solution with the highest Cu concentration were significantly different from all the others (Fig. 1). The lichens exposed to the polluted area incorporated more Cu from the less-concentrated metallic solutions than the samples retrieved from the nonpolluted area. On the contrary, when the samples were immersed in the more-concentrated metallic solutions, it was the thalli retrieved from the nonpolluted area that incorporated more Cu. These findings could be related to the presence of atmospheric pollutants that might have altered the normal synthesis of lichen compounds that act as binding sites of metallic ions, as suggested by Sarret et al. (1998). Another possible explanation is that the higher levels of atmospheric Pb in the polluted area, as was suggested by the accumulation of Pb in the lichen thalli retrieved from this area, could have been strongly attached to the binding sites in the cell wall thus preventing the binding of other cations. In any case, it seems that the thalli that were not damaged are able to incorporate Cu more efficiently than the thalli exposed and damaged by air pollutants, which is in accordance to the higher levels of Cu found in lichens retrieved from the nonpolluted area. This hypothesis is supported by observations of Chettri et al. (1997) that showed a higher Cu uptake by living than by dead thalli.

The content of Ni in the thalli of *U. amblyoclada* was significantly different between treatments and sampling areas. As the interaction was also significant, the mentioned factors were analyzed separately (Fig. 2). The content of Ni in lichen thalli increased simultaneously with its concentration in the solution and the thalli soaked in the most concentrated $Ni^{2+}$ solution were significantly different from all other treatments. The content of Ni was higher in the thalli retrieved from the nonpolluted area than that measured in thalli from the polluted area when the concentration of this cation in the solution was less than 1 mM. This could be explained either by an increase in the incorporation of

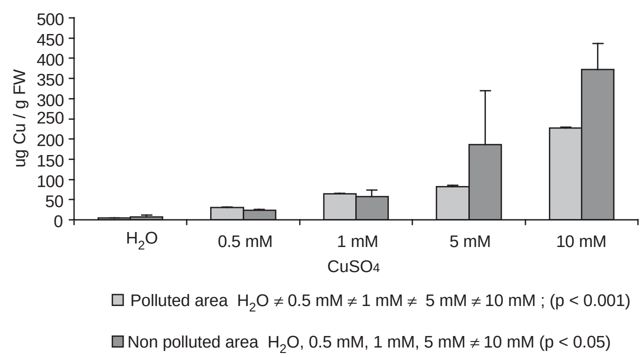


Fig. 1. Copper incorporation of *U. amblyoclada*, previously exposed to polluted and nonpolluted areas in Cordoba city, Argentina, from different concentrations of a $CuSO_4$ solution. Bars represent the mean ± SD.

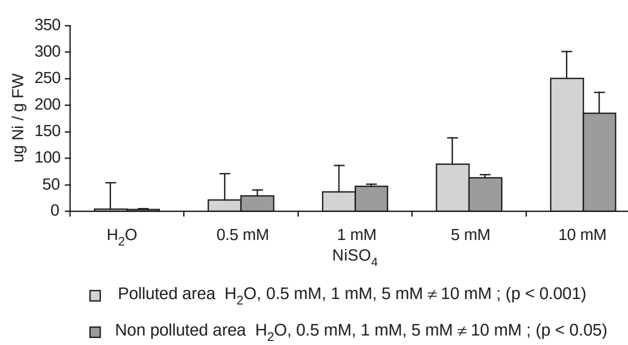


Fig. 2. Nickel incorporation of *U. amblyoclada*, previously exposed to polluted and nonpolluted areas in Cordoba city, Argentina, from different concentrations of a $NiSO_4$ solution. Bars represent the mean ± SD.

Ni in undamaged thalli or by a decrease in the ionic binding sites of the cell wall already occupied by other elements with a greater affinity or damaged by air pollutants. When the thalli were soaked in concentrated $Ni^{2+}$ solutions, its content was higher in lichens retrieved from the polluted area. These results suggest that when the concentration of Ni in the thalli exceeds 5 mM, the normal activity of the cellular membrane is altered and cations can be incorporated without restrictions. This is more evident in thalli retrieved from the polluted area because when they are soaked in the metallic solution they are probably already damaged by atmospheric pollutants, leading to an additive effect between the factors. Similarly, Hyvärinen Roitto et al. (2000) found that only the thalli treated with a concentrated $Ni^{2+}$ solution (6 mM) showed prejudicial effects on some indicators of membrane permeability.

Significant differences were observed in the Pb content in lichen thalli in relation to both the treatment with metallic solutions and the transplanting area. The uptake of Pb increased as did its concentration in the solution. As expected, thalli retrieved from the polluted area had higher Pb content than thalli retrieved from the nonpolluted area (Fig. 3). In many lichen species, it has been observed that $Pb^{2+}$ is mainly complexed to the fungal cell wall (Kramer et al., 1996; Sarret et al., 1998). On the other hand, it has been suggested that the uptake of some metals such as Pb and U in lichens is higher in dead than in living thalli (Chettri et al., 1997). One would expect the accumulation of metals in dead tissue if the living plasma membrane was responsible for preventing the entrance of metals to the cell. In agreement with this hypothesis, the increased content of Pb cations in thalli retrieved from the polluted area could be due to both the altered integrity of cell membranes promoted by air pollutants and the presence of higher environmental levels of this cation.

No significant differences were observed in the content of Zn in thalli related to either the metallic solutions or the transplanting area (Fig. 4). Considering that the maximum limit for metal uptake is regulated by the ionic exchange capacity of the cell wall, the high Zn concentration found in baseline thalli of *U. amblyoclada* could be limiting the incorporation of new cations. A slower uptake of physiologically important metallic cations such as $Zn^{2+}$ and $Mg^{2+}$ was also found by Brown and Beckett (1984). Also, the complexes formed by the lichen acids with heavy metals have different stabilities and the complexes formed by $Zn^{2+}$ are weak and unstable (Chettri et al., 1997). In the present study, the Zn content corresponding to thalli immersed in the most concentrated solutions was lower than the Zn content corresponding to thalli immersed in less concentrated solutions, suggesting that Zn also forms weak complexes with cell wall ligands and consequently leaches when its concentration outside the thalli is low.

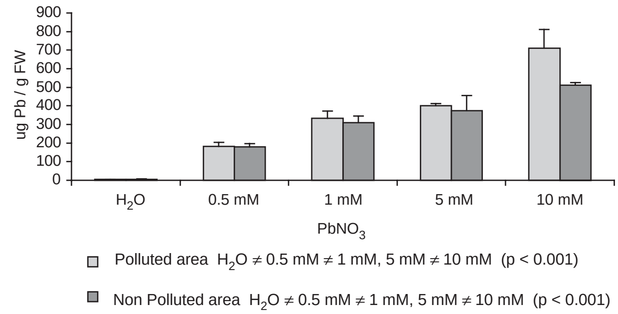


Fig. 3. Lead incorporation of *U. amblyoclada*, previously exposed to polluted and nonpolluted areas in Cordoba city, Argentina, from different concentrations of a $PbNO_3$ solution. Bars represent the mean ± SD.

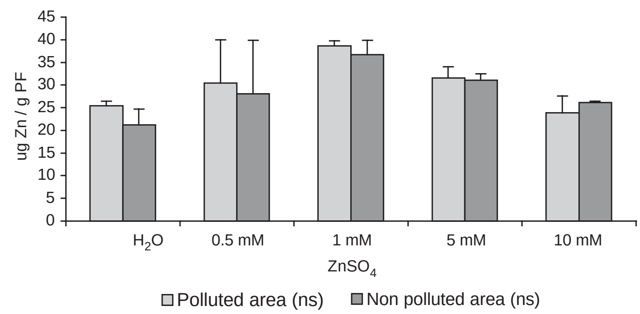


Fig. 4. Zinc incorporation of *U. amblyoclada*, previously exposed to polluted and nonpolluted areas in Cordoba city, Argentina, from different concentrations of a $ZnSO_4$ solution. Bars represent the mean ± SD.

If that is the case with *U. amblyoclada,* the levels of Zn quantified in the thalli do not reflect its real environmental proportion.

### 3.4. Differential uptake of metallic cations

Lichen thalli were immersed in different metallic solutions but with the same cation concentration. However, the uptake rate was different for every cation in the thalli retrieved from both sampling zones (Fig. 5). The uptake of Cu, Ni, and Pb increased with the concentration of the corresponding cation in the solution. The incorporation of Pb was two and three times higher than that of other cations with the same concentration in the solution followed by Cu and then Ni. These findings suggest a selective metal uptake in *U. amblyoclada* that could be related to the high concentrations of usnic acid deposited on the upper cortex of thalli, characteristic of this genus.

The same pattern was not found for Zn uptake, as the highest concentration was found in lichens immersed in medium-concentrated solutions. This finding could be explained by both the higher Zn level in baseline thalli which prevents the binding of newly incorporated

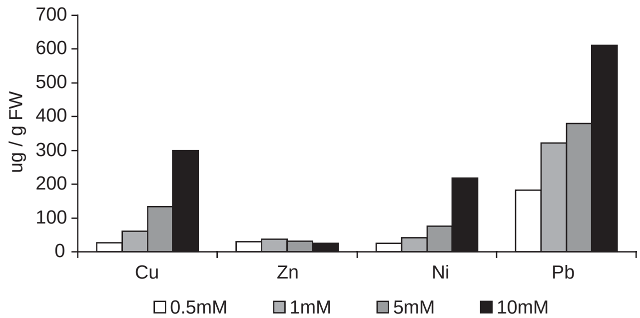


Fig. 5. Uptake rates of Cu, Ni, Pb, and Zn from metallic solutions in *U. amblyoclada* thalli transplanted to polluted and nonpolluted areas in Cordoba city, Argentina.

cations and by the formation of weak binding complexes with cell wall ligands.

Taken together, the results of this study allow us to conclude that urban environmental conditions can seriously damage *U. amblyoclada* thalli by altering several mechanisms related to the normal uptake and incorporation of heavy metals. The highest uptake levels correspond to Pb, which probably has a greater affinity for the lichen cell wall exchange sites and is strongly attached to binding sites, forming stable complexes. The second uptake level corresponds to Cu, followed by Ni and finally Zn. This sequence is in accordance to the characteristics of B class cations, whose toxicity increases with increasing B class characteristics (Cabral, 2003) and is similar to other results reported for other lichen species or mosses.

The competitive mechanism of cation uptake in *U. amblyoclada* should be taken into consideration when this species is used as a biomonitor, especially when estimating the concentration of some elements in areas where high Pb or Cu levels are suspected. Moreover, the high levels of secondary products characteristic of the *Usnea* genus could be responsible for the selective uptake of cations and for a possible tolerance to their uptake.

## Acknowledgements

This work was partially supported by the Agency of Scientific and Technology Promotion, the Brazilian Synchrotron Light Source, and the Secretary of Science and Technology of the National University of Córdoba, Argentina.

## References

Branquinho, C., Brown, D.H., Catarino, F., 1997. The cellular location of Cu in lichens and its effects on membrane integrity and chlorophyll fluorescence. Environ. Exp. Bot. 38, 165–179.

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

Cabral, J.P., 2003. Copper toxicity to five *Parmelia* lichens in vitro. Environ. Exp. Bot. 49, 237–250.

Cañas, M.S., Carreras, H.A., Orellana, L., Pignata, M.L., 1997. Correlation between environmental conditions and foliar chemical parameters in *Ligustrum lucidum* Ait. exposed to urban air pollutants. J. Environ. Manag. 49, 167–181.

Carreras, H.A., Pignata, M.L., 2001. Comparison among air pollutants, meteorological conditions and some chemical parameters in the transplanted lichen *Usnea amblyoclada*. Environ. Pollut. 111, 45–52.

Carreras, H.A., Gudiño, G.L., Pignata, M.L., 1998. Comparative biomonitoring of atmospheric quality in five zones of Córdoba city (Argentina) employing the transplanted lichen *Usnea* sp. Environ. Pollut. 103, 317–325.

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.

Chettri, M.K., Cook, C.M., Vardaka, E., Sawidis, T., Lanaras, T., 1998. The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. Environ. Exp. Bot. 39, 1–10.

Conti, M.E., Cecchetti, G., 2001. Biological monitoring: lichens as bioindicators of air pollution assessment—a review. Environ. Pollut. 114, 471–492.

Egger, R., Schleé, D., Türk, R., 1994. Changes of physiological and biochemical parameters in the lichen *Hypogimnia physodes* (L.) Nyl. due to the action of air pollutants—a field study. Phyton 34, 229–242.

Garty, J., 2000. Environment and elemental content content of lichens. In: Markert, B., Fiese, K. (Eds.), Trace Elements. Their Distribution and Effects in the Environment. Trace Metals in the Environment Series. Elsevier Science, Oxford, pp. 277–322.

Garty, J., Karary, Y., Harel, J., 1992. Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen *Ramalina duriaiei* (DeNot.) Bagl. Environ. Exp. Bot. 32, 229–241.

Garty, J., Kauppi, M., Kauppi, A., Garty, J., 1997. The production of stress ethylene relative to the concentration of heavy metals and other elements in the lichen *Hypogimnia physodes*. Environ. Toxicol. Chem. 16, 2402–2408.

Garty, J., Levin, T., Cohen, Y., Lehr, H., 2002. Biomonitoring air pollution with the desert lichen *Ramalina maciformis*. Physiol. Plant. 115, 267–275.

González, C.M., Casanovas, S.S., Pignata, M.L., 1996. Biomonitoring of air pollutants from traffic and industries employing *Ramalina ecklonii* (Spreng.) Mey. and Flot. in Córdoba, Argentina. Environ. Pollut. 91, 269–277.

Gries, C., 1996. Lichens as indicators of air pollution. In: Nash, III, T.H. (Ed.), Lichen Biology. University Press, Cambridge, pp. 240–254.

Hyvärinen Roitto, M., Ohtonen, R., Markkila, A., 2000. Impact of wet deposited nickel on the cation content of a mat-forming lichen *Cladina stellaris*. Environ. Exp. Bot. 43, 211–218.

Kauppi, M., Kauppi, A., Garty, J., 1998. Ethylene produced by the lichen *Cladina stellaris* exposed to sulphur and heavy metal containing solutions under acidic conditions. New Phytol. 106, 697–706.

Kramer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., Andrew, C., Smith, J., 1996. Free histidine as a metal chelator in plants that accumulate nickel. Nature 379, 635–638.

Levin, A.G., Pignata, M.L., 1995. *Ramalina ecklonii* as bioindicator of atmospheric pollution in Argentina. Can. J. Bot. 73, 1196–1202.

Loppi, S., Ivanov, D., Boccardi, R., 2002. Biodiversity of epiphytic lichens and air pollution in the town of Siena (Central Italy). Environ. Pollut. 116, 123–128.

Manrique, E., Balaguer, L., Barnes, J., Davidson, A.W., 1993. Photoinhibition studies in lichens using chlorophyll fluorescence analysis. Bryologist 96, 443–449.

McKinney, J., 1993. Metal bioavailability and disposition kinetics research needs workshop July 18–19, 1990. Toxicol. Environ. Chem. 38, 1–71.

Monaci, F., Bargagli, R., Gasparo, D., 1997. Air pollution monitoring by lichens in a small medieval town of central Italy. Acta Bot. Neerl. 46, 403–412.

Riga-Karandinos, A.N., Karandinos, M.G., 1998. Assessment of air pollution from a lignite power plant in the plain of Megalopolis (Greece) using as biomonitors three species of lichens; impacts on some biochemical parameters of lichens. Sci. Total Environ. 215, 167–183.

Sarret, G., Manceau, A., Cuny, D., 1998. Mechanisms of lichen resistance to metallic pollution. Environ. Sci. Technol. 32, 3325–3330.

Sawidis, T., Marnasidis, A., Zachariadis, G., Stratis, J., 1995. A study of air pollution with heavy metals in Thessaloniki City (Greece) using trees as biological indicators. Arch. Environ. Contam. Toxicol. 28, 118–124.

Scerbo, R., Possenti, L., Lampugnani, L., Ristori, T., Barale, R., Barghigiani, C., 1999. Lichen (*Xanthoria parietina*) biomonitoring of trace element contamination and air quality assessment in Livorno Province (Tuscany, Italy). Sci. Total Environ. 241, 91–106.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A., Galun, M., 1996. Comparative studies on *Xanthoria parietina*, a pollution-resistant lichen, and *Ramalina duriaei*, a sensitive species. II. Evaluation of possible air pollution-protection mechanisms. Lichenologist 28, 367–383.

Tarhanen, S., Holopainen, T., Oksanen, J., 1997. Ultraestructural changes and electrolyte leakage from ozone fumigated epiphytic lichens. Ann. Bot. 80, 611–621.

Turton, H.E., Dawes, I.W., Grant, C.M., 1997. *Sacharomices cerevisae* exhibits a yAP-1 mediated adaptative response to malondialdehyde. J. Bacteriol. 179, 1096–1101.

Von Arb, C., Brunold, C., 1989. Lichen physiology and air pollution. I. Physiological responses of in situ *Parmelia sulcata* among air pollution zones within Biel, Switzerland. Can. J. Bot. 68, 35–42.