Maximum values of Ni^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} in the biomonitor *Tillandsia capillaris* (Bromeliaceae): Relationship with cell membrane damage

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ABSTRACT

Although several plants belonging to the Bromeliaceae family have been used as heavy metal accumulators in biomonitoring studies, their accumulation ability has not been investigated. The present study obtained the accumulation rates of Ni^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} in leaves of *Tillandsia capillaris* and revealed their effects on lipid peroxidation by measuring the Malondialdehyde content (MDA). Leaves of *T. capillaris* were exposed to different metallic solutions of Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} cations. After this exposure period, the accumulation of these ions was measured by Total Reflection X-Ray Fluorescence (TXRF) analysis with Synchrotron Radiation, and the MDA content was calculated. Data sets were evaluated by a one-way analysis of variance (ANOVA) and a fitted regression hyperbola model. The results showed significant differences in the accumulation efficiencies of the cations under study. In addition, the enrichment factor (EF) estimated for these cations was higher for Ni^{2+}, suggesting a greater affinity of the plant with this element. Over time, all the metals under study caused significant increases in the MDA content, indicating their toxicity effects even in the most diluted solutions used in this study.

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1. Introduction

Contamination of the air, soil and water by metals is a major environmental problem, especially when considering that metals cannot be degraded and that any cleanup usually requires their removal (Lasat, 2002). The presence of metals from anthropogenic sources in the environment can be evaluated by instrumental monitoring or using biomonitors such as mosses, plant leaves or lichens (Monnet et al., 2005) with the monitoring of air pollution by living organisms providing low-cost information on the nature and quantity of pollutants in the environment (Markert et al., 1997).

Epiphytic plants have a great advantage over other plants in the assessment of airborne metal pollution in that their uptake of metals occurs from the air. The commonest epiphytic location is on trees, with virtually all the water and mineral nutrients being provided by the atmosphere. For this reason, their elemental tissue content reflects well the atmospheric levels of some toxic elements (Figueiredo et al., 2001).

The foliar morphology of most species of *Tillandsia* is dominated by an indumentum of trichomes (scales) obscuring the epidermal leaf surfaces. This foliar trichome undoubtedly conditions their physiology as it allows the efficient absorption of scarce nutrients and water available in the atmosphere into the leaf (Benz and Martin, 2006), which has conferred the epiphytic *Tillandsia* species with the ability of surviving in situations where high solar irradiation and constant winds prevail. This critical adaptation that *Tillandsia* species present for inhabiting the epiphytic niche was named extreme atmospheric epiphytes by Benzing in 1976 (Scatena and Segecin, 2005).

Tillandsia epiphytism permits a strong independence from the soil, thereby the only function of the adventitious roots is to adhere to a substratum (Papini et al., 2009). Regarding the bioindicating capacity of the epiphytic vascular plants of the *Tillandsia* genus, *Tillandsia usneoides* has proved to be an efficient atmospheric accumulator of Hg (Amado Filho et al., 2002; Calasans and Malm, 1997; Malm et al., 1995, 1998) and fluoride in rain water (Strehl and Arndt, 1989). Also, *Tillandsia aeranthos* and *Tillandsia recurvata* have been used to assess atmospheric levels of sulfur and different kinds of metals (class A, borderline and class B) in industrialized and residential areas (Figueiredo et al., 2006; Flores, 1987; Schrimpff, 1984). In Argentina, there are many species of *Tillandsia*, among which *Tillandsia capillaris* has been studied as a biomonitor of atmospheric quality (Pignata et al., 2002), and *Tillandsia permutata*, *Tillandsia tricholepis*, *Tillandsia retorta* have been used as both passive and active biomonitors (Bermudez et al., 2009; Wannaz et al., 2006, 2008; Wannaz and Pignata, 2006).

Although many investigations have focused on the uptake, retention, localization, release, tolerance, and toxicity of different elements using biomonitors, most of these were performed using

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lichen species (Branquinho et al., 1997; Chettri et al., 1998; Kauppi et al., 1998; Carreras et al., 2005). So far, no studies have been undertaken under laboratory controlled conditions for species of the *Tillandsia* genus with regard to metal accumulation. Such work could provide information about maximum accumulation rates, as well as the physiological response of these plants in the presence of metals at concentrations higher than the environmental ones.

The accumulation of large amounts of metals in plants brings about considerable metabolic changes. Metal ions may directly interfere with the metabolic activities by altering the conformation of proteins, such as enzymes, transporters or regulator proteins, owing to their strong affinities as ligands to the sulfhydryl and carboxylic groups (Van Assche and Clijsters, 1990). Moreover, some ions with strong redox properties, e.g. Cu (Weckx and Clijsters, 1996), but even those lacking them, e.g. Zn (Weckx and Clijsters, 1997; Dietz et al., 1999) and Cd (Olmos et al., 2003), are known to initiate membrane lipid peroxidation and stimulate the production of reactive oxygen species.

High concentrations of metals can be very toxic for plants, resulting in varied effects on the plant physiology (Sandalio et al., 2001; Schützendübel et al., 2001). Among other observations, oxidative stress was found to be directly related to high concentrations of metals (Demirevska-Kepova et al., 2004; Murzaeva, 2004; Schützendübel and Polle, 2002).

Lipid peroxidation in plants is usually detected and quantified by measuring malondialdehyde (MDA) and the other carbonyl by-products of lipid peroxidation, which form a coloured complex in the reaction with thiobarbituric acid (Dean et al., 1993; Ames et al., 1993). In this study, it was hypothesized that the species *T. capillaris* is a good accumulator of heavy metals, which produce adverse effects on plant physiology.

The present study obtained the maximum accumulation rates of Ni^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} in leaves of *T. capillaris* and predicted by lipid peroxidation whether these metals could cause membrane damage.

2. Materials and methods

2.1. Plant material

T. capillaris Ruíz & Pav. f. *capillaris* plants were collected in Intiyaco ($31°57'21''$S; $64°42'1''$W), which is considered to be an unpolluted site located in the west of the province of Córdoba. The area was selected considering the abundance of this species, and therefore the collection of samples had a very low impact on the population density. Any foreign materials and particles in the samples were eliminated both manually and with compressed air.

2.2. Sample treatment

To evaluate the ability of *T. capillaris* to incorporate Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} from metallic solutions, plants collected in Intiyaco were soaked for 45 min in Milli-Q water (control treatment) or in the following salt solutions: $CuSO_4 \cdot 5H_2O$, $NiSO_4 \cdot 6H_2O$, $Pb(NO_3)_2$ and $ZnSO_4 \cdot 7H_2O$, at concentrations of 0.5, 1, 2.5, 5 and 10 mM, respectively. The metal concentrations used in treatments were chosen taking into account that many of the Tillandsia species live in semi-arid areas where the dry deposition of particulate matter on the leaf surfaces is important, so when rain events occur or plants are exposed to higher levels of humidity (dew point), the concentrations of the dissolved cations on the surface of the leaves could be much higher than the corresponding values in rainwater (wet deposition). In addition, the choice of high concentrations was aimed at ensuring that the study would allow us to reach the highest accumulation values (saturation level) for each metal in the species.

Other authors, Pandey and Sharma (2002), exposed cabbage plants to an excess (500 mM) of Co_2, Ni_2 and Cd_2 in sand culture and observed a decrease in the chlorophyll content, a decrease in the activities of the enzymes (catalase and peroxidise), and a decreased water potential and transpiration rate.

Due to the fact that *T. capillaris* has a Crassulacean Acid Metabolism (Vianna et al., 2011), the samples ($n = 12$) were immersed in the metallic solutions at night (between 9:45 and 10:30 pm) in order that the *T. capillaris* leaves had their stomas open, which would favour the uptake of cations. Nocturnal stomatal opening in CAM plants is a very important survival mechanism and has a major effect on water economy in these plants (Van Heerden et al., 2007). Carbon dioxide enters the mesophyll leaf tissue through the open stomata at night, and in the reaction which is catalyzed by phosphoenol/pyruvate carboxylase (PEPC) in the cytosol it combines with phosphoenol pyruvate (PEP) to form oxolaacetate. Thus, in CAM plants, the fixation of CO_2 to malic acid takes place at night. During the day, the stomata are closed and malic acid is remobilized and decarboxylated (Jeon et al., 2006).

Once the exposure time (45 min) was over, the plants were removed from the solutions and washed three consecutive times with Milli-Q water. They were then placed on tissue paper at room temperature for three days in an isolated environment, in order to avoid pollution by trace elements. Once dry, three pools of leaves were separated for each treatment, with part of the vegetal material being stored at $-15°C$ and the remaining material being taken to constant weight in a stove at $50°C$.

2.3. Analysis of heavy metals

Leaves of *Tillandsia* plants from each treatment were dried to constant weight in an oven at $50 \pm 2°C$, and a 2 g dry weight sample of this material was used for multi-elemental analysis by Total Reflection X-Ray Fluorescence (TXRF) using Synchrotron Radiation. This dry material was ground and reduced to ashes at $500°C$ for 4 h, which were then digested with HCl (18%):HNO_3 (3:1) at $25 \pm 2°C$ and the solid residues separated by centrifugation. Finally, the volume was adjusted to 25 ml with Milli-Q water, and 10 ppm of a Ge solution was added as an internal standard. Aliquots of 5 μl were taken from this solution and dried on an acrylic support. Standard solutions with known concentrations of Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (with Ge as an internal standard) were prepared for the calibration of the system.

Samples were measured for 200 s, using the total reflection setup mounted at the X-ray fluorescence beamline of the National Synchrotron Light Laboratory (LNLS), Campinas, SP, Brazil. A polychromatic beam approximately 5 mm wide and 0.1 mm high was used for excitation. For the X-ray detection, a Si (Li) detector was used with an energy resolution of 165 eV at 5.9 keV.

2.4. Estimation of malondialdehyde (MDA) content

After each treatment with Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, leaf samples of 150 mg fresh weight (FW) were homogenized in 2.5 ml of distilled water. An equal volume of 0.5% TBA (2-thiobarbituric acid) in 20% trichloroacetic acid (TCA) solution was added and the sample was incubated at $95°C$ for 30 min. The reaction was stopped by placing the reaction tubes in an ice bucket. Samples were filtered and read at 532 nm, and the non-specific absorption value at 600 nm was read and subtracted. The amount of malondialdehyde (MDA) present was calculated using $\varepsilon = 155\ mM^{-1}\ cm^{-1}$ (Kosugi et al., 1989), which was determined for each of the subsamples ($n = 3$ for each treatment). The dry weight/fresh weight (DW/FW) ratio of the samples was calculated using the concentration of MDA in nmol g^{-1} DW.

2.5. Statistical analyses

Results were expressed as the mean values of two independent determinations, corresponding to each of the treatments carried out. A regression analysis was performed between the content of metals in the solution and the content of metals in *T. capillaris*, with metal concentrations being submitted to an analysis of variance (ANOVA). Post hoc comparisons were made using the Least Significant Difference (LSD) test, with a value of $p < 0.05$ being considered to be a significant difference.

3. Results and discussion

3.1. Incorporation of heavy metals

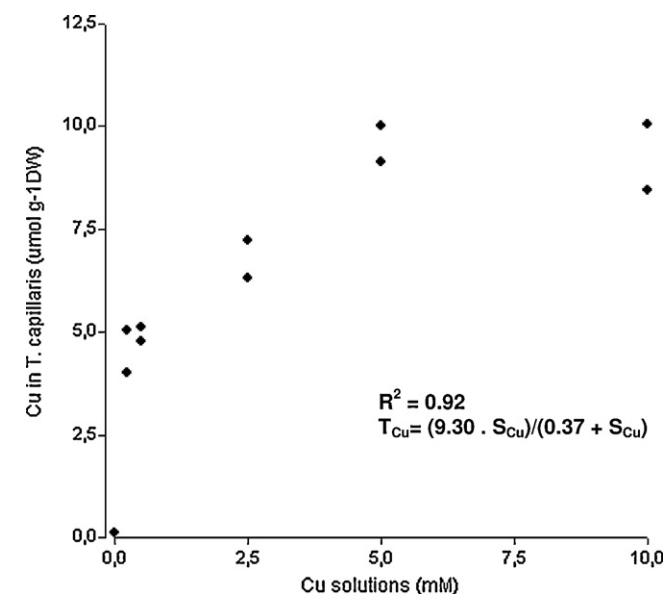In Figs. 1–4, we can observe the curves of the incorporation of metals in *T. capillaris* leaves immersed in solu-

Fig. 1. Concentration of Cu^{2+} in *T. capillaris* leaves immersed in different $CuSO_4$ solutions.

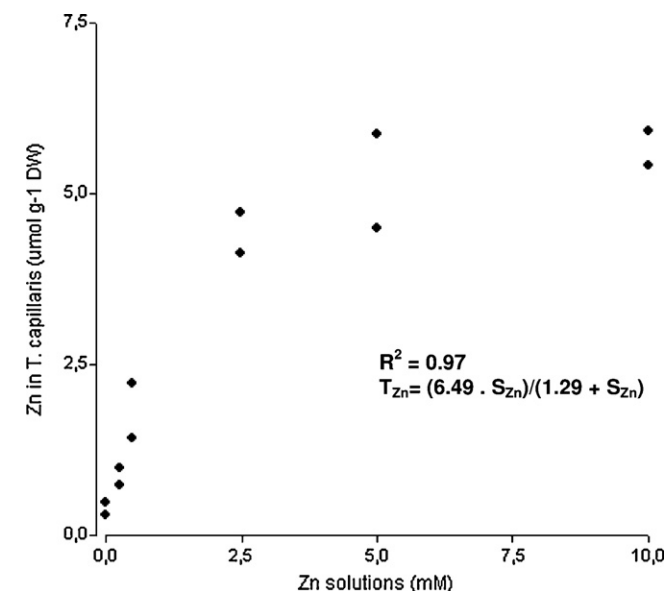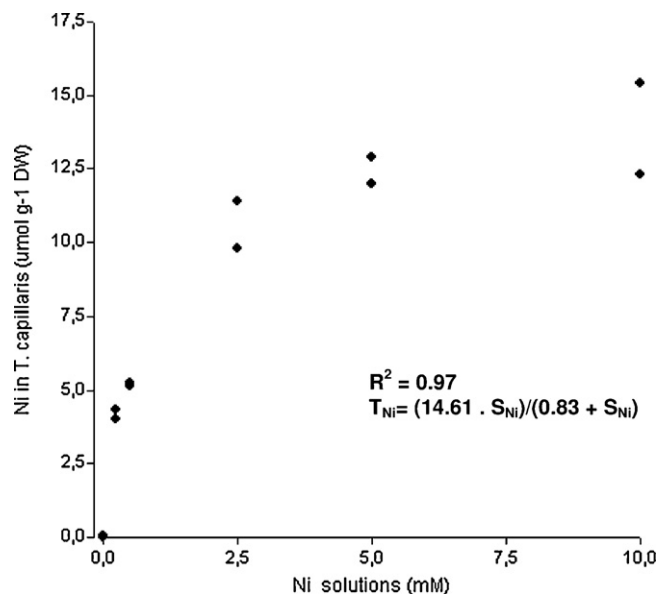**Fig. 2.** Concentration of Zn^{2+} in *T. capillaris* leaves immersed in different $ZnSO_4$ solutions.

Fig. 3. Concentration of Ni^{2+} in *T. capillaris* leaves immersed in different $NiSO_4$ solutions.

tions of various concentrations of Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, respectively.

The concentration of these elements rose significantly in *T. capillaris* leaves as their concentrations in the solutions increased to saturation level, with these accumulation rates being higher than those found by other authors who used this species as a biomonitor (Pignata et al., 2002; Wannaz et al., 2006, 2008; Wannaz and Pignata, 2006).

In every case, we observed that the curve of incorporation of these metals was a hyperbola according to the following equation:

$$T_x = \frac{a \cdot S_x}{b + S_x}$$

where T_x is the concentration of element x ($\mu M/gDW$) in *T. capillaris*, S_x is the concentration of element x (mM) in the solution where the
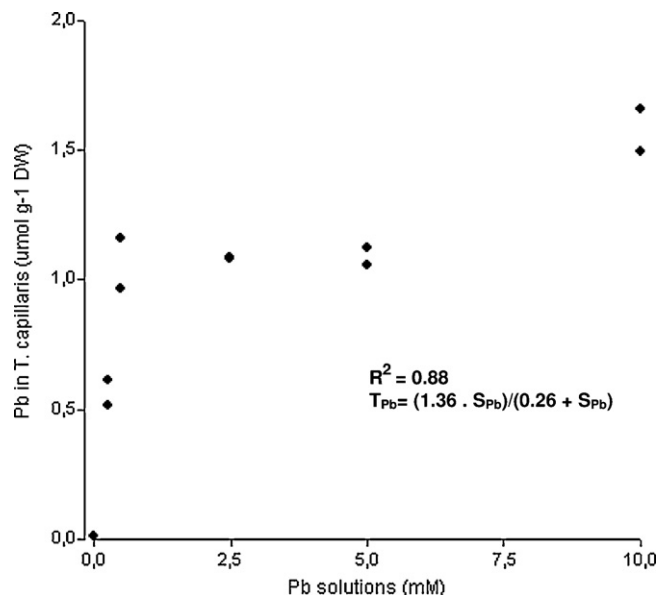

Fig. 4. Concentration of Pb^{2+} in *T. capillaris* leaves immersed in different $Pb(NO_3)_2$ solutions.

Table 1
Concentration of MDA in *T. capillaris* leaves immersed in different solutions.

Treatment	MDA (nmol g^{-1} DW) (mean ± SD)	MDA (nmol g^{-1} DW) (mean ± SD)	MDA (nmol g^{-1} DW) (mean ± SD)	MDA (nmol g^{-1} DW) (mean ± SD)
H$_2$O (control)	51.27 ± 4.61b	51.27 ± 4.61b	51.27 ± 4.61c	51.27 ± 4.61b
Solutions	Cu solutions	Ni solutions	Pb solutions	Zn solutions
Concentrations				
0.25 mM	108.87 ± 6.82 a	92.99 ± 24.05 ab	79.66 ± 12.32 b	124.64 ± 45.89 a
0.50 mM	94.41 ± 23.49 a	102.36 ± 23.44 a	95.34 ± 19.73 ba	123.89 ± 7.10 a
2.50 mM	102.65 ± 29.42 a	107.78 ± 9.23 a	82.10 ± 13.51 b	113.74 ± 15.33 a
5.00 mM	96.04 ± 17.90 a	107.85 ± 29.84 a	97.34 ± 10.04 ab	111.31 ± 7.97 a
10.00 mM	112.14 ± 24.10 a	116.30 ± 34.85 a	107.95 ± 18.47 a	138.50 ± 15.92 a
ANOVA (*p* value)	0.0293	0.0577	0.0061	0.0047

T. capillaris leaves were immersed, and *a* and *b* are the constants of the equation. By estimating the first derivative of this function, we were able to obtain the value of the maximum concentration of the solution, after which the accumulation rate decreased in *T. capillaris* for that metal. This value was estimated according to the following formula:

$$S_x \left(\sqrt{a \cdot b} \right) - b$$

These results show the concentration value of each metal in the solution, with saturation occurring in *T. capillaris* in the order Pb < Cu = Zn < Ni.

By replacing the values of the maximum concentration of solutions, after which there was a saturation or decrease in the accumulation rate in the hyperbola equation, the following maximum values (Tmax) of accumulation in *T. capillaris* leaves were obtained for the four heavy metals:

Tmax$_{Cu}$ = 7.45 μM/gDW (473.38 ppm)
Tmax$_{Ni}$ = 11.14 μM/gDW (654.03 ppm)
Tmax$_{Pb}$ = 0.76 μM/gDW (157.45 ppm)
Tmax$_{Zn}$ = 3.59 μM/gDW (234.59 ppm)

The accumulation capacity (or accumulation efficiency) in *T. capillaris* leaves was the highest for Ni, followed by Cu, Zn and finally by Pb. Similarly, a previous study performed on the accumulation efficiency of heavy metals in *T. usneoides* and *Parmotrema praesorediosum* found that *T. usneoides* was clearly more effective in accumulating Ni than lichen. These authors also mentioned that *T. usneoides* was a particularly effective bioaccumulator of atmospheric pollutants, both gaseous and particulate, which may be attributed to its pendulous growth form with a high surface area to volume ratio. These features result in the plant being in intimate contact with the atmosphere, making it at least as an effective accumulator of atmospherically transported pollutants as the lichen *P. praesorediosum*, which is also epiphytic on the same species of tree (Pyatt et al., 1999).

T. capillaris also has a high surface area to volume ratio, which contributes to a higher efficiency in the accumulation of elements. However, we were able to observe in the present study that the accumulation of Ni was higher than that of Cu, Pb and Zn, possibly showing that there is selectivity in the way *T. capillaris* accumulates metals. The maximum concentration values estimated for these four metals were higher than those found in two passive biomonitoring studies (Pignata et al., 2002; Wannaz et al., 2006) using *T. capillaris*, and were also higher than those found in an active biomonitoring study performed with four species of *Tillandsia*, including *T. capillaris* (Wannaz and Pignata, 2006). The high capacity of *T. capillaris* to bioaccumulate these metals confirms the usefulness of this species for active as well as passive biomonitoring studies, since even in very polluted areas the maximum saturation

levels for the metals under study cannot be reached, as was shown in the present study. In addition, the fact that the maximum levels were also higher than the ones found in other species of *Tillandsia* (Brighigna et al., 1997; Husk et al., 2004; Bi et al., 2001), suggests that *T. capillaris* may be particularly efficient in accumulating heavy metals.

3.2. Enrichment factor (EF)

The enrichment factor for each metal was estimated by dividing the maximum accumulation value (before the decrease in the accumulation rate) by the concentration value of each metal in the samples of the control treatment.

The EF values for the metals in *T. capillaris* leaves were:

EF$_{Cu}$ = 67; EF$_{Ni}$ = 371; EF$_{Pb}$ = 58; EF$_{Zn}$ = 9.2

implying that the order of the enrichment factors with respect to their values in the control experiments was: Ni > Cu = Pb > Zn. These EF values were higher than those obtained in previous studies (Wannaz and Pignata, 2006), suggesting that it is possible to use this species for biomonitoring studies of the metals without underestimating their concentrations in the environment.

3.3. Relation between lipid peroxidation and metal content

The MDA content quantified in leaves (Table 1) exposed to different concentrations of Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} was significantly higher in all the treatments compared to the control samples, which coincides with results previously obtained by Pignata et al. (2002). These authors suggested that the high levels of MDA accounted for the high concentrations of Cu, Zn and Pb. Furthermore, there is a large amount of information regarding the physiological effects of metals, emphasizing the inhibition of photosynthesis and the membrane damage occurring in lichens (Nieboer et al., 1979; Burton et al., 1981; Garty et al., 1992) and higher plants (Woolhouse, 1983; Ernst et al., 1992; Cavalcanti et al., 2004; Dhir et al., 2004; Guo et al., 2007).

Despite the fact that the MDA content in all the treatments was significantly higher than the control treatment, there were no relevant differences observed between the treatments. Although heavy metals such as Cu and Zn are essential for normal plant growth, elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms (Cakmak and Horst, 1991; Koca et al., 2006). For instance, copper is an essential redox-active transition metal, which acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Yruela, 2005). However, at elevated concentrations of >20–30 μg g^{-1} DW

(Marschner, 1995), copper becomes toxic to plants and alters the membrane permeability, chromatin structure, protein synthesis, enzyme activities, photosynthesis and respiratory processes, and may even induce senescence (Fernandes and Henriques, 1991; Yruela, 2005).

The leaves exposed to solutions of Ni^{2+} presented MDA concentrations significantly higher than those treated with water. Nevertheless, no differences were observed in the content of MDA among the various Ni^{2+} solutions used (0.25 mM, 0.50 mM, 2.5 mM, 5 mM and 10 mM). Pb^{2+} cation, as in the case of Ni^{2+}, caused a significant increase in the MDA concentration in all the treatments compared to the control treatment.

The responses of plants to heavy metal toxicity may result from the binding of the metals to the sulphydryl groups of proteins, leading to an inhibition of activity or the disruption of structures with the displacement of essential elements, such as Zn, Mg, Ca and Fe, thus causing further deficiency effects. It has been previously shown that heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, probably resulting in oxidative stress (Hall, 2002; Metwally et al., 2005). The fact that a significant increase was observed in the MDA content in samples treated with different metal concentrations supports these findings.

4. Conclusions

The efficiency of the metal accumulation in *T. capillaris*, given by the concentration of cations in the solutions in which plants were exposed and their concentrations in the leaves, showed the following gradient: Ni > Cu > Zn > Pb. Regarding the enrichment factor, the following order was observed: Ni > Cu = Pb > Zn, which indicates a higher affinity of the species towards Ni. It was also observed that all the metals used in this study (Cu, Ni, Pb and Zn) caused a significant increase in the MDA content, thus indicating their toxicities.

Acknowledgements

References

Amado Filho, G.M., Andrade, L.R., Farina, M., Malm, O., 2002. Hg localisation in *Tillandsia usneoides* L. (Bromeliaceae), an atmospheric biomonitor. Atmos. Environ. 36, 881–887.

Ames, B., Shigenaga, M., Hagen, T., 1993. Oxidants, antioxidants, and the degenerative diseases of ageing. Proc. Natl. Acad. Sci. U.S.A. 90, 7915–7922.

Benz, B., Martin, C.E., 2006. Foliar trichomes, boundary layers, and gas exchange in 12 species of epiphytic *Tillandsia* (Bromeliaceae). J. Plant Physiol. 163, 648–656.

Bermudez, G.M.A., Rodriguez, J.H., Pignata, M.L., 2009. Comparison of the air pollution biomonitoring ability of three *Tillandsia* species and the lichen *Ramalina celastri* in Argentina. Environ. Res. 109, 6–14.

Bi, M., Austin, E., Smith, B.W., Winefordner, J.D., 2001. Elemental analysis of Spanish moss using laser ablation inductively coupled plasma mass spectrometry. Anal. Chim. Acta. 435, 309–318.

Branquinho, C., Brown, D.H., Catarino, F., 1997. The cellular location of Cu in lichen and its effects on membrane integrity and chlorophyll fluorescence. Environ. Exp. Bot. 37, 165–179.

Brighigna, L., Ravanelli, M., Minelli, A., Ercoli, L., 1997. The use of an epiphyte (*Tillandsia caput-medusae morren*) as bioindicador of air pollution in Costa Rica. Sci. Total Environ. 198, 175–180.

Burton, M.A.S., Lesueur, P., Puckett, K.J., 1981. Copper, nickel and thallium uptake by the lichen *Cladina rangiferina*. Can. J. Bot. 59, 91–100.

Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase catalase and peroxidase activities in root tips of soybean (Glycine max.). Physiol. Plant. 83, 463–468.

Calasans, C.F., Malm, O., 1997. Elemental mercury contamination survey in a chlor-alkali plant by the use of transplanted Spanish moss. *Tillandsia usneoides* (L). Sci. Total Environ. 208, 165–177.

Carreras, H.A., Wannaz, E.D., Pérez, C.A., Pignata, M.L., Carreras, H.H., Wannaz, E.D., Perez, C., Pignata, M.L., 2005. The role of urban air pollutants on the heavy metals accumulation performance of *Usnea amblyoclada*. Environ. Res. 97, 50–57.

Cavalcanti, F.R., Oliveira, J.T.A., Martins-Miranda, A.S., Viégas, R.A., Silveira, J.A.G., 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol. 163, 563–571.

Chettri, M.K., Cook, C.M., Vardaka, E., Sawides, 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.

Dean, R.T., Gieseg, S., Davies, M., 1993. Reactive species and their accumulation on radical-damaged proteins. Trends Biol. Sci. 18, 437–441.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R., Feller, U., 2004. Biochemical changes in barley plants after excessive supply of copper and manganese. Environ. Exp. Bot. 52, 253–266.

Dietz, K.J., Krämer, U., Baier, M., 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: From Molecules to Ecosystems. Springer Verlag, pp. 73–97.

Dhir, B., Sharmila, P., Saradhi, P.P., 2004. Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. Aquat. Toxicol. 66, 141–147.

Ernst, W.H.O., Verkleij, J.A.C., Schat, H., 1992. Metal tolerance in plants. Acta Bot. Neerl. 41, 229–248.

Fernandes, J.C., Henriques, F.S., 1991. Biochemical, physiological and structural effects of excess copper in plants. Bot. Rev. 57, 246–273.

Figueiredo, A.M.G., Saiki, M., Ticianelli, R.B., Domingos, M., Alves, E.S., Market, B., 2001. Determination of trace elements in *Tillandsia usneoides* by neutron activation analysis for environmental biomonitoring. J. Radioanal. Nucl. Chem. 249, 391–395.

Figueiredo, A.M.G., Nogueira, C.A., Saiki, M., Milian, F.M., Domingos, M., 2006. Assessment of atmospheric metallic pollution in the metropolitan region of Sao Paulo, Brazil, employing *Tillandsia usneoides* L. as biomonitor. Environ. Pollut. 145, 279–292.

Flores, F.E.V., 1987. O uso de plantas como bioindicadores de poluçiao no ambiente urbano-industrial: experiencias en Porto Alegre, RS Brasil. Tübinger Geogr. Stud. 96, 79–86.

Garty, J., Karary, Y., Harel, J., 1992. Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. Environ. Exp. Bot. 32, 229–241.

Guo, T.G., Zhang, G.P., Zhang, Y.H., 2007. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. Colloids Surf. B 57, 182–188.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53, 1–11.

Husk, G.J., Weishampel, J.F., Schlesinger, W.H., 2004. Mineral dynamics in Spanish moss, *Tillandsia usneoides* L. (Bromeliaceae), from Central Florida, USA. Sci. Total Environ. 321, 165–172.

Jeon, M.W., Ali, M.B., Hahn, E.J., Paek, K.Y., 2006. Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM Doritaenopsis plantlets under relative humidity and air temperature. Environ. Exp. Bot. 55, 183–194.

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.

Koca, H., Ozdemir, F., Turkan, I., 2006. Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. Biol. Plant. 50, 745–748.

Kosugi, H., Jojima, T., Kikugawa, K., 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24, 873–881.

Lasat, M., 2002. The hazardous substance research centers program: an EPA perspective. Environ. Prog. 21, 2–3.

Malm, O., Castro, M.B., Bastos, W.R., Branches, F.J.P., Guimaraes, J.R.D., Zuffo, C.E., Pfeiffer, W.C., 1995. An assessment of mercury pollution in different goldmining areas, Amazon, Brazil. Sci. Total Environ. 175, 127–140.

Malm, O., De Freitas Fonseca, M., Hissnauer, M.P., Rodríguez, B.W., Neves Pinto, F., 1998. Use of epiphyte plants as biomonitors to map atmospheric mercury in a gold trade center city, Amazon, Brazil. Sci. Total Environ. 213, 57–64.

Markert, B., Oehlmann, J., Roth, M., 1997. In: Subramanian, K.S., Iyengar, G.V. (Eds.), General Aspects of Heavy Metal Monitoring by Plants and Animals. American Chemical Society, Washington, DC, pp. 18–29.

Marschner, H., 1995. Mineral Nutrition in Higher Plants. Academic Press, London.

Metwally, A., Safronova, V.I., Belimov, A.A., Dietz, K.J., 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J. Exp. Bot. 56, 167–178.

Monnet, F., Bordas, F., Deluchat, V., Chatenet, P., Botineau, M., Bauru, M., 2005. Use of the aquatic lichen *Dermatocarpon luridum* as bioindicator of copper pollution: accumulation and cellular distribution tests. Environ. Pollut. 138, 455–461.

Murzaeva, S.V., 2004. Effect of heavy metals on wheat seedlings: activation of antioxidant enzymes. Appl. Biochem. Microbiol. 40, 98–103.

Nieboer, E., Richardson, 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* II. Potassium efflux studies. New Phytol. 82, 621–632.

Olmos, E., Martinez-Solano, J.R., Piqueras, A., Hellin, E., 2003. Early steps in the oxidative burst induced by cadmium in cultured tobacco cells (BY-2 line). J. Exp. Bot. 54, 291–301.

Pandey, N., Sharma, C.P., 2002. Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Sci. 163, 753–758.

Papini, A., Tani, G., Di Falco, P., Brighigna, L., 2009. The ultrastructure of the development of Tillandsia (Bromeliaceae) trichome, and details of the wing cells degeneration. Flora 205 (2), 94–100.

Pignata, M.L., Gudiño, G.L., Wannaz, E.D., Pla, R.R., González, C.M., Carreras, H.A., 2002. Atmospheric quality and distribution of heavy metals in Argentina employing *Tillandsia capillaris* as a biomonitor. Environ. Pollut. 120, 59–68.

Pyatt, F.B., Grattan, J.P., Lacy, D., Pyatt, A.J., Seaward, R.D., 1999. Comparative effectiveness of *Tillandsia usneoides* L. and *Parmotrema praesorediosum* (Nyl.) hale as bio-indicators of atmospheric pollution in Louisiana. Water Air Soil Pollut. 111, 317–326.

Sandalio, L.M., Dalurzo, H.C., Gómez, M., Romero-Puertas, M.C., Del Rio, L.A., 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plant. J. Exp. Bot. 52, 2115–2126.

Scatena, V.L., Segecin, S., 2005. Anatomia foliar de *Tillandsia L.* (Bromeliaceae) dos Campos Gerais, Paraná, Brasil. Rev. Bras. Bot. 28 (3), 635–649.

Schrimpff, E., 1984. Air pollution patterns in two cities in Colombia S.A. according to trace substance content of an epiphyte (*Tillandsia recurvata* L.). Water Air Soil Pollut. 21, 279–315.

Schützendübel, A., Schwanz, P., Teichmann, T., Gross, K., Langenfeld-Heyser, R., Godbold, D.L., Polle, A., 2001. Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots Pine roots. Plant Physiol. 127, 887–898.

Schützendübel, A., Polle, A., 2002. Plant response to abiotic stress: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53, 1351–1365.

Strehl, T., Arndt, U., 1989. Alterations in *T. aeranthos* and *T. recurvata* (Bromeliaceae) fumigated with HF and SO_2. Iheringia Ser. Bot. Porto Alegre 39, 3–17.

Van Assche, F., Clijsters, H., 1990. Effects of metals on enzyme activity in plants. Plant Cell Environ. 13, 195–206.

Van Heerden, F.R., Horak, R.M., Maharaj, V.J., Vleggaar, R., Senabe, J.V., Gunning, P.J., 2007. An appetite suppressant from *Hoodia* species. Phytochemistry 68, 2545–2553.

Vianna, A.N., Gonçalves, D., Brandão, F., De Barros, R.P., Amado Filho, G.M., Meire, R.O., Torres, J.P., Malm, O., D'Oliveira Júnior, A., Andrade, L.R., 2011. Assessment of heavy metals in the particulate matter of two Brazilian metropolitan areas by using *Tillandsia usneoides* as atmospheric biomonitor. Environ. Sci. Pollut. Res. 18, 416–427.

Wannaz, E.D., Carreras, H.A., Pérez, C.A., Pignata, M.L., 2006. Assessment of heavy metal accumulation in two species of *Tillandsia* in relation to atmospheric emission sources in Argentina. Sci. Total Environ. 361, 267–278.

Wannaz, E.D., Pignata, M.L., 2006. Calibratrion of four species of *Tillandsia* as air pollution biomonitors. J. Atmos. Chem. 53, 185–209.

Wannaz, E.D., Harguinteguy, C.A., Jasan, R., Plá, R.R., Pignata, M.L., 2008. Identification of atmospheric trace-element sources by passive biomonitoring employing PCA and variogram analysis. Int. J. Environ. Anal. Chem. 88 (4), 229–243.

Weckx, J., Clijsters, H., 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol. Plant. 96, 506–512.

Weckx, J., Clijsters, H., 1997. Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem. 35, 405–410.

Woolhouse, H.W., 1983. Toxicity and tolerance in the response of plants to metals. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Responses to the Chemical and Biological Environment. Spring Verlag, Berlin, pp. 245–300.

Yruela, I., 2005. Copper in plants. Braz. J. Plant Physiol. 17, 145–156.