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Distribution of atmospheric trace elements and assesment of air quality in Argentina employing the lichen, *Ramalina celastri*, as a passive biomonitor: detection of air pollution emission sources

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Abstract: *Ramalina celastri* was used as a passive biomonitor to study the relationship between elemental accumulation, emission sources and physiochemical parameters used as air pollution biomarkers in Argentina. The concentration of 27 elements was determined in the thalli by Neutron Activation Analysis (NAA). The content of photosynthetic pigments, peroxidation products, water and sulphur was determined and a Pollution Index (PI) was calculated. Factor analysis was used to locate the possible emission sources of elements. Overall, the elemental concentrations were similar to other biomonitoring studies. The higher levels of arsenic were related to the soil particulate matter, which is characteristically rich in this element. High uranium concentrations were found near a uranium mine and elevated levels of zinc were found in areas congested with heavy traffic. Although there is no direct relationship found between the physiological parameters and the elemental concentrations, the geographical distribution of the PI allowed to detect areas with increased lichen damage.

Keywords: arsenic; biomonitoring; lichen; neutron activation analysis; pollution index; uranium; zinc.

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1 Introduction

Lichens have been used in biomonitoring studies of trace elements deposition in urban and industrial areas for decades. Lichens have certain characteristics that make them ideal biomonitoring organisms, among which we can outline the following: they are perennial species; they do not possess roots or other special organs for the uptake of nutrients; they lack a cuticle and they have a worldwide distribution. This is the reason why they have been defined as 'permanent control systems' for the assessment of air pollution (Sloof, 1995). Although lichens are recognised as effective air quality biomonitors (Kauppi and Halonen, 1992; Garty, Kauppi and Kauppi, 1996; González, Casanovas and Pignata, 1996), not all species are equally sensitive to the different contaminants. Therefore, if the response of a specific lichen species to pollutants is properly characterised and calibrated, it can then be considered a biomonitor (Markert, 1993).

Regarding heavy metal biomonitoring, lichens operate as ion exchange resins, absorbing metal ions into the extracellular matrix and consequently releasing H^+ ions or other low binding affinity metal ions (Richardson and Nieboer, 1981; Richardson, 1992). These features are highly relevant when deciding upon the use of lichens for biomonitoring airborne metal pollutants (Garty, 2001) and several studies have been performed concerning the accumulation of these metals in many different lichen species (Monaci, Bargagli and Gasparo, 1997; Scerbo et al., 1999; Loppi, Ivanov and Boccardi, 2002). Unlike the process of extracellular uptake, intracellular uptake is a slow process that increases with time and levels of exposure. Intracellular uptake is species-dependent and reflects the availability of intracellular functional groups with the metal-binding capabilities (Garty, 2001).

The accumulation of the metals in epiphytic organisms not only depends on their characteristics but also depends on the atmospheric availability of the elements. It is therefore fairly difficult to calibrate sampling methods that will provide small samples of the lichen population, with similar average composition. However, calibration studies are very important for both economic and practical reasons, as biomonitoring is a highly appropriate method for establishing wide regional monitoring systems. In addition, the atmospheric deposition of heavy metals from different sources is variable in both time and space, making it very difficult and expensive to obtain the detailed information from large areas using instrumental monitoring (Genoni, Parco and Santagostino, 2000).

Biomonitoring studies assume that the concentration of heavy metals found in the lichens reflect the proportion of those elements in the environment (Chettri et al., 1997). However, lichens react not only to a single pollutant but also to a complex mixture of contaminants that influence its accumulation and physiological responses (Carreras et al., 2005). *Ramalina celastri* is one of the most common lichen species in the province of Córdoba (central Argentina) and its physiological response has been successfully used for biomonitoring the air quality in several transplantation studies (Levin and Pignata, 1995; González, Casanovas and Pignata, 1996; Gonzalez et al., 1998; González and Pignata, 1999; González, Pignata and Orellana, 2003). Furthermore, in a previous study on passive biomonitoring, physiological damage was detected in *R. celastri* and was attributed to anthropogenic activities related to urban industrial emissions and pesticides (Pignata et al., 2004). Hence, the objectives of the present research are to establish the origin of the elements accumulated in *R. celastri* thalli, examine the relationships between the accumulation of trace elements and the physiological response in this lichen and detect the areas of poor atmospheric quality in Córdoba, Argentina.

2 Materials and methods

2.1 Study area and sampling procedure

The 50,000 km^2 study area was located in the central region of Argentina and defined by a quadrilateral with the following coordinates: to the West, 31º 25′ 21″ S, 65º 24′ W; to the East, 31º 41′ 15″ S, 62º 38′ 34″ W; to the north, 30º 36′ S, 63º 15′ W; and to the south, 32º 52′ S, 64º 10′ 12″ W. The morphology of the land is highly variable, ranging from a mean altitude of about 250 m in the southeast to more than 2,500 m in the mid-west. There are cities (large and medium-sized) and many small villages in the area. Most of the industrial plants (mainly metallurgical, petrochemical or chemical) and factories (food, vegetable oil and cement) are located in the centre and south of the study area, which is also the region with the highest records of population density.

For sampling purposes, the study area was divided into squares, each one measuring 25 by 25 km (80 sampling sites in all). Samples of *R. celastri* (Spreng.) Krog. and Swinsc. were collected at each intersection point whenever present. Altogether , a total of 61 sites were sampled; the collection sites were located at least 500 m away from main roads and densely populated areas and at least 300 m from streets with lower traffic density (Figure 1).

Each sample consisted of 15–20 individuals, randomly collected along the four cardinal directions within a 100 × 100 m area and no further than 100 m from the geographically referenced point. Only if it had not rained in the area for the previous 5 days, the samples were collected with plastic gloves, which were used to avoid any risk of sample contamination (Sloof, 1993).

Back at the laboratory, part of the lichen material was separated to determine the water content [Ratio of Dry Weight to Fresh Weight (DW/FW ratio)]. Another fraction of the sample material was stored in plastic vials at −15°C in complete darkness to be used for the physiochemical determinations. The remaining material was prepared for the metal determinations. The chemical determinations were performed in three independent sub-samples taken from each pool of the lichen samples.

2.2 Quantification of the physiochemical parameters

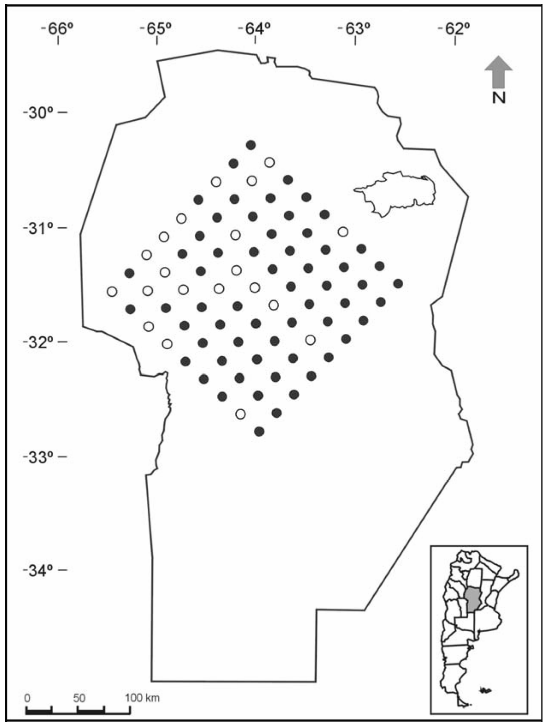
2.2.1 DW/FW ratio

The DW/FW ratio of the samples was determined by drying 1 g of fresh material at 60 ± 2°C until it reaches a constant weight. The results were expressed in g DW g^{-1} FW.

2.2.2 Chlorophyll determination

The concentrations of chlorophyll and phaeophytin were calculated on a dry weight basis (Carreras and Pignata, 2001). The chlorophyll b/chlorophyll a (Chl-b/Chl-a) ratios were also calculated. The results are expressed in mg g^{-1} DW.

Figure 1 Localisation of the study area and the individual sampling points in the survey undertaken in Córdoba, Argentina. ●Sample sites for *R. celastri*. ○Sample sites in which *R. celastri* was not found



2.2.3 Sulphur content

The amount of SO_4^{2-} in the solution was determined by the acidic suspension using $BaCl_2$ (González and Pignata, 1994). The results are expressed in mg of total sulphur g^{-1} DW.

2.2.4 Peroxidation products

The malondialdehyde content (MDA) was measured by a colorimetric method (Heath and Castillo, 1988). The amount of MDA present was calculated using $\varepsilon = 155\ mM^{-1}\ cm^{-1}$ (Kosugi, Jojima and Kikugawa, 1989). Results are expressed in μmol g^{-1} DW.

Hydroperoxy conjugated dienes (HPCD) were determined according to (Levin and Pignata, 1995) and the results were expressed in μmol g^{-1} DW.

2.2.5 Pollution index

A Pollution Index (PI) was calculated according to González, Casanovas and Pignata (1996), using the following equation:

$$PI = [(Phaeoph\text{-}a/Chl\text{-}a) + (S/S_M)]\ [(MDA/MDA_M) + (HPCD/HPCD_M)]$$

where Phaeoph-a is the concentration of phaeophytin-a in mg g^{-1} DW; Chl-a is the concentration of chlorophyll-a in mg g^{-1} DW; S is the content of sulphur in mg g^{-1} DW; MDA is the MDA concentration in µmol g^{-1} DW and HPCD is the HPCD concentration in µmol g^{-1} DW. The subscript 'M' in the denominator indicates the arithmetic mean calculated from all the sampling sites.

2.2.6 Neutron activation analysis

The lichen samples were washed with double distilled water following a standard procedure and left to dry at room temperature in a clean area. The samples were then ground in a mortar with liquid nitrogen and freeze-dried for 24 hours. About 300 mg of freeze-dried material was pelleted and wrapped-up in aluminium foil for irradiation together with two certified reference materials, NIST 1633b Coal Fly Ash and IAEA V-10 Hay Powder or IAEA Lichen 336.

The irradiations were undertaken at the RA-3 reactor (thermal flux 3×10^{13} cm^{-2} s^{-1}, 4.5 MW) of the Atomic Centre of Ezeiza (CNEA – Argentine National Commission of Atomic Energy) for 7 hours. Instrumental NAA was performed by taking two measurements after 6 and 30 days of decay using GeHP detectors (30% efficiency, 1.8 keV resolution for the 1332.5 keV ^{60}Co peak). The concentrations of As, Ba, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Na, Nd, Rb, Sb, Sc, Se, Sm, Ta, Tb, Th, U, Yb and Zn were calculated using software developed at the NAA laboratory. The precision of the method was estimated in 12 sets of three replicates. Control charts (*z*-values) (Bode and Van Dijk, 1997) were used to inspect the normalised concentrations in a series of measurements of one control sample for every element analysed. All the values were within the $|z| \geq 3$ range. NIST SRM 1547 Peach Leaves, 679 Brick Clay and 2709 San Joaquín Soil were used as control samples.

2.3 Data analysis

Descriptive statistics and statistical analyses were based on the mean value of the determinations performed in the three sub-samples from each sampling site. Twenty-eight elements and eight physiological parameters were selected for the factor analysis that was performed using SPSS version 10.0. The factors were generated using the Principal Component Analysis (PCA) and then rotated with Varimax rotation. Keeping the factors with eigenvalues over one, a total of eight factors were chosen that accounted for approximately 85% of the total variance, indicating that the results are statistically consistent. Also, Pearson's coefficients of correlation were calculated to study the relationships between the chemical elements and the physiological parameters.

A mapping procedure was used to evaluate the distribution of the elements that were suspected of having an anthropogenic origin. The Kriging and Contour plot derivation was performed using Surfer 5.00. The Kriging method was used to interpolate the altitudinal values (m.a.s.l.) with the sampling sites. Then, based on the extrapolated values, the Contour plots of the altitudes of the study area were traced.

3 Results and discussion

3.1 Statistics summary

Tables 1 and 2 show the mean, minimum and maximum values and the coefficient of variation of the elements as well as the physiochemical parameters measured in the *R. celastri* samples of the study area (n = 61).

Table 1 Mean (μg g^{-1} DW and mg g^{-1} DW for sulphur), range and Coefficient of Variation (CV) of the elemental concentration in *R. celastri* samples from Córdoba, Argentina (n = 61)

Element	*Mean*	*Range (minimum–maximum)*	*CV*
As	1.823	1.032–3.830	0.374
Ba	14.460	5.251–37.970	0.463
Br	1.885	0.581–3.620	0.377
Ca	3527.8	392.41–18425.3	1.019
Ce	2.531	0.770–4.920	0.409
Co	0.980	0.169–0.980	0.422
Cr	1.514	0.612–2.889	0.370
Cs	0.302	0.090–0.890	0.501
Eu	0.051	0.016–0.120	0.433
Fe	1202.4	397.88–2848.4	0.431
Hf	0.165	0.046–0.370	0.434
K	3225.1	2090.6–8164.3	0.301
La	1.303	0.428–3.39	0.470
Lu	0.018	0.006–0.050	0.489
Na	535.25	175.04–3837.4	1.119
Nd	1.514	0.579–3.340	0.461
Rb	4.879	1.602–8.640	0.358
S	1.318	0.829– 1.941	0.167
Sb	0.080	0.027–0.690	1.139
Sc	0.446	0.140–1.090	0.444
Se	0.219	0.089–0.380	0.320
Sm	0.267	0.267–0.081	0.545
Ta	0.035	0.011–0.090	0.453
Tb	0.040	0.011–0.080	0.392
Th	0.396	0.107–0.850	0.419
U	0.134	0.030–0.790	1.008
Yb	0.108	0.038–0.310	0.510
Zn	63.796	11.825–233.8	0.876

Table 2 Physiochemical parameters measured in *R. celastri* samples from Córdoba, Argentina ($n = 61$)

Chemical parameter	*Mean*	*Range (minimum–maximum)*	*CV*
DW/FW	0.891	0.845–0.930	0.020
Chl-a (mg g^{-1} DW)	1.337	0.711–2.266	0.261
Chl-b (mg g^{-1} DW)	0.414	0.222–0.686	0.275
Phaeoph-a (mg g^{-1} DW)	1.463	0.804–2.422	0.225
MDA (μmol g^{-1} DW)	0.152	0.115–0.211	0.147
HPCD (μmol g^{-1} DW)	9.993	6.165–14.166	0.179
Chl-b/Chl-a	0.314	0.231–0.471	0.151
Phaeoph-a/Chl-a	1.125	0.860–2.123	0.164
PI	4.258	2.679–6.637	0.154

The concentrations of most of the elements analysed in this study were similar to those observed in other countries (Nimis et al., 2000; Zhang et al., 2002; Bergamaschi et al., 2004). However, the levels of As were much higher than the concentrations cited by other authors in non-polluted sites (Zhang et al., 2002; Bergamaschi et al., 2004). The soil and underground water of some regions of Argentina is rich in As (Nicolli et al., 1989; Cabrera, Blarasin and Villalba, 2001; Farías et al., 2003) and this element is related to the presence of basic soils, typical of the Pampa plains. Regarding Na, the maximum values that were registered are higher than the values measured in a previous study using this species in the same area (Jasan et al., 2004). Elevated concentrations of U were also detected in some sites and were similar to the values described for samples of *Hypogymnia physodes* collected near a uranium mine in Slovenia (Jeran, Byrne and Batic, 1995).

The levels of Zn measured in *R. celastri* were higher than the values described for lichens collected in the natural areas of Europe (Bergamaschi et al., 2004) and China (Zhang et al., 2002) and were similar to the levels registered in lichens from highly industrialised and contaminated areas in Italy (Scerbo et al., 1999). It is important to note that the study area, except for the city of Córdoba, has a low population density and scarce industrial development compared to the countries in which similar concentrations of Zn were registered. On the other hand, we can associate these elevated levels of Zn to vehicle emissions as observed by Garty (1993) in Israel, as they were registered in sampling sites near urban areas with scarce industrial development. Christensen and Guinn (1979) suggested that the particles emitted by automobile tyres are the main source of Zn in urban areas. On the other hand, Ward (1989) found that Zn was one of the elements that increased together the density of traffic and suggested that the emission of this element is not only originated by the tyres but also from lubrication oils and brake pads. The physiological parameters and the values for S are similar to those reported for this species in previous studies (González, Casanovas and Pignata, 1996; González and Pignata, 1999).

3.2 Factor analysis

The results of the factor analysis are shown in Table 3. Considering the composition factor, F1 can be assigned to soil. Three factors comprise physiological parameters (F2, F5 and F7); F3 (Ca, As, U) regards elements associated to emission sources as for example: a limestone quarry that is present in the area (Ca), loessic soils (As) and a uranium mine (U); F4 (Zn, Sb, Br) is apparently associated to anthropogenic urban emission sources. The remaining factors include K and Se (F6) and S (F8).

The pattern detected for the first two factors (soil and pigment variation) could be essentially evidencing an adaptive response of the species to the environmental characteristics of the study area.

Factor 3 seems to associate elements with the same emission sources. However, this is not correct because, even though As is originated from loessic soils, it could also be originated by long range of transport of industrial contaminants. Ca is originated in a lime production plant, while the presence of U in this factor is related to the presence of an important uranium resource in this study area. Nonetheless, these sites are geographically close to each other and U and As evidence a similar geographical distribution (Figures 3 and 6).

The main contributor to Factor 4 is Zn, which is possibly related, as mentioned earlier, to vehicular traffic emissions. Br and Sb, also included in this factor, could be originated by the same sources of emission or could be the result of long range of transport (Bergamaschi et al., 2004).

Factor 5 reflects the oxidative damage of the biomonitor and Factor 6 evidences an association between K and Se. As K is a macronutrient, its levels are high in lichens. However, none of these elements was found to be associated to the soil marking elements (F1). Factor 7 is only described by the DW/FW ratio and Factor 8 is defined only by sulphur. The content of sulphur in lichens is generally related to vehicular traffic emission sources (González, Casanovas and Pignata, 1996; Carreras, Gudiño and Pignata, 1998; González et al., 1998; González and Pignata, 1999; Carreras and Pignata, 2002; González, Pignata and Orellana, 2003). Notwithstanding, in the present study S does not appear to be related to any other element and therefore its origin could be completely different to the rest. We think that the elevated level of S in the area is related to bush fires and agricultural practices using sulphur-containing fertilisers and pesticides that are still currently used in these agricultural regions of Argentina.

3.3 Relationship between the physiological parameters and altitude

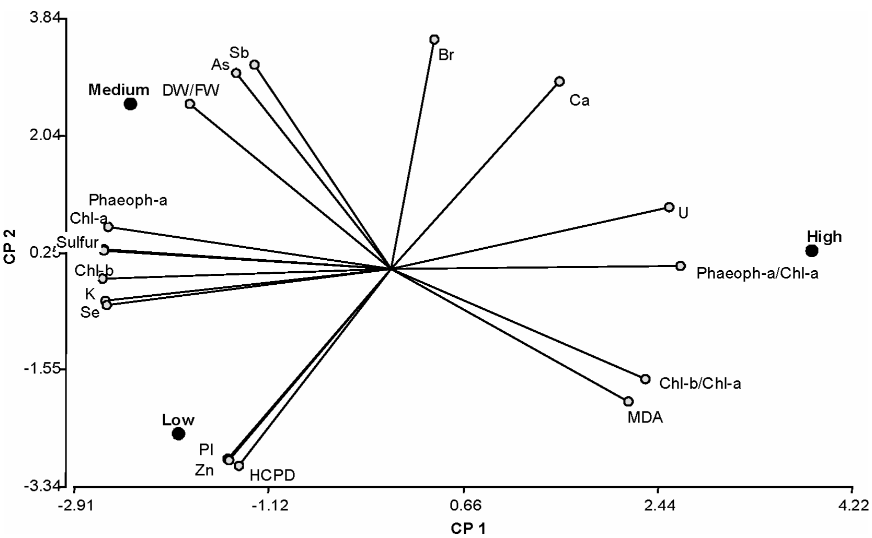
As the study area is characterised by the presence of areas with great altitudinal differences, the degree of association between the chemical parameters measured in *R. celastri* and altitude was evaluated. For this purpose, a PCA was undertaken using the different categories of altitude as classification criteria. Three different altitudinal categories were established in the study area: high, > 900 m.a.s.l.; medium, 450–900 m.a.s.l.; and low, < 450 m.a.s.l. The elements included in the first factor (F1, Table 3) were excluded for this analysis as they are related to soil.

Table 3 Eigenvectors obtained in the factor analysis (principal components) of physiochemical parameters and elements measured in *R. celastri*

	Component							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Sc	**0.953**	0.183	–	–	–	0.149	–	–
Th	**0.952**	–	–	–	–	0.188	–	–
Hf	**0.950**	–	–	–	–	0.203	–	–
Fe	**0.946**	0.188	–	–	–	0.161	–	–
Ce	**0.938**	–	0.219	–	–	0.141	–	–
Eu	**0.925**	0.118	0.230	–	–	–	–	–
Co	**0.917**	0.202	–	–	–	0.198	–	–
La	**0.894**	–	0.328	0.146	–	–	0.162	–
Lu	**0.891**	–	0.284	–	–	–0.116	0.131	0.126
Cr	**0.883**	0.117	–0.121	0.195	–	–	–	0.103
Yb	**0.874**	0.109	0.188	0.103	–	–	0.207	0.156
Ta	**0.869**	–	–	0.137	–	0.161	–0.236	–0.108
Sm	**0.848**	–	0.384	0.112	–	–0.186	0.158	–
Ba	**0.837**	0.256	0.145	0.197	–	–	–	–
Tb	**0.786**	–	0.206	–	–	–	–	–
Cs	**0.779**	–	0.105	0.123	–	0.347	–0.204	–0.238
Nd	**0.745**	0.298	0.212	–0.130	–0.105	–	0.158	0.109
Rb	**0.709**	–	0.427	–	–	0.268	–0.152	–0.273
Na	**0.644**	–	–	0.456	–0.267	0.106	0.125	–0.114
Chl-a	0.224	**0.954**	–	–	–0.110	–	–	–
Chl-b	0.214	**0.891**	–	–	0.297	–	0.148	–
Phaeoph-a	–	**0.845**	–	0.178	–	0.283	0.166	0.183
HPCD	0.156	**0.556**	–0.362	–0.406	–0.222	–	–0.137	–0.242
Phaeoph-a/Chl-a	–0.255	**–0.521**	0.234	0.131	0.170	0.435	0.318	0.176
U	0.103	–0.150	**0.871**	–	–	0.124	–	–
As	0.397	–	**0.720**	0.233	–	–	0.156	0.158
Ca	0.414	0.115	**0.578**	0.366	0.385	–0.134	–	–
Zn	–	–	–	**–0.845**	–0.210	–	–	–
Sb	0.387	0.198	–	**0.597**	–	–0.102	0.324	–
Br	0.165	–	0.364	**0.579**	–0.254	–	0.178	–0.284
MDA	–	–	–0.138	0.129	**0.790**	–	–0.256	–0.113
Chl-b /Chl-a	–	–	0.182	–	**0.789**	0.103	0.367	–0.146
K	0.392	0.215	–	–	–	**0.663**	–	–
Se	0.498	0.276	0.143	–0.107	–	**0.534**	–	–0.309
DW/FW	0.102	0.108	–	0.119	–	–	**0.820**	–0.205
Sulphur	0.121	–	–	–	–0.240	–	–0.244	**0.855**
Total Var (%)	42.60	9.96	7.89	6.31	5.22	4.36	4.35	3.89
Eigenvalues	15.34	3.59	2.84	2.27	1.88	1.57	1.56	1.40

The results of this analysis are presented in a biplot (Figure 2). As can be observed, there was a positive association between Phaeoph-a/Chl-a, Chl-a/Chl-b, MDA and high altitude. The same was observed for U. Low altitude was positively associated with PI, Zn and HPCD; whereas the individual pigments, As, Sb, S, K and Se were associated with medium altitudinal levels.

Figure 2 Variable scores based on the two principal components of PCA for *R. celastri* using three altitudinal levels (low; medium; high) as clasification criterion



The elevated concentrations of U in *R. celastri* associated with high altitudinal areas, as well as its higher coefficient of variation (Table 1), indicates the presence of atmospheric contamination by this element, which could be attributed to the abandoned uranium mine (Schlagintweit Uranium Mine, Los Gigantes, Córdoba, Argentina). At present, there is neither an additional data available in Argentina confirming this situation (uranium levels in air, soil or water) nor any other information about the measures taken by government organisations concerning a programme of environmental sanitation to process the wastes deposited there. It must be noted that, at the mining site, the debris and waste piles have remained open and untreated for more than two decades. The high content of uranium in *R. celastri* could also reflect a historical origin of its accumulation, as described by Sloof (1993). Hence, the presence of U in particulate atmospheric matter must be investigated further.

Pearson's analysis of correlation was undertaken to evaluate whether the physiological response of *R. celastri* is correlated with the trace elements accumulated in the thalli (data not shown). A negative correlation between U and Chl-a and a positive correlation between U and the Phaeoph-a/Chl-a ratio was observed. The decrease of Chl-a concentration together with an increase in the Phaeoph-a/Chl-a ratio has been described to indicate the presence of lichen damage by pollution in this particular species (Levin and Pignata, 1995; González, Casanovas and Pignata, 1996; González and Pignata, 1999). However, to the current day there have been no reports of any harmful effects of U on the integrity of the photosynthetic pigments in lichens. Furthermore, the evidence provided by this study is not enough to distinguish whether the degradation of

Chl-a is specifically due to high levels of U or to a co-variation between U and other factors associated to the environmental conditions of the sites in which it is found in increased concentrations as, for example, higher levels of UV radiation (due to high altitude) or the presence of photochemical oxidants such as O_3 produced by the transportation of gaseous contaminants from urban environments (of which Br could be a marker). It is important to note that this association was observed in sites with altitudes over 1,500 m.a.s.l.

The association between the high levels of Zn, PI and HPCD and low altitude confirms the emission of Zn and other pollutants in urban areas causing lichen damage and oxidation of membrane lipids in *R. celastri*. The increase of PI and HPCD has been previously observed in atmospheric contaminant biomonitoring studies using this same species (Levin and Pignata, 1995; González, Casanovas and Pignata, 1996; Gonzalez et al., 1998; González, Pignata and Orellana, 2003). Together, our results show that in these low altitude areas the level of atmospheric contamination is higher.

The DW/FW ratio was positively correlated (Pearson's coefficient, data not shown) with As, Sb and Br, which suggests that the loss of humidity could be associated with the presence of volatile elements that affect the water balance of the lichen thalli. HPCD was positively correlated with Zn corroborating the fact that this metal or other gaseous contaminants originated by anthropogenic emission sources are associated with higher levels of membrane lipid peroxidation in *R. celastri*. This same effect was reported for *Usnea amblyoclada* exposed to high environmental levels of Zn (Carreras and Pignata, 2002). The concentration of MDA was positively correlated with Fe and Co and negatively correlated with Nd. No significant correlation was found between the PI and any of the elements measured in the bioindicator, in agreement with the previous results by Jasan et al. (2004), who hardly found any correlation between the accumulation of trace elements and physiological damage in this species.

3.4 Mapping

Mapping the distribution of some elements allowed the detection of their emission sources and their range of dispersion within the study area. Accordingly, three areas were detected with high concentrations of As in the bioindicator (Figure 3), all of which had loessic soils (Farías et al., 2003). One of these areas was located in the southeast region of the study area, an agricultural area with loessic soils; another was located to the south, near the city of Río Tercero that has important industrial and petrochemical industries; the third was located in the northwest.

The highest concentrations of sulphur in the biomonitor (Figure 4) were found in agricultural areas (northeast, southeast and south of the study area), indicating that its origin is related to the use of fertilisers and pesticides as well as with the industrial activities of the city of Río Tercero. High levels of Zn (Figure 5) were found near the city of Córdoba and its access roads (east and northeast). The distribution of U (Figure 6) clearly shows that the main source of this element was the uranium mine mentioned earlier. The distribution map for Br (Figure 7) shows that there are increased concentrations of this element in high altitudinal areas (possibly related with long range of transport) and in the south of the study area where the chemical industries are found (among them a chlor-alkali plant). The geographical distribution of the PI (Figure 8) shows that the biomonitor suffered greater damage in the agricultural areas. It is important to recall that the lichen was not present in any urban and industrial areas and that the samples were collected at least 500 m away from these sites.

Figure 3 Geographical distribution of the content of arsenic (μg g^{-1} DW) in *R. celastri* and the altitudinal levels of the survey area

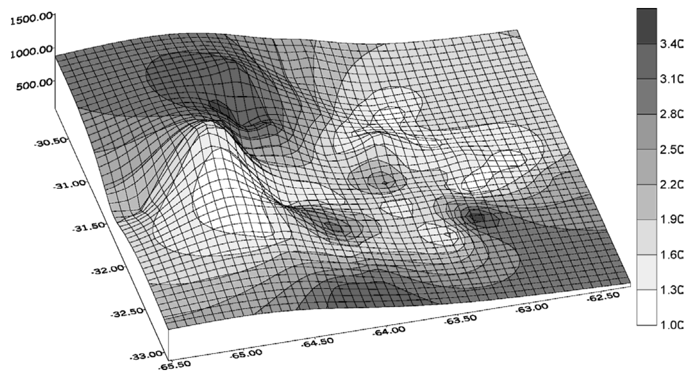


Figure 4 Geographical distribution of the content of sulfur (mg g^{-1} DW) in *R. celastri* and the altitudinal levels of the survey area

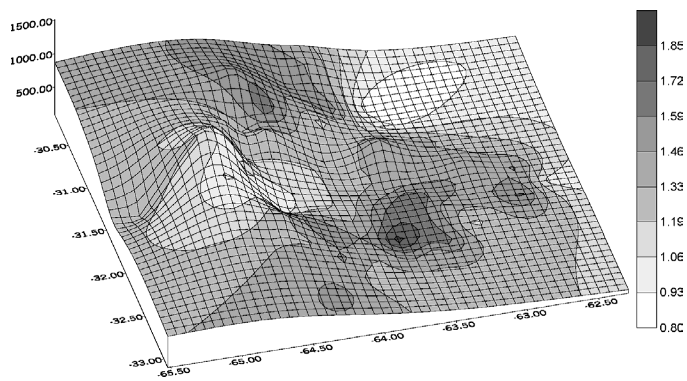


Figure 5 Geographical distribution of the content of zinc (μg g^{-1} DW) in *R. celastri* and the altitudinal levels of the survey area

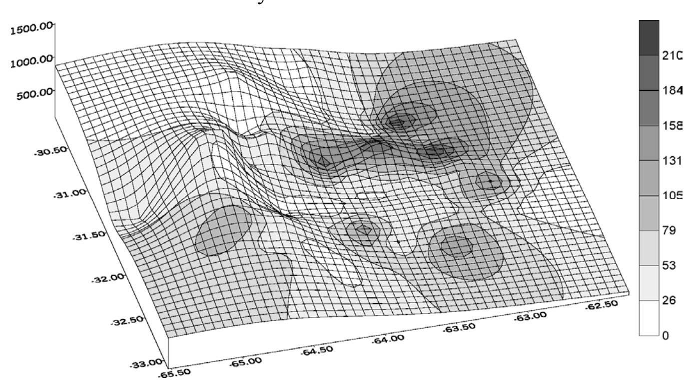


Figure 6 Geographical distribution of the content of uranium (μg g^{-1} DW) in *R. celastri* and the altitudinal levels of the survey area

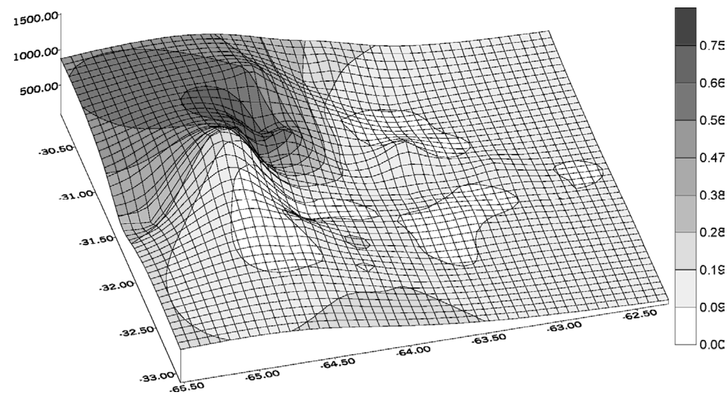


Figure 7 Geographical distribution of the content of bromine (μg g^{-1} DW) in *R. celastri* and the altitudinal levels of the survey area

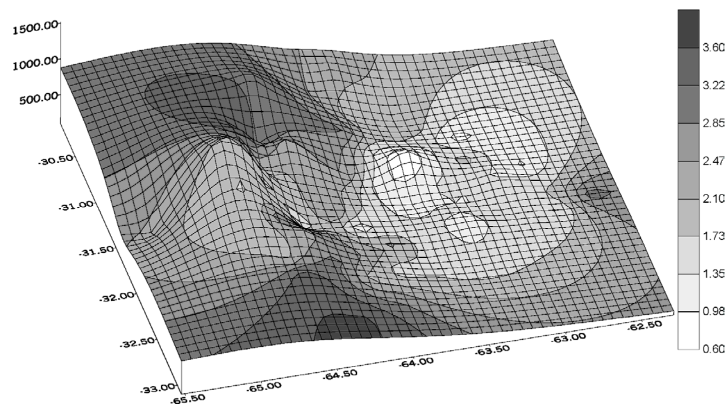
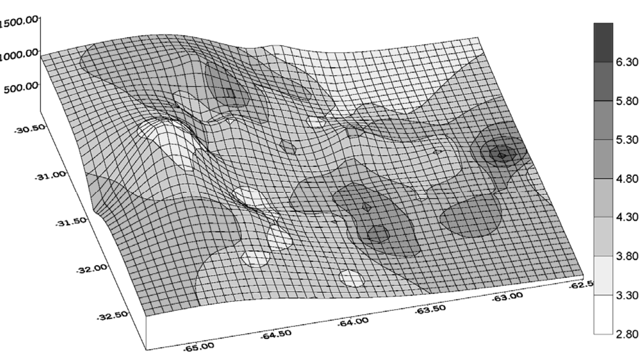


Figure 8 Geographical distribution of the Pollution Index values for *R. celastri* and the altitudinal levels of the survey area



4 Conclusion

The present study has revealed the capacity of *R. celastri* to detect, on a regional scale, the emission sources of certain contaminant elements. Furthermore, it evidences the lichen's ability to estimate the potential damage of these pollutants and other air pollutants, for a bioindicator and hence their threat to human health. Hence, the PI values that were calculated allowed different levels of atmospheric quality to be established.

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