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Assessment of human health risk related to metals by the use of biomonitors in the province of Córdoba, Argentina

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Metal accumulation in epiphytes is correlated with human respiratory diseases.

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ABSTRACT

The evaluation of metal contents in the environment is of vital importance for the assessment of human exposure. Thus the species *Usnea amblyoclada*, *Ramalina celastri* and *Tillandsia capillaris* were tested as bioaccumulators of transition metals in the urban area of Córdoba city, Argentina. The level of metals on biomonitors was compared to that of total deposition samples. All three species discriminated zones within the urban area of Córdoba city with different pollution levels; they revealed high levels of Zn in the downtown area and confirmed high levels of some transition metals in an industrial area. The correlation analysis revealed that the lichen *R. celastri* had the highest correlation rates with total deposition samples, suggesting it is a valuable biomonitor of atmospheric pollution. A significant relationship was also observed between respiratory diseases in children and the contents of metal accumulated in *R. celastri* and *T. capillaris*, indicating their usefulness when assessing human exposure to metals.

1. Introduction

Metals are classified among the most dangerous groups of anthropogenic environmental pollutants due to their toxicity and persistence in the environment. Consequently, the evaluation of the levels of metal deposition is of vital importance for the assessment of human exposure.

Lichens were recognized as potential indicators of air pollution as early as the 1860s in Europe and elsewhere (Freitas, 1985; Freitas et al., 1999; Gao, 1988; Garty et al., 1988; Garty, 1993; Nyangababo, 1987). Since then, lichens are being increasingly used in air pollution studies because they are largely dependent on the environment to which they are exposed for the uptake of chemical substances. Besides, they can accumulate and retain many trace elements in concentrations that vastly exceed their physiological requirements due to their relatively large surface area and slow growth (Markert et al., 1996) and they can also tolerate these high concentrations by sequestering elements extracellularly as oxalate crystals or lichen acid complexes (Nieboer et al., 1978). Therefore, they have been widely used as bioindicators of class B or borderline metals and trace elements in national and regional surveys (Brooks, 1995; Garty, 2000), either by sampling of the organism in situ or by using the transplantation technique.

Regarding the bioindicating capacity of 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, 1981). In Argentina there are many species of *Tillandsia*, among which *Tillandsia capillaris* has been studied as biomonitor of atmospheric quality in the province of Córdoba (Pignata et al., 2002) and *Tillandsia permutata*, *Tillandsia tricholepis*, *Tillandsia retorta* have been used as passive and active biomonitors (Wannaz et al., 2006, 2008; Wannaz and Pignata, 2006).

Urban air pollution is a well-known phenomenon; however, in the last few years special care was devoted to suspended particulate matter due to the fact that evidence is consistent in showing adverse health effects from exposures that are currently experienced by urban populations in both developed and developing countries. Long-term exposure to particulate matter air pollution has been associated with increased mortality from respiratory and cardiovascular diseases and from lung cancer (Pope et al., 2002). Besides, evidence suggests that no threshold levels have been identified below which adverse health effects do not occur (Health Effects Institute, 2004). Particulate matter may include a broad range of chemical species. Particularly, all kind of metals and carbon

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compounds, indicators of anthropogenic production processes, are found in association to fine particles (Bergamaschi et al., 2007).

Since the association between air pollutants and acute and chronic health effects has been proved, many biomonitoring surveys have been performed throughout the world because of their low cost and ability to perform high density sampling allowing the measurement of a wide range of pollutants (Wappelhorst et al., 2002; Sarmento et al., 2007). However, since most of these studies were conducted in the developed countries, there is still a need for studies in cities from developing countries, where characteristics of air particulate matter, meteorological conditions, and socio-demographic status of local residents may be different from those of developed countries.

The aims of the present paper are to compare the capacity of three epiphytic species to accumulate some transition metals (class B and borderline) and to assess their performance as sentinels of human exposure to metals by correlating them with some health indicators.

2. Methodology

2.1. Study area

The present study was undertaken in the city of Córdoba, located in the centre of Argentina (31°24′S, 64°11′W) at an altitude of approximately 400 m above sea level (Fig. 1). It has a population of 1.3 million and an irregular topography. Its general structure is funnel-shaped, with an increasing positive slope from the centre towards the surrounding areas. This somewhat concave formation reduces the air circulation and causes frequent thermal inversions both in autumn and winter. The climate is sub-humid, with an average annual rainfall of 790 mm, concentrated mainly in summer. The mean annual temperature is 17.4 °C and the prevailing winds come from the NE, S and SE.

The main sources of air pollution in the downtown area of Córdoba city are automobile sources (Stein and Toselli, 1996). The traffic pattern holds a strong relationship with all the primary pollutants measured (CO, NO_x and PM10) which is another reason further justifying the assumption that the pollution in the city is mainly due to automobile sources (Olcese and Toselli, 2002). It is important to notice that the use of leaded gasoline in Argentina has now been reduced for over a decade; however, elevated levels of lead are still measured associated to soils near main roads (Pignata et al., 2007). Córdoba city also has an important industrial development of mainly metallurgic and mechanical industries which are located in surrounding areas.

2.2. Biological material, plant transplantation and total deposition samples

All biological material was collected from unpolluted sites. *Usnea amblyoclada* (Müll. Arg.) Zahlbr was collected from Los Gigantes, 70 km West of Córdoba city; *Ramalina celastri* (Spreng.) Krog & Swinsc and *T. capillaris* Ruíz & Pav. f. *capillaris* were collected from a natural reserve located 40 km to the North of the city.

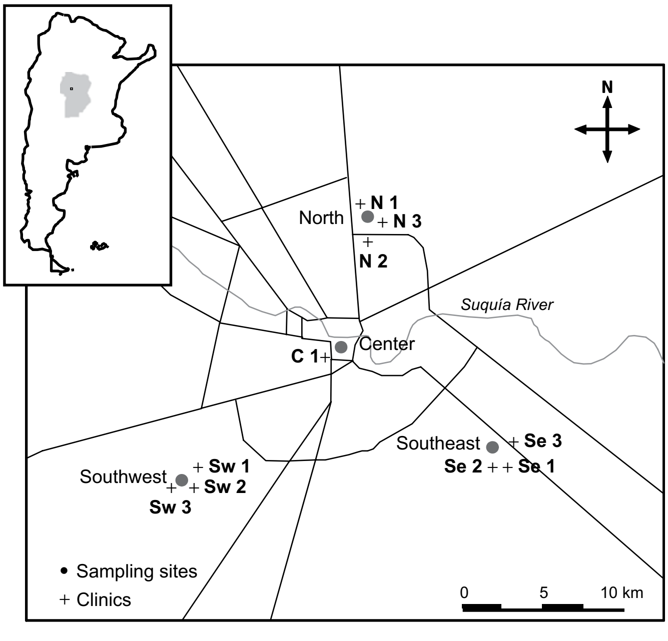

Fig. 1. Location of the sampling sites (●) and clinics (+) in Córdoba, Argentina.

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 to a nylon rope on posts 3 m above the ground in each sampling area. *Tillandsia* bags containing 200 g plant material were prepared according to González and Pignata (1994); six bags were hung at each site.

Transplantation started in June 2002. This period corresponds to the end of autumn in which the lowest rainfall values and temperatures are recorded. Six months later (December) the bags were collected and taken to the laboratory. Three assays were taken from each sample for all the determinations.

In order to establish the initial state of the samples before transplantation a basal sample from the original collection site was analysed for each species. These samples were then used as controls to compare the transplanted samples.

For the evaluation of the deposit flux, suitable experimental devices were adapted from those described previously (Hewitt and Rashed, 1991) in order to collect wet and dry depositions. The device consisted of a polypropylene bottle with a funnel (20 cm diameter) that was located 3 m above ground level so as to avoid soil interference and was covered with a PVC laminated net to prevent the filtering of insects or leaves. The devices were hung where the plant material was transplanted and were collected 6 months later coinciding with the collection of the plant samples. Before exposing the devices they were washed with an acidified solution (HNO_3, 5% V/V).

2.3. Description of the transplantation areas

Four areas in the city of Córdoba were selected for transplantation (Fig. 1), two of them corresponded to highly contaminated places: one by traffic (Centre) and the other by industrial activities (Southeast). The other two sites corresponded to residential areas where high levels of atmospheric contamination are not expected. A detailed description of the sampling areas is presented in Wannaz and Pignata (2006).

In each sampling area only the clinics located over a range of 1.5 km from the sampling point were selected for the present study.

2.4. Elemental analysis of the plant samples

The concentrations of Mn, Fe, Co, Ni, Cu, Zn and Pb were analysed in thalli of *U. amblyoclada*, *R. celastri* as well as in leaves of *T. capillaris*.

Lichen samples (0.5 g dry weight) and *T. capillaris* samples (3 g dry weight) were ground and reduced to ashes at 500 °C for 4 h. The ashes were digested with HCl (18%):HNO_3 (3:1), the solid residue was separated by centrifugation and the volume was adjusted to 25 mL with Milli-Q water. After this, 10 ppm of a Ge solution was added to be used as internal standard. Aliquots of 5 μL were taken from this solution and dried on an acrylic support. As a quality control, blanks and samples of the standard reference material "Hay IAEA-V-10" were prepared in the same way (Table 1). Standard solutions with known concentrations of different elements and Ge as an internal standard were prepared for the calibration of the system.

The samples were measured for 200 s, using the total reflection set up mounted at the X-ray fluorescence beamline of the National Synchrotron Light Laboratory (LNLS), in Campinas, Brazil. For the excitation, a polychromatic beam approximately 0.3 mm wide and 2 mm high was used. For the X-ray detection, a Si(Li) detector with an energy resolution of 165 eV at 5.9 keV was used. Results are expressed as μg/g dry weight.

2.5. Elemental analysis of the total deposition samples

The total deposition samples (wet plus dry) of each corresponding area were evaporated until dry and the residue was heated in an oven at 500 °C for 4 h. The ashes were treated in the same way as those of the plant samples in order to prepare the metallic acid solutions to be analysed by TRXF. Results are expressed as μg/m^2. These data were already presented in Wannaz and Pignata (2006).

Table 1
Analysis of the reference material Hay IAEA-V-10 by X-ray fluorescence with total reflection

	Recommended value (95% C.I.)	Recovered value
Fe	186 (177–190)	182
Mn	47 (44–51)	49
Co	0.13 (0.11–0.14)	0.15
Ni	4.2 (3.8–4.9)	3.5
Cu	9.4 (8.8–9.7)	8.7
Zn	24 (23–25)	22
Pb	1.6 (0.8–1.9)	0.87

Values are expressed in mg kg^{-1} of dry mass.

Table 2
Mean values (±S.D.) of metal concentrations measured in *Usnea amblyoclada*, baseline and transplanted samples in Córdoba city, Argentina

	Baseline	Mean ± SD				ANOVA (*p*)
		Centre	Southeast	North	Southwest	
Mn	35.45 ± 8.48					
6 Months		40.25 ± 8.97b	46.99 ± 2.71b	63.82 ± 13.18a	39.78 ± 5.40b	<0.01
Fe	226.1 ± 10.7					
6 Months		527.9 ± 38.2b	919.2 ± 352.6a	401.4 ± 31.31b	452.1 ± 102.7b	<0.05
Co	0.072 ± 0.004					
6 Months		0.177 ± 0.095a	0.238 ± 0.139a	0.065 ± 0.021b	0.071 ± 0.002b	<0.05
Ni	1.05 ± 0.18					
6 Months		1.94 ± 0.32b	2.67 ± 0.37a	2.62 ± 0.10a	1.73 ± 0.15b	<0.01
Cu	4.26 ± 0.56					
6 Months		6.80 ± 1.13bc	11.16 ± 3.26a	5.40 ± 1.14c	9.49 ± 1.56ab	<0.05
Zn	42.30 ± 15.46					
6 Months		182.5 ± 77.2a	46.50 ± 11.55b	29.51 ± 6.81b	42.50 ± 9.14b	<0.001
Pb	3.28 ± 5.36					
6 Months		14.82 ± 1.23a	13.69 ± 3.96ab	10.04 ± 3.85bc	9.71 ± 1.03c	<0.05

Results of the ANOVA between the different sampling areas. Mean values on each row followed by the same letter do not differ significantly ($p < 0.05$).

2.6. Health data

The city of Córdoba has two systems of medical care – public and private. We decided to use the public one since its data are representative of subjects that live near the clinics, that is, they should be mostly affected by the local conditions of air pollution. Besides, only data from children aged 6 years old were analysed since they are not affected by work-related pollutants that may act as confusing factors.

The number of children that attended the clinics located in the near surroundings (1.5 km) of the sampling points, during the period January–December 2002, was obtained from the recorded database of municipal health services. The population under study comprised a total number of 17,652 children – 51.8% male and 48.2% female. The frequency of some respiratory diseases (pharyngitis; tonsillitis; asthma; laryngitis; allergic rhinitis) in children was determined with respect to the total number of patients that attended each sanitary district. These frequencies were employed to build up a mean for each sampling area. To determine probable connections between total deposition and human health, the incidence of diseases was correlated to the concentration of transition metals in the biomonitors.

2.7. Statistical analysis

The significance of differences was checked by a one-way analysis of variance (ANOVA). Whenever ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means by least significant difference (LSD) test was performed. The relationship between data was analysed for Pearson's correlation. Analysis was performed with SPSS version 7.5.

3. Results and discussion

3.1. Metal content in the total deposition samples

Wannaz and Pignata (2006) reported significantly high deposition values of Zn in the city centre, which was related to vehicular traffic, since it is the main source of air pollutants in this area (Stein and Toselli, 1996). Other authors also mentioned the vehicular traffic as a source of Zn (Caussy et al., 2003; Viard et al., 2004). Monaci et al. (2000) also emphasized that Ba and Zn are the new tracers of vehicle emissions instead of Pb. On the other hand, high levels of Mn, Fe, Co, Ni, Cu and Pb were measured in the industrial area and can be associated to the emission of industrial sources.

3.2. Metal content in biomonitors

All three biomonitor species employed indicated that following exposure, accumulation or severe accumulation of all elements occurred. This is particularly true for Pb which showed severe accumulation in *R. celastri*.

Table 2 shows metal values measured in the lichen *U. amblyoclada* exposed in each sampling area and in baseline material. Higher values of Fe, Co, Cu, Ni and Pb were observed in lichens exposed in the Southeast area. The samples exposed in the North area had a significantly higher content of Mn, which could be tracer of both eolic dust particles as well as vehicular traffic, since this element has recently been used as a substitute for Pb in additives (Ardeleanu et al., 1999). The highest value of Zn was measured in lichens exposed in the centre area while higher values of Pb were observed in both, the centre and Southeast areas. As it is widely known, the main emission source of Pb into the earth's atmosphere was the combustion of gasoline. Therefore, despite its phasing-out

Table 3
Mean values (±S.D.) of metal concentrations measured in *Ramalina celastri*, baseline and transplanted samples in Córdoba city, Argentina

	Baseline	Mean ± SD				ANOVA (*p*)
		Centre	Southeast	North	Southwest	
Mn	25.80 ± 6.75					
6 Months		36.53 ± 13.98b	212.9 ± 19.8a	56.42 ± 18.89b	43.59 ± 6.98b	<0.001
Fe	887 ± 192					
6 Months		1310 ± 692b	12,246 ± 1420a	1081 ± 203b	1124 ± 251b	<0.001
Co	0.30 ± 0.09					
6 Months		0.68 ± 0.42b	4.50 ± 1.54a	0.55 ± 0.21b	0.70 ± 0.42b	<0.05
Ni	1.50 ± 0.66					
6 Months		1.80 ± 0.01d	11.71 ± 1.58a	2.01 ± 0.38c	2.10 ± 0.45b	<0.001
Cu	11.32 ± 3.30					
6 Months		15.60 ± 4.43b	175.5 ± 35.6a	10.41 ± 2.01b	15.33 ± 3.59b	<0.001
Zn	43.99 ± 20.27					
6 Months		303.4 ± 80.8a	260.3 ± 13.9a	114.2 ± 22.1b	40.2 ± 15.6c	<0.001
Pb	2.13 ± 0.75					
6 Months		3.23 ± 0.50b	46.88 ± 12.43a	3.73 ± 0.24b	4.38 ± 0.76b	<0.001

Results of the ANOVA between the different sampling areas. Mean values on each row followed by the same letter do not differ significantly ($p < 0.05$).

Table 4
Mean values (±S.D.) of metal concentrations measured in *Tillandsia capillaris*, baseline and transplanted samples in Córdoba city, Argentina

	Baseline	Mean ± SD				ANOVA (*p*)
		Centre	Southeast	North	Southwest	
Mn	64.02 ± 1.30					
6 Months		107.41 ± 9.89b	166.7 ± 10.4a	93.50 ± 3.23c	58.88 ± 7.99d	<0.001
Fe	929.0 ± 182.1					
6 Months		1852 ± 186b	3790 ± 281a	1812 ± 288b	1193 ± 66c	<0.001
Co	0.408 ± 0.163					
6 Months		0.707 ± 0.094b	1.42 ± 0.174a	0.599 ± 0.061b	0.457 ± 0.03c	<0.001
Ni	1.04 ± 0.11					
6 Months		1.73 ± 0.64b	3.52 ± 0.26a	1.36 ± 0.17c	0.98 ± 0.19d	<0.001
Cu	4.89 ± 0.67					
6 Months		9.26 ± 0.14b	64.08 ± 4.75a	7.91 ± 1.10bc	4.86 ± 0.43c	<0.001
Zn	14.87 ± 0.59					
6 Months		36.61 ± 1.51b	92.60 ± 8.72a	32.71 ± 6.67b	15.51 ± 1.41c	<0.001
Pb	3.18 ± 0.93					
6 Months		9.00 ± 0.79b	27.33 ± 0.45a	7.57 ± 0.52c	3.33 ± 1.12d	<0.001

Results of the ANOVA between the different sampling areas. Mean values on each row followed by the same letter do not differ significantly ($p < 0.05$).

and the observed decreasing trend in the atmosphere, Pb remains a significant urban air pollutant in developing countries.

In the lichen *R. celastri*, significant higher values of all elements were measured in samples transplanted to the Southeast area (Table 3). As it was observed in *U. amblyoclada*, the levels of Zn were also higher in the centre of town. Previous studies that used *R. celastri* as a passive biomonitor in a large area in the province of Córdoba (Pignata et al., 2004, 2007) showed concentrations lower than the ones found in the present study.

The presence of transition metals in *T. capillaris* was already reported in Wannaz and Pignata (2006); however, to allow the comparison with lichen species in the present paper the results are expressed in μg/g DW. It was observed that the accumulation of metals in *T. capillaris* (Table 4) was similar to the one observed in *R. celastri*, with significantly higher values of all elements in samples exposed in the Southeast area. This species has been assessed several times before as biomonitor of mining activities (Wannaz et al., 2008), industrial activities (Pignata et al., 2002; Wannaz et al., 2006) and vehicular emissions (Wannaz et al., 2006). Moreover, in a previous study it was proved to be a better bioaccumulator than other three species from the same *Tillandsia* genus (Wannaz and Pignata, 2006).

The results obtained suggest that both lichens species as well as *Tillandsia* species can be successfully used to monitor air pollution. However, several other factors should be considered before taking a decision on the preferred biomonitor species, such as background elemental concentration, selective uptake or detoxificant mechanisms.

3.3. *Correlation between atmospheric total deposition and metal content in the biomonitors*

In order to compare the ability of these epiphytic species to accumulate some transition metals, exposed to control ratios (EC ratios) were calculated for each species according to Frati et al. (2005), since some of them showed significant variations in the concentration of elements in baseline material. Bergamaschi et al. (2007) proposed that the calculation of EC ratios allows for the correction of the differential initial concentration of elements, which may be influenced by their morphology and natural occurrence of soil dust. According to this ratio, *R. celastri* was the species with the highest accumulation capacity while *U. amblyoclada* had the lowest one (Table 5). Several studies revealed that lichens may selectively accumulate extracellular elements and metabolize or eliminate those elements that enter the cell wall (Branquinho et al., 1997; Chettri et al., 1997). The calculation of EC ratios also evidenced this ability of lichens species.

Thus, in *U. amblyoclada* the highest EC ratio corresponded to Pb which can be related to a selective cation uptake as was informed previously by Carreras et al. (2005). The authors attributed this finding to a greater affinity between Pb cations and the lichen cell wall exchange sites that are probably strongly attached to binding sites, forming stable complexes.

In *R. celastri* the highest EC ratio was observed for Pb, too. In general, the ratios obtained in the present study are higher than the ones obtained by Cercasov et al. (2002) in a biomonitoring performed with *Ramalina farinacea* exposed in an urban–industrial area in Padua (Italy) and the ones informed by Garty et al., 2003 in a study performed with *Ramalina lacera* around a coal-fired power station in Israel.

Regarding *T. capillaris* the highest EC ratio corresponded to Cu and Pb. Similarly, Brighigna et al. (1997) found higher content of Pb and Cu in different *Tillandsia* species exposed at urban sites.

The correlations between the elements accumulated on biomonitors and the composition of total deposition samples are presented in Table 6. A good positive correlation between all the species and total deposition samples for Fe, Cu and Pb was observed. The content of Co in total deposition samples correlated with that measured in *U. amblyoclada* and *T. capillaries* and the Ni content correlated with the one measured in *R. celastri* and *T. capillaries*. Regarding Zn, both lichen species showed a significant correlation with total deposition. Finally, *R. celastri* was the only

Table 5
Enrichment factor (exposed/control) ratios of the transition metals quantified in the three species of biomonitors

	Usnea amblyoclada					*Ramalina celastri*					*Tillandsia capillaris*				
	Centre	Southeast	North	Southwest	Mean EC	Centre	Southeast	North	Southwest	Mean EC	Centre	Southeast	North	Southwest	Mean EC
Mn	1.14	1.33	1.8	1.12	1.35	1.42	8.25	2.19	1.67	3.38	1.67	2.6	1.46	0.92	1.66
Fe	2.33	4.07	1.76	2	2.54	1.48	13.81	1.22	1.27	4.45	1.99	4.07	1.95	1.28	2.32
Co	2.46	3.31	0.9	0.99	1.92	2.29	15.15	1.85	2.36	5.41	1.73	3.48	1.47	1.12	1.95
Ni	1.85	2.54	2.5	1.65	2.14	1.2	7.81	1.34	1.4	2.94	1.66	3.38	1.3	0.94	1.82
Cu	1.6	2.62	1.27	2.23	1.93	1.38	15.5	0.92	1.35	4.79	1.89	13.1	1.62	0.99	4.4
Zn	4.31	1.1	0.7	1	1.78	6.9	5.92	2.6	0.91	4.08	2.46	6.22	2.2	1.04	2.98
Pb	4.5	4.17	3.06	2.96	3.68	1.52	22.01	1.75	1.75	6.76	2.83	8.59	2.38	1.05	3.71

Table 6
Correlation analysis between the metal content of the total deposition samples and the metal content measured in the biomonitors

Total deposition	*U. amblyoclada*	*R. celastri*	*T. capillaris*
Mn	ns	0.87***	ns
Fe	0.79***	0.99***	0.94***
Co	0.74***	ns	0.75***
Ni	ns	0.83***	0.90***
Cu	0.56*	0.92***	0.93***
Zn	0.93***	0.66*	ns
Pb	0.55*	0.92***	0.98***

ns, Not significant; significant with *$p < 0.05$; ***$p < 0.001$.

species that showed a significant relationship for Mn. The fact that *R. celastri* showed significant correlations with almost all elements measured in total deposition samples suggests that this species is the one that better reflects the metal concentrations found in the environment.

Although a quantitative relationship between biomonitors and total deposition samples faces some difficulties, the results obtained indicate that it is possible to draw important conclusions about air quality using lichens and particularly *R. celastri* as qualitative indicator.

3.4. Correlation analysis between atmospheric deposition and frequency of diseases

The fact that a good correlation was observed between the total deposition samples and one of the species employed as biomonitor suggests that the information obtained using biomonitors could be employed to study human exposition risk (Fuga et al., 2007). Some of the transition metals measured in the present study are essential to human biological function, such as Mn and Cu; however, they can be toxic when ingested or inhaled at elevated concentrations. It was demonstrated that an increased copper content can lead to coronary diseases, arteriosclerosis and damage to the central nervous system (Goyer, 1997). Some others, like Pb, are toxic and have shown to negatively impact the neurological development of children (Wasserman et al., 2004). Other metals can remain in the body for years and their association with health impacts is unknown.

In the city of Córdoba it was possible to obtain diseases data disaggregated by sanitary districts (Table 7). Thus, a correlation analysis was performed to explore the relationship between the incidence of various respiratory health outcomes, the composition of total deposition samples and the transition metals accumulated in the biomonitors (Table 8). This analysis was made with the total deposition samples and accumulation on biomonitors corresponding to each study area, and the percentages of diseased children obtained from the clinics located over a range of 1.5 km from the sampling point where the biomonitors were exposed. In *R. celastri* and *T. capillaris* significant associations for almost all the elements were measured with all the diseases analysed. On the contrary, in *U. amblyoclada* significant associations were observed only for the content of Co, Cu and Fe accumulated in thalli. The significant correlations found with the lichen and not with total deposition samples may be due to the high accumulation capacity of this species, as determined with the EC values.

Table 7
Percentage of diseased children aged under six, calculated on the basis of the total number of people treated at clinics situated in each study area, for each of the respiratory diseases selected

	Centre (%)	Southeast (%)	North (%)	Southwest (%)
Pharyngitis	0.026	0.317	0.089	0.222
Tonsillitis	2.484	3.762	1.363	2.496
Asthma	0.554	1.072	0.210	0.656
Laryngitis	0.211	0.267	0.144	0.187
Allergic rhinitis	0.000	0.019	0.000	0.009

Table 8
Correlation analysis between the percentage of diseased children under six years old and the metal content in total deposition samples or biomonitors

	Total deposition	*U. amblyoclada*	*R. celastri*	*T. capillaris*
Pharyngitis	Mn***; Fe***	Fe*; Cu***	Mn***; Fe***; Co*; Ni***; Cu***; Pb***	Fe*; Co*; Ni*; Cu**; Zn*; Pb*
Tonsillitis	Mn***; Fe***; Co**; Ni***; Cu***; Pb***	Fe**; Co*; Cu***	Mn***; Fe***; Co**; Ni***; Cu***; Pb***	Mn**; Fe**; Co***; Ni***; Cu***; Zn***; Pb***
Asthma	Mn***; Fe***; Co*; Ni***; Cu***; Pb***	Fe**; Co*; Cu***	Mn***; Fe***; Co**; Ni***; Cu***; Pb***	Mn*; Fe**; Co***; Ni***; Cu***; Zn**; Pb***
Laryngitis	Mn***; Fe***; Co***; Ni***; Cu***; Pb***	Fe***;Co**; Cu*	Mn***; Fe***; Co**; Ni***; Cu***; Pb***; Zn*	Mn***; Fe***; Co***; Ni***; Cu***; Zn***; Pb***
Allergic rhinitis	Mn***; Fe***; Ni*; Cu**; Pb**	Fe**; Cu***	Mn***; Fe***; Co***; Ni***; Cu***; Pb***	Fe**; Cu**; Ni**; Cu***; Zn**; Pb**

Only the positive significant correlations are shown.
Significant with *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Despite the fact that some of these associations may be randomly allocated (e.g. we cannot explain the correlations between Co and diseases in *R. celastri*, because the content of Co in thalli did nor correlate with the content of Co measured in deposition samples), the fact that the correlation pattern included all the elements from total deposition samples that showed significant associations with diseases suggests the suitability of this biomonitor to study the impact of metals on human health.

Associating a specific environmental contaminant with a specific health outcome is difficult, and we do not assume the transition metals measured in this study are the cause of the diseases analysed. While direct causation cannot be established from chemical data alone, information on metal concentrations in the atmosphere may help to identify whether unfavorable environmental conditions exist.

4. Conclusion

The combination of biomonitoring and total deposition samples allows for the assessment of air quality at various locations. Although their relationship may be difficult to measure, it was possible to draw important conclusions regarding the bioindicators that best reflect the concentration of some transition metals present in the environment.

On the other hand, this is the first study comparing the level of air pollution, determined by biomonitoring, with the incidence of various types of diseases, in Argentina. The significant correlations found between metal concentrations in lichens and the incidence of some human health outcomes only provide indications as to the probable causes of the disease but never a definite connection. Although we are aware of the difficulties arising on observational data because of the possibility of bias caused by extraneous variables, the exploratory analyses presented here highlight the possibility offered by biomonitoring methods in measuring a wide range of pollutants that may be harmful for human health.

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