
Volume 109
Number 1
January 2009
ISSN 0013-9351
ELSEVIER
Environmental Research
A Multidisciplinary Journal of Environmental Sciences, Ecology, and Public Health
Editor-in-Chief: Ellen K. Silbergeld
www.elsevier.com/locate/envres

Environmental Research 109 (2009) 6–14

Contents lists available at ScienceDirect

Environmental Research

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

# Comparison of the air pollution biomonitoring ability of three *Tillandsia* species and the lichen *Ramalina celastri* in Argentina

Gonzalo M.A. Bermudez, Judith H. Rodriguez, María L. Pignata *

*Instituto Multidisciplinario de Biología Vegetal (IMBIV), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Cátedra de Química General, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Avda, Vélez Sársfield 1611, Ciudad Universitaria (X5016 GCA) Córdoba, Argentina*

ARTICLE INFO

*Article history:*
Received 28 April 2008
Received in revised form
26 August 2008
Accepted 29 August 2008
Available online 31 October 2008

*Keywords:*
Active biomonitoring
Air pollution sources
Heavy metals
Physiological response

ABSTRACT

Bioaccumulation ability and response to air pollution sources were evaluated for *Tillandsia capillaris* Ruíz and Pav. f. *capillaris*, *T. recurvata* L., *T. tricholepis* Baker and the lichen *Ramalina celastri* (Spreng.) Krog. and Swinsc. Epiphyte samples collected from a non contaminated area in the province of Córdoba were transplanted to a control site and three areas categorised according to agricultural, urban and industrial (metallurgical and metal-mechanical) emission sources. Bioindicators were exposed for 3-, 6- and 9-month periods. A foliar damage index was established for *Tillandsia* and a pollution index for the lichen, and S, Fe, Mn and Zn concentrations were determined. An order of efficiency for the species and conditions studied is proposed taking into account heavy metal accumulation: *T. recurvata* > *T. tricholepis* > *R. celastri* > *T. capillaris*. All species studied showed Mn to be related to agricultural activity and Fe to industries and soil particles, and Zn was related to urban and industrial sources. As far as physiological response is concerned, *T. tricholepis* and *T. capillaris* were more sensitive to agricultural activities, whereas *T. recurvata* was sensitive to urban and industrial sources, and only partially to agricultural sources. No relationship was found for *R. celastri*.

## 1. Introduction

Heavy metals have been a major concern for human and environmental health in recent years because they can mobilize, disperse and to some extent produce toxic effects, which in turn can lead to growth reduction and decline in crop yield (Adriano, 1986, 1992). In this context, lichens and mosses are widely used in biomonitoring studies of air pollution, either as bioindicators of air quality or as bioaccumulators of atmospheric deposition (Conti and Cecchetti, 2001; Wolterbeek, 2002; Sczepaniak and Biziuk, 2003, and references therein). These organisms can be easily sampled, are low-cost and allow wide areas to be monitored. Lichens do not have root systems, nor do they have waxy cuticles, and thus they are strongly dependant on wet and dry deposition for their mineral nutrients. Likewise, some epiphyte plants, especially those from the *Tillandsia* genus, can also uptake and accumulate elements in their tissues because they obtain nutrients directly from the air. Therefore, their elemental composition and physiological status largely reflect the atmospheric input of air pollutants such as toxic gases and heavy metals (Figueiredo et al., 2001).

In Argentina, studies on the multielemental composition of the environment by means of bioindicators have mainly been undertaken using not only lichens (González et al., 2003; Pignata et al., 2004, 2007; Jasan et al., 2004; Carreras et al., 2005), but also species of the *Tillandsia* genus (Pignata et al., 2002; Wannaz and Pignata, 2006; Wannaz et al., 2006). For practical purposes, the suitability of various lichen species to monitoring heavy metal air pollution has become of special interest to determine which species is the most suitable as a biomonitor of an environmental condition (Cercasov et al., 2002; Minganti et al., 2003; Bergamaschi et al., 2007). In this context, comparisons between lichens and mosses have been performed in an increasing number of studies (Tretiach et al., 2007; Bargagli et al., 2002; Basile et al., 2008). However, comparison of mosses and lichens with vascular plants are scarce (Chiarenzelli et al., 2001; Salemaa et al., 2004). Furthermore, the compared suitability of both epiphyte plants and lichens as biomonitors of either physiological response or heavy metals accumulation remains uncertain.

Employing *T. capillaris* as a passive biomonitor, Pignata et al. (2002) and Wannaz et al. (2006) demonstrated that Zn was related to urban and industrial areas and Mn to agricultural activity. The first condition was also proved in an earlier active biomonitoring study (Wannaz and Pignata, 2006), while the relationship of Mn to agricultural activity still needs to be tested. Likewise, Fe content in epiphytes was ascribed to the contribution of soil particles (Pignata et al., 2002; Wannaz et al., 2006) and

---

* Corresponding author. Fax: +54 351 4332097.
*E-mail address:* pignata@com.uncor.edu (M.L. Pignata).

0013-9351/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2008.08.014

industrial activities (Pignata et al., 2002; Wannaz and Pignata, 2006), but the level of Fe accumulation in epiphytes transplanted to agricultural areas remains unknown.

Passive biomonitoring studies using *Ramalina celastri* have associated high Zn thallus content with motor vehicle traffic and industrial and agricultural activity (Pignata et al., 2004, 2007) whereas soil contribution was recognised as the main source of Fe (Pignata et al., 2007). However, the physiological response of *R. celastri* to heavy metal accumulation in active biomonitoring still remains unclear (Jasan et al., 2004). Knowledge about this phenomenon may provide insight into the conditions that affect lichen sensitivity to different air pollution sources.

The aim of this paper was to determine the best suitability between *T. capillaris*, *T. recurvata*, *T. tricholepis* and *R. celastri* for different air pollution sources by comparing physiological response to heavy metal accumulation.

## 2. Materials and methods

### 2.1. Biological material and sample preparation

Plants of *T. capillaris* Ruíz and Pav. f. *capillaris*, *T. recurvata* L., *T. tricholepis* Baker and thallus of the lichen *Ramalina celastri* (Spreng.) Krog. and Swinsc. were collected from trunks of standing trees in the Totoral Department, Province of Córdoba (between 30° 75′17″ and 30° 05′31″S, and 64° 05′41″ and 64° 16′46″W) These areas are considered to be unpolluted and represent the initial (baseline) conditions of these species.

Bags containing 300 g of *Tillandsia* individuals and 10 g of *R. celastri* were prepared according to González and Pignata (1994) and transplanted simultaneously to three areas (Fig. 1) with different atmospheric pollution emission sources on 25 August 2003, namely, traffic (Córdoba city center), metallurgical and metal-mechanical industries (South–East of Córdoba city) and agrochemicals and agricultural activities (Río Primero). In addition, bags were also exposed in an area considered to be low-polluted which was defined as a control site (Mendiolaza).

Nine bags were placed at 3 m above ground level in each area for 3, 6 and 9 months (i.e., from 25 August 2003 to 25 November 2003, 25 February 2004 and 25 May 2004, respectively). Table 1 shows precipitation and temperature data of these periods for each sampling area. After exposure, one bag of each species was retrieved from each study area. Part of the sample was separated to determine water content. Another fraction was stored in plastic vials at −15 °C in complete darkness to be used for physiological determination. Remaining material was prepared for metal determination.

### 2.2. Sampling points

#### 2.2.1. City area (traffic, metallurgical and metal-mechanical industries)

The city of Córdoba is located in the center of the Argentine Republic (31°24′S, 64°11′W) at an approximate altitude of 400 m above sea level. Climate is sub-humid and average annual precipitation is 790 mm, concentrated principally in summer. Population is around 1.3 million and topography is irregular. Its general structure is funnel shaped, with an increasing positive slope from the center towards the surrounding areas. This somewhat concave formation reduces air circulation and causes frequent thermal inversions both in autumn and winter. The main source of air pollution in the downtown area is from mobile sources (Stein and Toselli, 1996; Olcese and Toselli, 2002). There is also an important industrial development of mainly metallurgic and mechanical industries located in peripheral areas.

Two city areas were chosen for transplantation (Fig. 1). The south east (SE) is a typically industrial area with metallurgic and metal-mechanic industries. The sampling site was located near to an industrial plant where metal parts are made (power transformers, tanks, radiators, covers, etc.). The downtown sampling site was located in a densely populated area including most public buildings, government offices and shops where almost all local bus lines run through. These sites were chosen because of the substantial damage they caused in transplanted *Tillandsia* species, as mentioned by Wannaz and Pignata (2006). Furthermore, both urban and industrial sites showed the highest metal concentration in total atmospheric deposition (Wannaz and Pignata, 2006).

#### 2.2.2. Río Primero (agricultural activities)

Río Primero is located in the central region of Córdoba province at 58 km from the city of Córdoba. Mean annual temperature is 18 °C, and average minimum and maximum temperatures are 10 °C and 25 °C, respectively. The frost season extends from early May until mid September. Annual precipitation reaches 800 mm (Ramírez Sosa and Alé, 1997). Deforestation for agricultural practice, mainly soybean crops, has led to the disappearance of a great number of woody formations, and there is a high risk of desertization (Ramírez Sosa and Alé, 1997). The city of Río Primero has a population of around 5000 inhabitants.

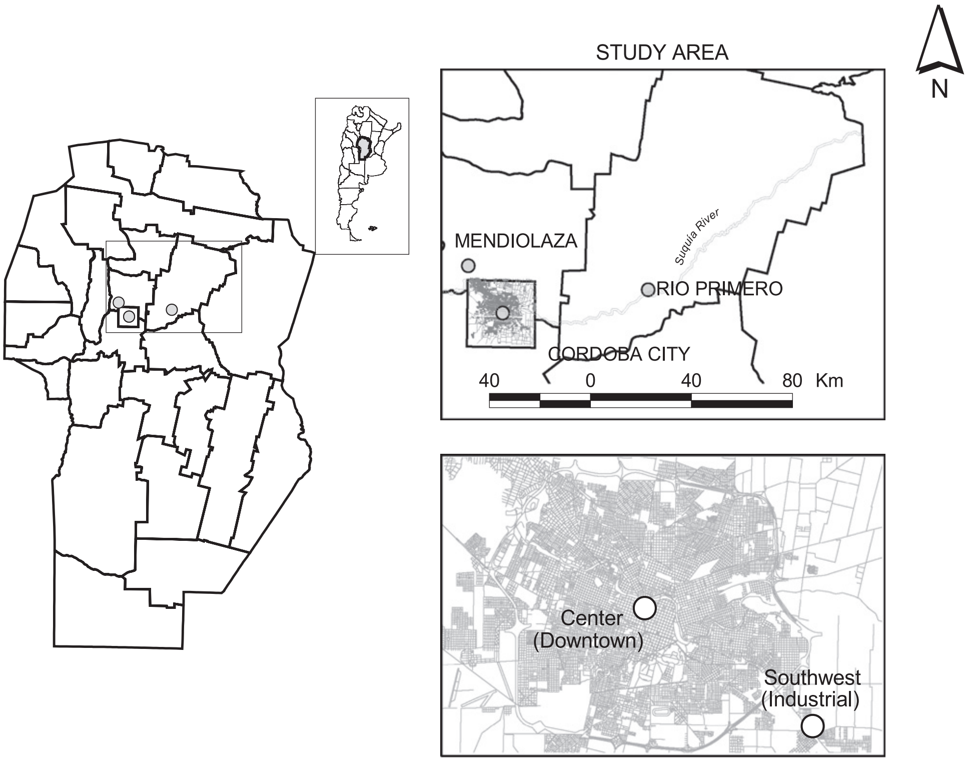

**Fig. 1.** Location of the four areas where biomonitors were transplanted in the Province of Córdoba in Argentina, i.e. Mendiolaza (*control site*), Río Primero (*agricultural activity*) and Córdoba city (detailed bottom square): center (*downtown: motor vehicle traffic*) and south east (*metallurgical and metal-mechanical industries*).

**Table 1**
Cumulative values of rainfall (mm) and mean, maximum and minimum values (°C, on a daily basis) in three transplantation areas from 25 August 2003 to 24 May 2004

| Periods | Areas of transplantation and air pollution emission sources | | | | | |
|---|---|---|---|---|---|---|
| | Córdoba City (*traffic, metallurgical and metal-mechanical industries*) | | Río Primero City (*agricultural activities*) | | Control (*low-polluted, control site*) | |
| | Rainfall (mm) | Mean *T* (°C) (min-max) | Rainfall (mm) | Mean *T* (°C) (min–max) | Rainfall (mm) | Mean *T* (°C) (min–max) |
| 25th August–24th November | 82.9 | 18.9 (12.9–27.1) | 53.5 | 16.9 (7.7–26.2) | 81.2 | 18.3 (11–26.1) |
| 25th November–24th February | 509 | 23.4 (17.6–30.0) | 232.5 | 22.9 (15.5–30.3) | 321 | 22.6 (16.5–28.9) |
| 25th February–24th May | 221.3 | 19.1 (13.6–24.6) | 203 | 17.3 (11.1–23.6) | 229 | 17.6 (12.8–23.6) |

#### 2.2.3. *Mendiolaza (control site)*

Mendiolaza is located 20 km NW of the City of Cordoba. Mean annual temperature is 15 °C and average annual precipitation ranges from 600 to 700 mm. The frost season extends from early May until mid September (Servicio Metereológico Nacional, 2007). Mendiolaza is part of the espinal phytogeographic region. This small location is sparsely populated and has no industrial or agricultural activity. Therefore, it is considered to be a "clean" control area.

### 2.3. *Physiological determinations*

Three sub-samples were taken at each sampling point. All concentrations were expressed on a dry weight basis ($g^{-1}$ DW).

Quantification procedures for chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*), phaeophityn *a* (Phaeoph-*a*), malondialdehyde (MDA), hydroperoxy conjugated dienes (HPCD), sulphur content (S), dry weight/fresh weight ratio (DW/FW) and other ratios consisted in measurements performed in the manner previously described by González et al., 1996).

A foliar damage index (FDI) was calculated for *Tillandsia* in order to combine the variation of each individual parameter indicating stress due to atmospheric pollution (Pignata et al., 2002). A pollution index (PI) was determined for *R. celastri* using the equation cited by González et al. (1996). These indexes had been statistically checked and used in previous biomonitoring studies of the same species. Equations were the following:

$$\mathrm{FDI} = [(\mathrm{Chl}\text{-}b/\mathrm{Chl}\text{-}a) + (\mathrm{S}/\mathrm{S_b})] \times [(\mathrm{MDA}/\mathrm{MDA_b}) + (\mathrm{HPCD}/\mathrm{HPCD_b})] \times (\mathrm{DW}/\mathrm{FW})$$

$$\mathrm{PI} = [(\mathrm{Phaeoph}\text{-}a/\mathrm{Chl}\text{-}a) + (\mathrm{S}/\mathrm{S_b})][(\mathrm{MDA}/\mathrm{MDA_b}) + (\mathrm{HPCD}/\mathrm{HPCD_b})]$$

where Chl-*b* is chlorophyll *b* concentration in mg $g^{-1}$ DW; Chl-*a* is chorophyll *a* concentration in mg $g^{-1}$ DW; Phaeoph-*a* is phaeophityn *a* concentration in mg $g^{-1}$ DW; *S* is sulphur content in mg $g^{-1}$ DW; MDA is malondialdehyde concentration in nmol $g^{-1}$ DW; HPCD is hydroperoxy conjugated dienes in μmol $g^{-1}$ DW; and DW/FW is the dry to fresh mass ratio. Parameters with subscript *b* in the denominator stand for arithmetic mean values calculated in the basal samples.

### 2.4. *Determination of heavy metal concentration*

Atomic absorption spectrophotometry (AAS) was used for analyzing heavy metals. A portion (2.5 g dry weight) of *Tillandsia* leaf samples and 1 g of *R. celastri* thallus were reduced in an oven at 500 °C for 7 h. Ashes were digested using a mixture of HCl (20%) and concentrated $HNO_3$ (5:1) V/V. Solid residue was separated by centrifugation and the sample was diluted with ultrapure water to a final volume of 25 ml. Finally, the sample was analyzed by AAS to determine Zn, Mn and Fe concentration. Analysis accuracy was estimated by the relative standard deviation of four replicates and was found to be 5–10% for all elements studied. Certified material IAEA/V-10 Hay Powder was analyzed every 10 samples to control the analytical method. Digestion blanks were prepared and analyzed in the same manner (Pfeiffer and Barclay-Estrup, 1992).

### 2.5. *EC ratios*

The ratio of the concentrations of each metal in exposed samples to that of control samples (exposed-to-control ratio) was used to calculate accumulation rates in lichen thalli and *Tillandsia* leaves (Frati et al., 2005).

### 2.6. *Statistical analysis*

Statistical analyses were based on the mean value of determinations performed on the three sub-samples obtained at each sampling point. A two-way analysis of variance (ANOVA) for each parameter was carried out considering the exposure period (three levels: 3, 6 and 9 months) and the transplantation area (four levels: control, urban, agricultural and industrial areas). There being no interaction, a one-way ANOVA was performed.

A pairwise comparison of means by least significant difference (LSD) was done whenever the ANOVA indicated substantial effects ($p<0.05$). An ANOVA was performed between exposure periods including baseline concentrations for accumulation parameters. Both FDI and PI were considered to be physiological response biomarkers.

## 3. Results and discussion

Tables 2–5 show mean values, standard errors and ANOVA results for chemical parameters and metal contents measured in *T. capillaris*, *T. recurvata*, *T. tricholepis* and *R. celastri*, respectively.

### 3.1. *FDI, PI and sulphur concentration*

#### 3.1.1. *T. capillaris*

The highest FDI values in *T. capillaris* were found in the agricultural area in every period assayed, indicating specific sensitivity of this species to the use of agrochemicals (Table 2). This result concurs with Pignata et al. (2002) who obtained high FDI values in a passive biomonitoring study in areas with intense agricultural activity. Furthermore, agricultural FDI values were very similar in both active and passive biomonitoring studies.

Sulphur dioxide is a by-product of coal or fuel oil combustion in many industrial processes and is present in vehicle exhaust. As expected, sulphur accumulation in *T. capillaris* after 6 months showed higher values in the industrial area than in the control site (Table 2). On the other hand, urban and agricultural sulphur accumulation in plants was significantly higher after a 9-month exposure period. Our results are in agreement with Wannaz and Pignata (2006), although the highest concentrations we observed might indicate a worsening of air pollution by sulphur oxides in the sites under study.

#### 3.1.2. *T. recurvata*

Physiological damage in the industrial area was the highest after a 3-month exposure period in the case of *T. recurvata*. Epiphytes transplanted to agricultural and urban areas showed higher FDI values than the control site after three and 6 months (Table 3). However, a recovery toward the initial or even lower physiological damage, with end values not statistically different from those of epiphytes transplanted to the control area in the last period of transplantation, shows an unclear temporal pattern of response that may probably be attributed to better weather conditions in this period (summer).

Sulphur concentrations found in *T. recurvata* were higher in epiphytes transplanted to urban and industrial areas (Table 3), which is in accordance with Graciano et al. (2003).

**Table 2**

Comparison of average concentration values (±SE) with the results of variance analysis (ANOVA) of chemical parameters and metals on *Tillandsia capillaris* Ruiz and Pav. f. *capillaris* between different areas and exposure periods

| | Mean±SE | | | | ANOVA[b] |
|---|---|---|---|---|---|
| | Control | Urban | Agricultural | Industrial | |
| FDI | | | | | |
| Baseline | 1.282±0.062 | 1.282±0.062 | 1.282±0.062 | 1.282±0.062 | |
| 3 months | 0.994±0.065 Bc | 1.275±0.077 Bb | 2.120±0.134 a | 1.390±0.183 b | *** |
| 6 months | 1.414±0.201 ABb | 1.951±0.280 Aa | 2.061±0.250 a | 1.605±0.123 ab | * |
| 9 months | 1.459±0.051 Ab | 1.113±0.192 Bb | 2.034±0.040 a | 1.186±0.066 b | ** |
| ANOVA[a] | * | * | ns | ns | |
| S | | | | | |
| Baseline | 1.518±0.043 C | 1.518±0.043 B | 1.518±0.043 B | 1.518±0.043 B | |
| 3 months | 2.180±0.043 Aa | 1.923±0.077 Ab | 1.920±0.025 Ab | 2.235±0.107 Aa | * |
| 6 months | 1.926±0.028 Bb | 1.960±0.014 Ab | 1.910±0.070 Ab | 2.226±0.040 Aa | ** |
| 9 months | 1.356±0.015 Dc | 1.964±0.190 Aa | 1.852±0.029 Aab | 1.515±0.112 Bbc | * |
| ANOVA[a] | *** | * | *** | *** | |
| Fe | | | | | |
| Baseline | 1511±177.5 B | 1511±177.5 B | 1511±177.5 | 1511±177.5 B | |
| 3 months | 2239±426.5 A | 1674±187.8 B | 1794±163.5 | 2519±181.2 A | ns |
| 6 months | 1671±106.1 Ba | 850.5±121.8 Cb | 1736±277.7 a | 1829±185.2 Ba | * |
| 9 months | 2201±153.5 A | 2543±358.4 A | 2379±24.62 | 2501±507.5 A | ns |
| ANOVA[a] | * | * | ns | * | |
| Mn | | | | | |
| Baseline | 81.25±17.68 B | 81.25±17.68 | 81.25±17.68 | 81.25±17.68 | |
| 3 months | 81.25±13.26 B | 85.94±17.79 | 98.44±24.31 | 104.7±15.47 | ns |
| 6 months | 118.3±8.431 A | 84.67±23.03 | 122.3±4.152 | 118.1±16.15 | ns |
| 9 months | 79.77±10.87 Bb | 90.68±4.124 b | 119.4±2.310 a | 96.95±10.17 b | * |
| ANOVA[a] | * | ns | ns | ns | |
| Zn | | | | | |
| Baseline | 21.17±4.989 | 21.17±4.989 B | 21.17±4.989 C | 21.17±4.989 B | |
| 3 months | 35.28±6.832 | 35.38±5.998 B | 35.28±6.200 B | 28.23±14.97 B | ns |
| 6 months | 30.55±7.377 | 62.31±28.29B | 21.21±5.430 C | 57.25±17.978 A | ns |
| 9 months | 37.39±11.24 c | 139.0±10.55 Aa | 51.27±20.04 Abc | 62.40±21.456 Ab | ** |
| ANOVA[a] | ns | ** | * | * | |

ns: not significant; FDI: foliar damage index; S: sulphur; Fe: iron; Mn: manganese; Zn: zinc.

* Significant at $p<0.05$.

** Significant at $p<0.01$.

*** Significant at $p<0.001$.

[a] ANOVA among times (in capital letters).

[b] ANOVA among areas.

### 3.1.3. *T. tricholepis*

*T. tricholepis* showed the highest FDI values in agricultural and urban areas (Table 4). Although the response to urban pollutants is consistent with Wannaz and Pignata (2006), it seems reasonable to assume that intense agricultural activity, as taken into account in this study, may have revealed a specific sensitivity of *T. tricholepis* to agrochemicals. It has been demonstrated that some *Tillandsia* species are sensitive to herbicides and fungicides (Caldiz and Beltramo, 1989; Bartoli et al., 1993).

In the case of *T. tricholepis*, sulphur concentration was significantly higher in the agricultural and urban areas than in the control site after 9 months (Table 4), and higher than those cited by Wannaz and Pignata (2006).

It is important to note that sulphur concentration in the three *Tillandsia* species transplanted to the agricultural area were higher than in the control site 9 months after transplantation. This result might indicate a strong influence of agricultural activities in Córdoba, which was described by Garty et al. (1988). These findings are likely to be the effect of the use of fertilizers containing sulphates such as $(NH_4)_2SO_4$ and insecticides containing sulphur like some cyclodienes (cyclic esters of sulphurous acid), as previously described by Pignata et al. (2007). This agrochemical is applied to soybean cultures and is frequently used in summer before harvesting, which happened concurrently with the 9-month transplantation period considered in this work. Furthermore, the use of low-quality fossil fuels, such as those employed in agricultural machinery at the time of harvesting, could act as an additional source of sulphur in Argentina.

### 3.1.4. *R. celastri*

As far as *R. celastri* is concerned, the PI showed differences only between exposure periods, and the highest values were recorded 6 months after transplantation (Table 5). *R. celastri* had shown physiological damage to be related to urban and industrial air pollution sources in earlier active biomonitoring studies (González et al., 1996, 2003). This effect was also cited for other species of the *Ramalina* genus (Garty et al., 2001, 2003). In the present work, the absence of significant differences between the control site and the agricultural, industrial and urban areas could be due to the presence of previous physiological damage in baseline samples. This may be connected to high phaeophytinization levels and lipid peroxidation products (data not shown), which would indicate an increase in oxidative processes in membranes. On the other hand, earlier work with this species conducted by our group showed that samples transplanted into control sites had PI values ranging from 1.7 and 1.9 (Levin and Pignata, 1995; González et al., 1996; Rodriguez et al., 2007). Damage shown by baseline samples used in this work might have been caused by low temperature in the collection area some weeks before sampling was done. This may

**Table 3**

Comparison of average concentration values (±SE) with the results of variance analysis (ANOVA) of chemical parameters and metals on *Tillandsia recurvata* L. between different areas and exposure periods

| | Mean±SE | | | | ANOVA[b] |
|---|---|---|---|---|---|
| | Control | Urban | Agricultural | Industrial | |
| FDI | | | | | |
| Baseline | 0.796±0.118 | 0.796±0.118 | 0.796±0.118 | 0.796±0.118 | |
| 3 months | 0.595±0.058 Bd | 2.139±0.171 Ab | 1.718±0.165 Ac | 2.495±0.149 Aa | *** |
| 6 months | 0.991±0.092 Ac | 1.434±0.131 Ba | 1.345±0.158 Bab | 1.155±0.050 Bbc | ** |
| 9 months | 0.986±0.053 Aab | **1.291±0.170 Ba** | 1.020±0.103 Bab | 0.668±0.131 Cb | * |
| ANOVA[a] | ** | ** | ** | *** | |
| S | | | | | |
| Baseline | 1.676±0.034 A | 1.676±0.034 B | 1.676±0.034 C | 1.676±0.034 C | |
| 3 months | 1.924±0.005 Aa | 1.678±0.098 Bb | 1.607±0.020 Cb | 1.935±0.060 Ba | * |
| 6 months | 1.750±0.145 Ac | 2.157±0.106 Aab | 1.859±0.029 Bbc | 2.200±0.061 Aa | * |
| 9 months | 1.283±0.057 Bb | 1.879±0.108 ABa | 1.992±0.031 Aa | 1.814±0.041 BCa | *** |
| ANOVA[a] | ** | * | *** | *** | |
| Fe | | | | | |
| Baseline | 1287±260.7 | 1287±260.7 B | 1288±260.7 B | 1287±260.7 B | |
| 3 months | 2376±243.1 b | 1683±290.7 Bc | 2145±199.8 Ab | 2861±218.8 Aa | * |
| 6 months | 1830±350.2 | 1679±466.6 B | 907.3±177.7 B | 1708±280.6 B | ns |
| 9 months | 1610±788.9 | 2615±319.2 A | 2790±313.7 A | 2906±478.6 A | ns |
| ANOVA[a] | ns | * | * | ** | |
| Mn | | | | | |
| Baseline | 34.37±4.419 B | 34.38±4.419 C | 34.38±4.42 B | 34.38±4.419 C | |
| 3 months | 74.80±2.850 Aa | 40.63±4.419 Cc | 64.06±2.210 Bb | 75.00±4.420 Ba | * |
| 6 months | 85.78±2.523 Ab | 82.07±6.145 Ab | 102.2±6.813 Aa | 88.16±1.146 Ab | * |
| 9 months | 31.95±16.71 Bc | 70.18±3.299 Bb | 118.2±19.79 Aa | 80.93±1.146 ABb | * |
| ANOVA[a] | ** | ** | * | ** | |
| Zn | | | | | |
| Baseline | 28.33±1.410 BC | 28.36±1.410 D | 28.36±1.410 | 28.36±1.410 C | |
| 3 months | 40.57±2.495 Ab | 31.85±0.141Cc | 35.28±4.989 bc | 51.16±2.495 Ba | * |
| 6 months | 20.16±4.977 Cc | 75.53±1.808 Aa | 38.35±11.16 b | 47.40±2.862 Bb | * |
| 9 months | 39.27±5.797 ABc | 59.63±0.730 Bab | 60.00±3.285 b | 67.52±1.094 Aa | * |
| ANOVA[a] | * | * | ns | *** | |

ns: not significant; FDI: foliar damage index; S: sulphur; Fe: iron; Mn: manganese; Zn: zinc.

* Significant at $p<0.05$.

** Significant at $p<0.01$.

*** Significant at $p<0.001$.

[a] ANOVA among times (in capital letters).

[b] ANOVA among areas.

account for the narrow difference observed in the response of *R. celastri* to environmental conditions in transplantation sites. When differences among periods occurred, these related to the recovery of physiological conditions possibly in response to better weather conditions.

### 3.2. *Heavy metal content and EC ratios*

#### 3.2.1. *T. capillaris*

The greatest percentage of metal content corresponded to Fe. It should be noted that Fe is the main element in Argentine soils (Gaiero et al., 2003). Several authors have shown that the distribution pattern of Fe and Mn originates mainly from soil particles (Adriano, 1986; Guevara et al., 1995; Loppi et al., 1999; Frati et al., 2005). We found that *T. capillaris* showed little difference for Mn between areas in the course of this study. The highest concentration was seen in the agricultural area 9 months after exposure. Similar results were found by Wannaz et al. (2006).

Fe and Zn concentration values in both the downtown area of Córdoba and the industrial area were similar to those cited by Wannaz and Pignata (2006) for the same sites and months of the year. This might indicate that metal content in *T. capillaris* leaves experiences a seasonal pattern of accumulation. Furthermore, a substantial loss of Fe was observed 6 months after exposure, when total precipitation was fairly higher than in the other two transplantation periods thus strengthening the above hypothesis. These results are in accordance with previous work performed with bioindicators (Cercasov et al., 2002; Frati et al., 2005; Bergamaschi et al., 2007; Ayrault et al., 2007), which acknowledges a "washing effect" by rain. Frati et al. (2005) pointed out that a balance between wet and dry deposition could account for differences between periods under conditions of constant pollution load. Consequently, different amounts of pollutants possibly cause direct toxicity to epiphytes. This leads to a high degree of biological stress and in turn alters element uptake. Decreasing metal concentration in tissues might also be explained by biological excretion caused not only by toxic or saturation effects but also by seasonally varying degrees of dilution in mass increments, resulting from both seasonal variations and differential air pollution effects on growth rates (Wolterbeek, 2002).

On the other hand, we found a higher Fe concentration in epiphytes than that mentioned by Wannaz et al. (2006) for agricultural, urban, and particularly industrial areas in all transplantation periods. This could be partially accounted for by the greater impact of specific air pollution sources on biomonitors in this study.

Table 6 shows EC ratios and the relative scale described by Frati et al. (2005). *T. capillaris* accumulated Mn in agricultural and

**Table 4**

Comparison of average concentration values (±SE) with the results of variance analysis (ANOVA) of chemical parameters and metals on *Tillandsia tricholepis Baker* between different areas and exposure periods

| | Mean±SE | | | | ANOVA$^b$ |
|---|---|---|---|---|---|
| | Control | Urban | Agricultural | Industrial | |
| FDI | | | | | |
| Baseline | 0.633±0.116 | 0.633±0.116 | 0.633±0.116 | 0.633±0.116 | |
| 3 months | 0.671±0.036 Bd | 1.843±0.072 Ab | 5.790±0.442 Aa | 1.413±0.078 Ac | *** |
| 6 months | 0.827±0.107 Ab | 1.203±0.048 Ba | 1.076±0.147 Ba | 0.876±0.012 Bb | ** |
| 9 months | 0.617±0.028 Bb | 0.885±0.055 Ca | 0.896±0.023 Ba | 0.670±0.092 Cb | * |
| ANOVA$^a$ | * | *** | *** | *** | |
| S | | | | | |
| Baseline | 1.665±0.075 B | 1.665±0.075 B | 1.665±0.075 B | 1.665±0.075 B | |
| 3 months | 2.033±0.061 A | 1.858±0.113 B | 1.904±0.083 AB | 1.878±0.063 B | ns |
| 6 months | 2.186±0.073 Aab | 2.350±0.031 Aa | 2.010±0.064 Ab | 2.354±0.069 Aa | ** |
| 9 months | 1.371±0.034 Cb | 1.850±0.057 Ba | 1.906±0.060 Aa | 1.322±0.138 Cb | ** |
| ANOVA$^a$ | *** | *** | * | *** | |
| Fe | | | | | |
| Baseline | 567.2±67.57 Cb | 567.2±67.57 Cb | 567.2±67.57 Ba | 567.2±67.57 Ba | |
| 3 months | 879.7±134.8 BCb | 865.7±104.4 Bb | 1428±238.6 Aa | 1588±246.4 Aa | * |
| 6 months | 1025±165.2 Bb | 990.5±157.1 Bb | 1244±205.7 Aab | 1679±280.6 Aa | * |
| 9 months | 1576±245.8 A | 1258±88.19 A | 1504±232.0 A | 1314±129.6 A | ns |
| ANOVA$^a$ | * | * | ** | * | |
| Mn | | | | | |
| Baseline | 31.25±4.419 BC | 31.25±4.419 BC | 31.25±4.419 C | 31.25±4.419 B | |
| 3 months | 26.56±2.210 C | 28.63±2.707 C | 53.13±13.26 BC | 39.06±2.210 B | ns |
| 6 months | 48.73±3.947 Ab | 53.93±4.001 Ab | 73.75±4.365 Aa | 69.76±6.847 Aa | ** |
| 9 months | 40.03±6.285 ABb | 44.25±10.49 ABb | 67.72±6.182 ABa | 35.56±1.029 Bb | * |
| ANOVA$^a$ | * | ** | * | *** | |
| Zn | | | | | |
| Baseline | 28.23±9.979 B | 28.23±9.979 B | 28.23±9.979 C | 28.23±9.979 C | |
| 3 months | 26.46±2.495 B | 31.40±5.495 B | 28.23±4.989 C | 38.81±9.980 C | ns |
| 6 months | 110.0±36.58 Aab | 160.6±47.80 Aa | 52.40±5.128 Bc | 89.04±3.292 Bbc | * |
| 9 months | 77.01±14.19 ABb | 127.2±4.865 Aa | 114.1±9.991 Aa | 119.6±5.852 Aa | * |
| ANOVA$^a$ | * | * | * | *** | |

ns: not significant; FDI: foliar damage index; S: sulphur; Fe: iron; Mn: manganese; Zn: zinc.

* Significant at $p<0.05$.

** Significant at $p<0.01$.

*** Significant at $p<0.001$.

[a] ANOVA among times (in capital letters).

[b] ANOVA among areas.

industrial areas. Wannaz et al. (2006) suggested that Mn could be related to agrochemicals (fertilizers and pesticides) since their use has noticeably grown in Argentina in recent years. *T. capillaris* showed severe Zn accumulation. It should be noted that Zn behaves as an atmosphile element (Loppi et al., 1997) in urban and industrial areas as seen in a previous study (Wannaz et al., 2006). Zinc is also considered to be related to the use of fertilizers (Loppi et al., 1999) or fungicides such as zinctetramethyl dithiocarbomated ethyl-bisdithiocarbamated (Novo et al., 1998). In the course of this study, however, *T. capillaris* only accumulated Zn in the agricultural area 9 months after exposure.

### 3.2.2. *T. recurvata*

Fe concentration in *T. recurvata* differed significantly from that in the control site only in the industrial area (Table 3), suggesting that industrial air pollution sources had a greater impact than soil contribution. Temporal variation was similar to the pattern described for *T. capillaris*. The highest Mn content was recorded in the agricultural area followed by urban and industrial areas 9 months after exposure. This difference between sites indicates that *T. recurvata* seems to accumulate Mn more effectively than *T. capillaris*. On the other hand, Zn concentration was higher in industrial and urban areas.

EC ratios observed in this study indicate that *T. recurvata* accumulated Fe and severely accumulated Mn at the end of the 9-month exposure period. The highest ratios corresponded to industrial and agricultural areas, respectively (Table 6). These results are in agreement with those obtained for *T. capillaries*. Yet, it should be pointed out that *T. recurvata* accumulated Zn stemming from agricultural activities somewhat more efficiently than *T. capillaris*.

### 3.2.3. *T. tricholepis*

Fe concentration in *T. tricholepis* was higher in agricultural and industrial areas (Table 4). Similar results were observed by Wannaz and Pignata (2006). As described earlier for *T. capillaris* and *T. recurvata*, Mn content in *T. tricholepis* was higher in the agricultural area, but with a slightly lower concentration than that mentioned by Wannaz and Pignata (2006). Zn was partially related to urban, agricultural and industrial air pollution sources.

Considering EC ratios shown in Table 6, it can be seen that *T. tricholepis* accumulated Mn, Zn and mainly Fe in the industrial area. This study seems to indicate that *T. tricholepis* is the best Mn accumulator in the agricultural area since it exhibited average or severe accumulation rates for this metal in all exposure periods. On the other hand, only Zn was observed to be accumulated in the

**Table 5**

Comparison of average concentration values (± SE) with the results of variance analysis (ANOVA) of chemical parameters and metals on *Ramalina celastri* (Spreng.) Krog. and Swinsc. between different areas and exposure periods

| | Mean ± SE | | | | ANOVA[b] |
|---|---|---|---|---|---|
| | Control | Urban | Agricultural | Industrial | |
| PI | | | | | |
| Baseline | 4.385 ± 0.668 | 4.385 ± 0.668 | 4.385 ± 0.668 | 4.385 ± 0.668 | |
| 3 months | 2.612 ± 0.120 B | 3.514 ± 0.157 B | 3.438 ± 0.073 | 2.115 ± 0.800 B | ns |
| 6 months | 4.210 ± 0.653 A | 4.105 ± 0.908 A | 3.683 ± 0.403 | 4.560 ± 0.720 A | ns |
| 9 months | 3.205 ± 0.197 B | 3.151 ± 0.321B | 3.113 ± 0.326 | 3.744 ± 0.126 A | ns |
| ANOVA[a] | ** | *** | ns | ** | |
| S | | | | | |
| Baseline | 1.828 ± 0.107 | 1.828 ± 0.107 | 1.828 ± 0.107 | 1.828 ± 0.107 | |
| 3 months | 1.896 ± 0.213 | 2.054 ± 0.117 | 2.137 ± 0.039 | 1.942 ± 0.065 | ns |
| 6 months | 1.648 ± 0.413 | 1.534 ± 0.575 | 1.960 ± 0.123 | 1.773 ± 0.260 | ns |
| 9 months | 2.018 ± 0.096 | 1.873 ± 0.140 | 2.202 ± 0.201 | 2.053 ± 0.208 | ns |
| ANOVA[a] | ns | ns | ns | ns | |
| Fe | | | | | |
| Baseline | 698.91 ± 79.28 B | 698.9 ± 79.28C | 698.9 ± 79.28 C | 698.9 ± 79.28 D | |
| 3 months | 1073 ± 47.57 A | 833.4 ± 26.43 BC | 953.1 ± 68.71 BC | 938.1 ± 89.86 C | ns |
| 6 months | 1347 ± 97.77 Aa | 979.2 ± 71.60 ABb | 1235 ± 167.7 Aa | 1203 ± 30.59 Ba | * |
| 9 months | 1335 ± 177.3 Ab | 1111 ± 75.25 Ab | 1223 ± 112.1 ABb | 1580 ± 103.6 Aa | * |
| ANOVA[a] | * | * | * | * | |
| Mn | | | | | |
| Baseline | 14.00 ± 4.272 B | 14.00 ± 4.272 B | 14.00 ± 4.272 C | 14.00 ± 4.272 C | |
| 3 months | 28.00 ± 3.443 A | 24.00 ± 5.657 A | 28.00 ± 2.891 B | 28.15 ± 0.212 B | ns |
| 6 months | 32.66 ± 4.141 A | 25.66 ± 4.041 A | 35.03 ± 6.154 A | 32.66 ± 4.449 AB | ns |
| 9 months | 32.66 ± 8.08 A | 28.05 ± 4.360 A | 37.33 ± 4.475 A | 35.01 ± 5.992 A | ns |
| ANOVA[a] | * | * | * | * | |
| Zn | | | | | |
| Baseline | 6.909 ± 1.954 B | 6.909 ± 1.954 C | 6.909 ± 1.954 C | 6.909 ± 1.954 C | |
| 3 months | 6.358 ± 1.788 Bb | 12.44 ± 1.704 BCa | 12.44 ± 1.930 BCa | 11.06 ± 1.772 Ca | * |
| 6 months | 20.26 ± 1.595 A | 23.94 ± 5.750 AB | 28.55 ± 5.164 AB | 35.93 ± 6.024 B | ns |
| 9 months | 21.18 ± 6.380 Ac | 28.55 ± 7.975 Abc | 38.67 ± 5.525 Aab | 46.04 ± 6.380 Aa | * |
| ANOVA[a] | * | * | ** | * | |

ns: not significant; PI: pollution index; S: sulphur; Fe: iron; Mn: manganese; Zn: zinc.

* Significant at $p < 0.05$.

** Significant at $p < 0.01$.

*** Significant at $p < 0.001$.

[a] ANOVA among times (in capital letters).

[b] ANOVA among areas.

urban area. These results are in agreement with previous data obtained for this species.

### 3.2.4. *R. celastri*

Increasing metal concentration in *R. celastri* was acknowledged after each exposure period. However, significant differences between exposure areas were ascertained only in the case of Fe and Zn (Table 5). The highest Zn content was observed in agricultural and industrial areas 9 months after transplantation. The use of fungicides containing Zn in agricultural areas has been reported in previous biomonitoring studies with *R. celastri* (Pignata et al., 2007). Zn content regarding industrial activity could relate to manufacturing processes of power transformers, which is the main activity in the area. Furthermore, EC ratios (Table 6) show that *R. celastri* performs as a good Zn accumulator in connection with air pollution sources currently under study. On the other hand, the longer the transplantation period, the more significantly Mn increased in the agricultural area. This result is very similar to that described above for *Tillandsia*. Pignata et al. (2007) have mentioned that Fe accumulation in *R. celastri* could be ascribed to soil contribution and that Fe uptake could additionally be influenced by industrial activities.

EC ratios indicate that a decrease of heavy metal content was noticed in all species except *R. celastri* both in urban and agricultural areas, particularly 6 months after transplantation. This period corresponded to the highest precipitation values recorded at the time (Table 1) thus strengthening the "washing effect" hypothesis referred to above.

## 4. Conclusion

The relationship of Mn to agricultural activity and Fe to industrial and agricultural activity was proved. Zn was the most specific-dependent heavy metal as it was related to urban activity (*T. capillaris* and *T. tricholepis*), urban and industrial activity (*T. recurvata*), and industrial and agricultural activity (*R. celastri*).

Considering physiological response, *T. tricholepis* and *T. capillaris* were more sensitive to agricultural activity while *T. recurvata* was more sensitive to urban and industrial activity. The FDI proved to be a good biomarker for physiological response of *Tillandsia* in all transplantation periods, but it was not necessarily related to the heavy metal concentration in tissue measured in this work. Therefore, clear physiological differences for *Tillandsia* species could be established 3 months after exposure. This work might demonstrate that agricultural activity is a source of air pollution having a great impact on the native flora. No relationship was found for *R. celastri*.

**Table 6**
EC ratios for elements assayed in three transplanted *Tillandsia* species and the lichen *R. celastri* exposed as of 24 August 2003 for 3–9 months

| Metal | Species | Time | Areas of exposure | | |
|---|---|---|---|---|---|
| | | | Urban | Agricultural | Industrial |
| Fe | *T. capillaris* | 3 months | 0.75 | 0.80 | 1.13 |
| | | 6 months | $0.51^{\dagger}$ | 1.04 | 1.09 |
| | | 9 months | 1.16 | 1.08 | 1.13 |
| | *T. recurvata* | 3 months | $0.71^{\dagger}$ | 0.90 | 1.20 |
| | | 6 months | 0.92 | $0.50^{\dagger}$ | 0.93 |
| | | 9 months | **1.63** | **1.73** | **1.81**$^{\ddagger}$ |
| | *T. tricholepis* | 3 months | 0.98 | **1.62** | ***1.81***$^{\ddagger}$ |
| | | 6 months | 0.97 | 1.21 | **1.64** |
| | | 9 months | 0.79 | 0.95 | 0.83 |
| | *R. celastri* | 3 months | 0.78 | 0.89 | 0.88 |
| | | 6 months | $0.73^{\dagger}$ | 0.92 | 0.89 |
| | | 9 months | 0.83 | 0.92 | 1.18 |
| Mn | *T. capillaris* | 3 months | 1.06 | 1.21 | **1.29** |
| | | 6 months | $0.72^{\dagger}$ | 1.03 | 1.00 |
| | | 9 months | 1.14 | **1.50** | 1.22 |
| | *T. recurvata* | 3 months | $0.54^{\dagger}$ | 0.86 | 1.00 |
| | | 6 months | 0.96 | 1.19 | 1.03 |
| | | 9 months | **2.20**$^{\ddagger}$ | **3.70**$^{\ddagger}$ | **2.53**$^{\ddagger}$ |
| | *T. tricholepis* | 3 months | 1.08 | **2.00**$^{\ddagger}$ | **1.47** |
| | | 6 months | 1.11 | **1.51** | **1.43** |
| | | 9 months | 1.11 | **1.69** | 0.89 |
| | *R. celastri* | 3 months | 0.86 | 1.00 | 1.01 |
| | | 6 months | 0.79 | 1.07 | 1.00 |
| | | 9 months | 0.86 | 1.14 | 1.07 |
| Zn | *T. capillaris* | 3 months | 1.00 | 1.00 | 0.80 |
| | | 6 months | **2.04**$^{\ddagger}$ | $0.69^{\dagger}$ | **1.87**$^{\ddagger}$ |
| | | 9 months | **3.72**$^{\ddagger}$ | **1.37** | **1.67** |
| | *T. recurvata* | 3 months | 0.79 | 0.87 | **1.26** |
| | | 6 months | **3.75**$^{\ddagger}$ | **1.90**$^{\ddagger}$ | **2.35** $^{\ddagger}$ |
| | | 9 months | **1.52** | **1.23** | **1.72**$^{\ddagger}$ |
| | *T. tricholepis* | 3 months | 1.19 | 1.07 | **1.47** |
| | | 6 months | **1.46** | $0.48^{\dagger}$ | 0.81 |
| | | 9 months | **1.65** | **1.48** | **1.55** |
| | *R. celastri* | 3 months | **1.96**$^{\ddagger}$ | **1.96**$^{\ddagger}$ | **1.74** |
| | | 6 months | 1.18 | **1.41** | **1.77**$^{\ddagger}$ |
| | | 9 months | **1.35** | **1.83**$^{\ddagger}$ | **2.17**$^{\ddagger}$ |

The $^{\dagger}$ symbol represents loss ($0.25>\text{EC}>0.75$), bold numbers indicate accumulation ($1.25<\text{EC}<1.75$), and bold numbers with the $^{\ddagger}$ symbol represent severe accumulation ($\text{EC}>1.75$) of heavy metals (Frati et al., 2005).

EC ratios allow the accumulation ability of different species to be normalized and hence enable their biomonitor behaviour to be compared. We propose an order of efficiency for the species and conditions studied taking into account heavy metal accumulation and severe accumulation events, i.e., *T. recurvata* >*T. tricholepis* >*R. celastri* >*T. capillaris*.

## Funding source

This work was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica (FONCyT) and the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECYT-UNC).

## Acknowledgements

We are thankful to the Comando de Regiones Aéreas del Servicio Meteorológico Nacional, Centro de Información Meteorológica, Fuerza Aérea Argentina. The authors, Bermudez and Rodriguez, are doctorate students in Biological Sciences at the National University of Córdoba and fellows at the CONICET.

## References

Adriano, D.C., 1986. Trace Elements in the Terrestrial Environment. Springer, New York, p. 533.

Adriano, D.C., 1992. Biochemistry of Trace Metals. Lewis Publications, Boca Raton, Florida, p. 514.

Ayrault, S., Clochiatti, R., Carrot, F., Daudin, L., Bennett, J., 2007. Factors to consider for trace element deposition biomonitoring surveys with lichen transplants. Sci. Total Environ. 372, 712–727.

Bargagli, R., Monaci, F., Borghini, F., Bravi, F., Agnorelli, C., 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on *Hypnum cupressiforme* and *Parmelia caperata* in a former mining district in Italy. Environ. Pollut. 116, 279–287.

Bartoli, C.G., Beltramo, J., Fernandez, L.V., Caldiz, D.O., 1993. Control of the epiphytic weeds *Tillandsia recurvata* and *Tillandsia aëranthos* with different herbicides. Forest Ecol. Manag. 59, 289–294.

Basile, A., Sorbo, S., Aprile, G., Conte, B., Castaldo Cobianchi, R., 2008. Comparison of the heavy metal bioaccumulation capacity of an epiphytic moss and an epiphytic lichen. Environ. Pollut. 151, 401–407.

Bergamaschi, L., Rizzio, E., Giaveri, G., Loppi, S., Gallorini, M., 2007. Comparison between the accumulation capacity of four lichen species transplanted to a urban site. Environ. Pollut. 148, 468–476.

Caldiz, D.O., Beltramo, J., 1989. Control of the epiphytic weeds *Tillandsia recurvata* and *Tillandsia aëranthos* with simazine. Forest Ecol. Manag. 28, 153–159.

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

Cercasov, V., Pantelica, V., Sa la gean, M., Caniglia, G., Scarlat, A., 2002. Comparative study of the suitability of three lichen species to trace-element air monitoring. Environ. Pollut. 119, 129–139.

Chiarenzelli, J., Aspler, L., Dunn, C., Cousens, B., Ozarko, D., Powis, K., 2001. Multi-element and rare earth element composition of lichens, mosses, and vascular plants from the central Barrenlands, Nunavut, Canada. Appl. Geochem. 16, 245–270.

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

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.

Frati, L., Brunialti, G., Loppi, S., 2005. Trace element deposition in repeated surveys: a case study from central Italy. J. Atmos. Chem. 52, 221–230.

Gaiero, D.M., Probst, J.L., Depetris, P.J., Bidart, S.M., Leleyter, L., 2003. Iron and other transition metals in Patagonian riverborne and windborne materials: geochemical control and transport to the southern South Atlantic Ocean. Geoch. Cosmochim. Ac. 67, 3603–3623.

Garty, J., Kardish, N., Hagemeyer, J., Ronen, R., 1988. Correlations between the concentrations of adenosine triphosphate, chlorophyll degradation and the amounts of airborne heavy metals and sulphur in a transplanted lichen. Arch. Environ. Con. Tox. 14, 601–611.

Garty, J., Weissman, L., Cohen, Y., Karnieli, A., Orlovsky, L., 2001. Transplanted lichens in and around the Mount Carmel national park and Haifa Bay industrial region in Israel: physiological and chemical responses. Environ. Res. 85, 159–176.

Garty, J., Tomer, S., Levin, T., Lehr, H., 2003. Lichens as biomonitors around a coal-fired power station in Israel. Environ. Res. 91, 186–198.

González, C.M., Pignata, M.L., 1994. The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulphur and heavy metals in a transplanted lichen. Chem. Ecol. 9, 105–113.

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.

González, C.M., Pignata, M.L., Orellana, L., 2003. Application of redundancy analysis for the detection of chemical response patterns to air pollution in lichen. Sci. Total Environ. 312, 245–253.

Graciano, C., Fernández, L.V., Caldiz, D.O., 2003. *Tillandsia recurvata* L. as a bioindicator of sulphur atmospheric pollution. Ecol. Austral. 13, 3–14.

Guevara, S.R., Arribére, M.A., Calvelo, S., Roman Ross, G., 1995. Elemental composition of lichens at Nahuel Huapi national park, Patagonia, Argentina. J. Radioanal. Nucl. Chem. 198, 437–448.

Jasan, R.C., Verburg, T.G., Wolterbeek, H.T., Plá, R.R., Pignata, M.L., 2004. On the use of the lichen *Ramalina celastri* (Spreng.) Krog and Swinsc. as an indicator of atmospheric pollution in the province of Córdoba (Argentina), considering both lichen physiological parameters and element concentrations. J. Radioanal. Nucl. Chem. 259, 93–97.

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

Loppi, S., Nelli, S., Ancora, S., Bargagli, R., 1997. Passive monitoring of trace elements by means of tree leaves, epiphytic lichens and bark substrate. Environ. Monit. Assess. 45, 81–88.

Loppi, S., Pirintsos, S.A., Dominicis, V., 1999. Soil contribution to the elemental composition of epiphytic lichens (Tuscany, Central Italy). Environ. Monit. Assess. 58, 121–131.

Minganti, V., Capella, R., Drava, G., De Pellegrini, R., Brunialti, G., Giordani, P., Modonesi, P., 2003. Biomonitoring of trace metals by different species of lichens (*Parmelia*) in North–West Italy. J. Atmos. Chem. 45, 219–229.

Novo, R.J., Cragnolini, C.L., Cavallo, R.L., Conces, M.Y., 1998. Fungicidas. Departamento de Protección Vegetal y Terapéutica Vegetal. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, pp. 8–48.

Olcese, L.E., Toselli, B.M., 2002. Simple modelling approaches to assess the impact of traffic air pollution: Córdoba case study. Int. J. Environ. Pollut. 22, 396–408.

Pfeiffer, H.N., Barclay-Estrup, P., 1992. The use of a single lichen species, *Hypogimnia physodes*, as an indicator of air quality in Northwestern Ontario. Bryologist 95, 3–41.

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

Pignata, M.L., González, C.M., Wannaz, E.D., Carreras, H.A., Gudiño, G.L., Martinez, M.S., 2004. Biomonitoring of air quality employing in situ *Ramalina celastri* in Argentina. Int. J. Environ. Pollut. 22, 409–429.

Pignata, M.L., Plá, R.R., Jasan, R.C., Martínez, M.S., Rodríguez, J.H., Wannaz, E.D., Gudiño, G.L., Carreras, H.A., González, C.M., 2007. Distribution of atmospheric trace elements and assessment of air quality in Argentina employing the lichen *Ramalina celastri* as a passive biomonitor: detection of air pollution emission sources. Int. J. Environ. Health 1, 29–46.

Ramírez Sosa, A.M., Alé, V. (Eds.), 1997. Informes Departamentales: Río Primero. Ministerio de la Provincia de Córdoba, Córdoba, p. 47.

Rodriguez, J.H., Carreras, H.A., Pignata, M.L., González, C.M., 2007. Nickel exposure enhances the susceptibility of lichens *Usnea amblyoclada* and *Ramalina celastri* to urban atmospheric pollutants. Arch. Environ. Contam. Toxicol. 53, 533–540.

Salemaa, M., Derome, J., Helmisaari, H.-S., Nieminen, T., Vanha-Majamaa, I., 2004. Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulphur deposition in Finland. Sci. Total Environ. 324, 141–160.

Sczepaniak, K., Biziuk, M., 2003. Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. Environ. Res. 93, 221–230.

Servicio Metereológico Nacional. Ministerio de Defensa, Secretaria de Planeamiento. URL 〈http://www.smn.gov.ar〉.

Stein, A.F., Toselli, B.M., 1996. Street level air pollution in Córdoba City, Argentina. Atmos. Environ. 30, 3491–3495.

Tretiach, M., Adamo, P., Bargagli, R., Baruffo, L., Carletti, L., Crisafulli, P., Giordano, S., Modenesi, P., Orlando, S., Pittao, E., 2007. Lichen and moss bags as monitoring devices in urban areas. Part I: Influence of exposure on sample vitality. Environ. Pollut. 146, 380–391.

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

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.

Wolterbeek, B., 2002. Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Environ. Pollut. 120, 11–21.