

## Biomonitoring of air quality employing *in situ* *Ramalina celastri* in Argentina

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**Abstract:** The lichen *Ramalina celastri* was employed as an *in situ* biomonitor for the study of air quality and heavy metal distribution in the central region of the Argentine Republic. The thallus content of copper, iron, manganese, cobalt, zinc, nickel and lead was determined by atomic absorption spectrophotometry. Pigments, hydroperoxy conjugated dienes, malondialdehyde and sulfur were also measured. Some of these parameters were used to calculate a pollution index. Geographical distribution patterns were obtained for the different metals by mapping. Thus, zinc shows high levels in industrial and agricultural zones. The highest nickel values were found in urban-industrial zones located at the centre and south of the study area. The same was observed for sulfur. The pollution index distribution map showed that major physiological damage in the biomonitor was found in central and southeastern areas that coincide with Córdoba City and agricultural zones. So, the effect would seem to be related to urban activities and the use of pesticides.

**Keywords:** Argentina, biomonitoring, heavy metals, malondialdehyde, pigments, pollution index, *Ramalina celastri*, sulfur.

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### Biographical notes

**María Luisa Pignata** graduated in biochemical sciences and subsequently obtained a PhD in chemical sciences at the Universidad Nacional de Córdoba,

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### Biographical notes

**María Luisa Pignata** graduated in biochemical sciences and subsequently obtained a PhD in chemical sciences at the Universidad Nacional de Córdoba,

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Argentina. She has worked since 1990 at the Universidad Nacional de Córdoba as professor for teaching and research in general chemistry for biological sciences, and is head of the group's research. Her research interest focuses on the biomonitoring of air pollution and the effects of pollutants on plants. Since 1996 she has been environmental chemistry professor for a Master of Environmental Engineering at the Universidad Tecnológica Nacional, Argentina. Her research goals emphasize multidisciplinary projects and collaboration among biology, chemistry, geology and environmental engineering disciplines, and involve research experiences for undergraduates. Applications include environmental biomonitoring, mapping of contaminants and detection of pollution sources.

**Claudia María Gonzalez** graduated in biological sciences and teaching in biological sciences and subsequently obtained a PhD in biological sciences at the Universidad Nacional de Córdoba, Argentina. Since 1989, she has worked at the Universidad Nacional de Córdoba as a part-time professor for teaching and research in general chemistry for biological sciences, where she studies the use of lichens as biomonitors. She has obtained fellowships of CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas) in the plant biochemistry area and of the SECyT (Secretaría de Ciencia y tecnología de la Universidad Nacional de Córdoba). Her research interest focuses on the biomonitoring of air pollution, effects of pollutants on plants and plant biochemistry.

**Eduardo Daniel Wannaz** graduated in 1999 at the Universidad Nacional de Córdoba, Argentina. He has a CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas) fellowship to obtain a PhD in biological sciences. He studies the atmospheric quality in Córdoba province by the intercomparison of three species of *Tillandsia*.

**Hebe Alejandra Carreras** graduated at the Universidad Nacional de Córdoba, Argentina. Since 1992, she has worked at the Universidad Nacional de Córdoba as a part-time professor, teaching and researching in the area of general chemistry for biological sciences. She has received many fellowships from different funding agencies, and now has a postdoctorate fellowship from CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas). She has published many papers on biomonitoring using both trees and lichen species. Her research interests focus on the biomonitoring of air pollution and the effects of pollutants, mainly heavy metals, on the biomonitors.

**Gustavo Luis Gudiño** graduated in 1993 at the Universidad Nacional de Córdoba, Argentina. He obtained a fellowship of the Secretaría de Extensión Universitaria of the Universidad Nacional de Córdoba for a study on the biomonitoring of air quality in Córdoba City using lichens. He has trained in environmental chemistry with applications in atmospheric pollution and mapping methods.

**María Soledad Martrínez** graduated in biochemical sciences in 1999 at the Universidad Nacional de Córdoba, Argentina. She works as a collaborator in the general chemistry research group headed by María Luisa Pignata.

## 1 Introduction

Human activities release large amounts of exchangeable elements into the environment and have become a major factor in altering biogeochemical cycles. This alteration is progressively affecting the long-established equilibrium between earth processes and biological evolution [1]. Increasing burdens of potentially toxic elements in organisms have highlighted the importance of establishing reliable monitoring systems at different scales. A reliable assessment of pollutant concentrations in such an extremely variable environment as the atmosphere needs a statistical approach based on a large number of samples. The high costs of establishing and managing automatic monitoring networks often limit the number of sampling stations and/or the number of pollutants considered. Thus, although reliable, instrumental recording data may be statistically weak [2]. For that reason it should be complemented with information obtained from bioindicators, which are comparatively cheap, enabling coverage of large and remote areas, and providing current and retrospective information on the integrated effects of atmospheric pollutants on a living organism and other environmental factors.

As far back as 1866, a study was published on epiphytic lichens used as bioindicators [3]. Since then, many studies have stressed the possibility of using lichens as biomonitors of air quality in view of their sensitivity to various environmental conditions, which can provoke changes in some of their components and/or specific parameters [4–9]. Indeed many physiological parameters are used to evaluate damage on lichens, such as photosynthesis [10], chlorophyll content and degradation [11, 12], decrease of ATP and variations in respiration levels [13], and changes in the level of endogenous auxins and ethene production [14].

Lichens are frequently used in monitoring the deposition of elements in urban and industrial areas because they are ideal for this purpose. Not only are they perennials, but they also accumulate impurities effectively thanks to their large surface area and the absence of cuticle. So their content of mineral substances almost solely represents material landing on the thallus as wet and dry deposition. In addition, there is no active translocation of absorbed substances outside the thallus. They have been defined as 'permanent control systems' for air pollution assessment [15].

The accumulation of metals in lichens depends on many factors, such as the availability of elements, the characteristics of the thallus already mentioned, and other such parameters as temperature, available moisture, substratum characteristics, etc. [16]. Contaminants deposit on lichens through normal and indirect precipitation. The latter includes mist, dew, dry sedimentation and gaseous absorption. Indirect precipitation occurs in highly stable atmospheric conditions and contains higher nutrient and contaminant concentrations of different orders of size when compared with normal precipitation [17].

To correctly evaluate the degree of contamination in an ecosystem, or to carry out biomonitoring operations, it is necessary to first establish the background level of the contaminant, both in the environment and in the biomonitor. The background level may be interpreted in different ways: it may be understood as a pre-industrial level (prior to any human activity), as a natural level (the average conditions of an area or a region where there may be human activity, but which is in good state of conservation), a standard level (based on global geographical references) or even a zero level (the concentration of an element in the environment or in an organism prior to the



development of a particular activity that is independent of the degree of conservation) [18].

*Ramalina celastri* (Spreng.) Krog. & Swinsc. is one of the most common epiphytic lichen species in Córdoba province, and their transplanted thalli have been often employed for biomonitoring purposes [19–21]. The present investigation was designed to assess the elemental content of this species, collected in a wide region from Córdoba province. Another goal was to estimate the atmospheric quality of this region, taking into consideration both the concentration of some elements in lichen thalli and the physiological response of the biomonitor.

## 2 Materials and methods

### 2.1 Study area and sampling

The study area of 50 000 km<sup>2</sup> was selected in the central region of the Argentine Republic, defined by a quadrilateral whose extreme points had the following coordinates:

- to the west, 31° 25' 21" S, 65° 24' W
- to the east, 31° 41' 15" S, 62° 38' 34" W
- to the north, 30° 36' S, 63° 15' W
- to the south, 32° 52' S, 64° 10' 12" W

Land morphology is highly variable, ranging from a mean altitude of about 250 m in the southeast to more than 2500 m in the mid-west. There are cities (large and medium sized) and many small villages in the area. Industrial plants (mainly metallurgical, petrochemical, or chemical), food, vegetable oil, and cement factories are mostly located in the centre and south where the population density is highest.

For sampling purposes, the chosen area was divided into a square pattern, with each square measuring 25 × 25 km (80 sampling points in the area). Where present, samples of *R. celastri* were collected at each intersection point. The total number of sampled points was 29 and the collection sites were located at least 500 m from major routes and highly 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, at a distance of no more than 100 m from the geographically referenced point. Extraneous material was removed from each sample and they were put into paper bags. The collection was done using plastic gloves to avoid any risk of sample contamination [22].

Sampling was conducted only when a condition of five days without rain was fulfilled. Once back at the laboratory, part of the plant material was separated to determine water content (dry weight to fresh weight ratio). The rest of the material was stored in plastic vials at –15 °C in complete darkness for chemical and physiological determinations, and then prepared for metal determinations. Chemical determinations

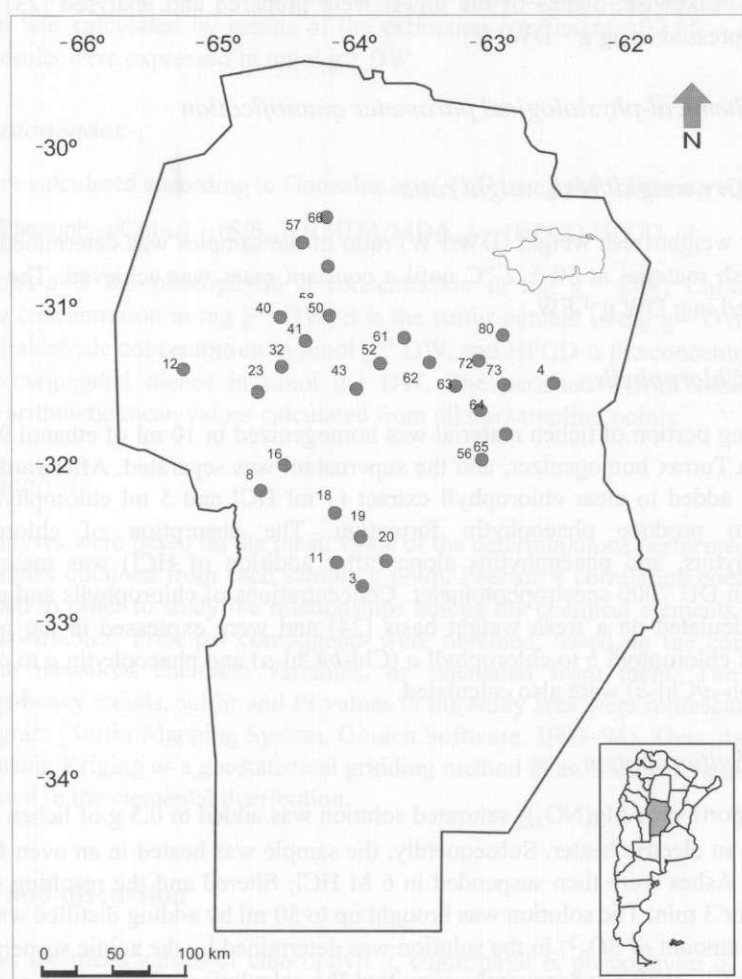
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were done in triplicate using independent sub-samples taken from samples corresponding to each pool.



**Figure 1** Localization of the study area and individual sampling points in the survey made in Córdoba, Argentina.

## 2.2 Atomic absorption spectrometry

An accurately weighed portion of each sample (1 g DW) was placed in a porcelain crucible and ashed at 500 °C for 2 h. Ashes were digested using a 5:1 mixture of HCl (6 M) and HNO<sub>3</sub> (conc.). The solid residue was centrifuged off and diluted to 50 ml with ultrapurified water.

Analyses for Fe, Mn, Zn, Pb, Cu, Ni and Co were conducted with a Buck 210-VGP atomic absorption spectrophotometer, using the air/acetylene flame for all elements. Metal concentrations were calculated on a dry weight basis.

The precision of analysis was estimated by the relative standard deviation of four replicates and was found to be 5–10% for all elements studied. To control the analytical method, the certified reference material IAEA/V-10 Hay Power was analysed every 10 samples. Likewise, blanks of the digest were prepared and analysed [23]. The results were expressed in  $\mu\text{g g}^{-1}$  DW.

### 2.3 Chemical-physiological parameter quantification

#### 2.3.1 Dry weight/fresh weight ratio

The dry weight/fresh weight (DW/FW) ratio of the samples was determined by drying 1 g of fresh material at  $60 \pm 2$  °C until a constant mass was achieved. The results were expressed in g DW  $\text{g}^{-1}$  FW.

#### 2.3.2 Chlorophylls

A 100 mg portion of lichen material was homogenized in 10 ml of ethanol 96% v/v with an Ultra Turrax homogenizer, and the supernatant was separated. Afterwards, HCl (0.06 M) was added to clear chlorophyll extract (1 ml HCl and 5 ml chlorophyll extract) in order to produce phaeophytin formation. The absorption of chlorophylls and phaeophytins, and phaeophytins alone (after addition of HCl) was measured with a Beckman DU 7000 spectrophotometer. Concentrations of chlorophylls and phaeophytins were calculated on a fresh weight basis [24] and were expressed in  $\text{mg g}^{-1}$  DW. The ratios of chlorophyll *b* to chlorophyll *a* (Chl-*b*/Chl-*a*) and phaeophytin *a* to chlorophyll *a* (Phaeoph-*a*/Chl-*a*) were also calculated.

#### 2.3.3 Sulfur content

A 5 ml portion of  $\text{Mg}(\text{NO}_3)_2$  saturated solution was added to 0.5 g of lichen material and dried in an electric heater. Subsequently, the sample was heated in an oven for 30 min at 500 °C. Ashes were then suspended in 6 M HCl, filtered and the resulting solution was boiled for 3 min. The solution was brought up to 50 ml by adding distilled water.

The amount of  $\text{SO}_4^{2-}$  in the solution was determined by the acidic suspension method using  $\text{BaCl}_2$ , which subsequently permitted the calculation of the sulfur content in each sample [19]. The concentration was expressed in mg of total sulfur  $\text{g}^{-1}$  DW.

#### 2.3.4 Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method [25]. A 50 mg portion of freeze-dried lichen was homogenized in 2.5 ml of distilled water. An equal volume of 0.5% TBA (2-thiobarbituric acid) in 20% trichloroacetic acid solution was added, and the sample was incubated at 95 °C for 30 min. The reaction was stopped by placing the reaction tubes in an ice-bath. Samples were then centrifuged at 10 000 g for 30 min. The supernatant was removed and read at 532 nm, and the value for non-specific absorption at 600 nm was read and subtracted from this. The amount of MDA present was calculated from the extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  [26]. Results were expressed in  $\mu\text{mol g}^{-1}$  DW.

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Hydroperoxy conjugated dienes (HPCD) were extracted by homogenization of the lichen material (50 mg) in 96% v/v ethanol at a ratio of 1 : 50 FW/v with an Ultra Turrax homogenizer. The absorption was measured at 234 nm in the supernatant, and its concentration was calculated by means of the extinction coefficient of  $2.65 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  [21]. Results were expressed in  $\mu\text{mol g}^{-1} \text{ DW}$ .

### 2.3.5 Pollution index

PI values were calculated according to Gonzalez *et al.* [27] using the following equation:

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

where Phaeoph-*a* is the phaeophytin *a* concentration in  $\text{mg g}^{-1} \text{ DW}$ ; Chl-*a* is the chlorophyll *a* concentration in  $\text{mg g}^{-1} \text{ DW}$ ; S is the sulfur content in  $\text{mg g}^{-1} \text{ DW}$ ; MDA is the malondialdehyde concentration in  $\mu\text{mol g}^{-1} \text{ DW}$ , and HPCD is the concentration of hydroperoxy conjugated dienes in  $\mu\text{mol g}^{-1} \text{ DW}$ . The parameters with subindex *m* represent the arithmetic mean values calculated from all the sampling points.

### 2.4 Data analysis

Statistical analyses were based on the mean value of the determinations performed on the three sub-samples obtained from each sampling point. Pearson's correlation coefficients were calculated in order to study the relationships among the chemical elements and the physiological variables. Principal components were obtained, based on the correlation matrix of the measured chemical variables, or calculated from them. The spatial distribution of heavy metals, sulfur and PI values in the study area were represented by a mapping program (Surfer Mapping System, Golden Software, 1993–94). Thus, data were transformed using Kriging as a geostatistical gridding method in an attempt to express the trends suggested in the elemental distribution.

## 3 Results and discussion

Table 1 shows the mean values of chlorophyll *a*, chlorophyll *b*, phaeophytin *a*, HPCD, MDA, sulfur, DW/FW, Chl-*b*/Chl-*a*, Phaeoph-*a*/Chl-*a*, PI and ANOVA results among sampling points. No significant differences were observed for Phaeoph-*a*/Chl-*a* and DW/FW. The mean concentrations of metals are reported in Table 2. Significant differences among sampling points were found for Cu, Zn, Ni, Mn and Fe.



**Table 1** Mean values (mean  $\pm$  S.D.) of parameters measured in *R. celastri* corresponding to sampling sites and results of variance analysis between sites.

Site	Chl- <i>a</i> (mg g <sup>-1</sup> DW)	Chl- <i>b</i> (mg g <sup>-1</sup> DW)	Phaeoph- <i>a</i> (mg g <sup>-1</sup> DW)	Chl- <i>b</i> /Chl- <i>a</i> (mg g <sup>-1</sup> DW)	Phaeoph- <i>a</i> /Chl- <i>a</i> (mg g <sup>-1</sup> DW)	MDA ( $\mu$ mol g <sup>-1</sup> DW)	HPCD ( $\mu$ mol g <sup>-1</sup> DW)	Sulfur (mg g <sup>-1</sup> DW)	DW/FW (g g <sup>-1</sup> DW)
3	1.199 $\pm$ 0.192	0.417 $\pm$ 0.056	1.346 $\pm$ 0.187	0.349 $\pm$ 0.012	1.126 $\pm$ 0.029	2.659 $\pm$ 0.443	1.069 $\pm$ 0.449	3.2436 $\pm$ 0.359	0.889 $\pm$ 0.014
4	0.941 $\pm$ 0.102	0.357 $\pm$ 0.097	1.259 $\pm$ 0.213	0.375 $\pm$ 0.063	1.358 $\pm$ 0.374	4.300 $\pm$ 0.413	0.930 $\pm$ 0.144	3.0466 $\pm$ 0.391	0.885 $\pm$ 0.026
8	0.594 $\pm$ 0.051	0.302 $\pm$ 0.090	0.863 $\pm$ 0.154	0.512 $\pm$ 0.154	1.449 $\pm$ 0.196	3.542 $\pm$ 1.115	1.018 $\pm$ 0.064	1.8366 $\pm$ 0.267	0.883 $\pm$ 0.004
11	1.110 $\pm$ 0.050	0.432 $\pm$ 0.001	1.239 $\pm$ 0.031	0.390 $\pm$ 0.017	1.117 $\pm$ 0.023	4.042 $\pm$ 0.325	1.184 $\pm$ 0.338	2.6346 $\pm$ 0.650	0.885 $\pm$ 0.016
12	1.556 $\pm$ 0.453	0.556 $\pm$ 0.034	1.938 $\pm$ 0.199	0.370 $\pm$ 0.086	1.281 $\pm$ 0.246	4.292 $\pm$ 0.151	2.100 $\pm$ 0.436	4.3216 $\pm$ 1.070	0.917 $\pm$ 0.041
13	1.040 $\pm$ 0.084	0.453 $\pm$ 0.047	1.415 $\pm$ 0.247	0.465 $\pm$ 0.015	1.416 $\pm$ 0.126	4.756 $\pm$ 8.658	1.452 $\pm$ 0.268	2.4676 $\pm$ 0.824	0.905 $\pm$ 0.027
16	1.042 $\pm$ 0.206	0.488 $\pm$ 0.304	2.030 $\pm$ 1.272	0.444 $\pm$ 0.185	1.853 $\pm$ 0.788	5.461 $\pm$ 1.324	0.429 $\pm$ 0.130	2.8966 $\pm$ 0.506	0.893 $\pm$ 0.018
18	1.016 $\pm$ 0.352	0.353 $\pm$ 0.120	1.094 $\pm$ 0.398	0.348 $\pm$ 0.021	1.072 $\pm$ 0.021	4.707 $\pm$ 0.928	1.620 $\pm$ 1.099	4.4036 $\pm$ 0.408	0.897 $\pm$ 0.027
19	1.661 $\pm$ 0.106	0.611 $\pm$ 0.090	1.748 $\pm$ 0.091	0.367 $\pm$ 0.040	1.052 $\pm$ 0.014	4.562 $\pm$ 0.752	0.749 $\pm$ 0.114	4.0226 $\pm$ 0.541	0.852 $\pm$ 0.028
20	2.014 $\pm$ 0.168	0.841 $\pm$ 0.105	2.367 $\pm$ 0.456	0.421 $\pm$ 0.087	1.170 $\pm$ 0.129	5.386 $\pm$ 0.113	0.924 $\pm$ 0.346	6.2686 $\pm$ 0.707	0.925 $\pm$ 0.001
23	1.656 $\pm$ 0.310	0.546 $\pm$ 0.136	1.785 $\pm$ 0.400	0.328 $\pm$ 0.021	1.074 $\pm$ 0.041	7.329 $\pm$ 2.514	1.203 $\pm$ 0.906	4.8226 $\pm$ 0.546	0.887 $\pm$ 0.003
32	1.041 $\pm$ 0.222	0.652 $\pm$ 0.102	1.086 $\pm$ 0.938	0.630 $\pm$ 0.036	0.969 $\pm$ 0.694	7.296 $\pm$ 0.100	0.977 $\pm$ 0.009	2.5426 $\pm$ 0.166	0.892 $\pm$ 0.023
40	2.094 $\pm$ 0.351	0.776 $\pm$ 0.220	2.597 $\pm$ 0.780	0.374 $\pm$ 0.104	1.246 $\pm$ 0.345	3.927 $\pm$ 0.349	0.758 $\pm$ 0.041	3.9676 $\pm$ 0.442	0.871 $\pm$ 0.002
41	1.573 $\pm$ 0.135	0.500 $\pm$ 0.215	1.683 $\pm$ 0.526	0.318 $\pm$ 0.108	1.070 $\pm$ 0.200	5.825 $\pm$ 0.321	0.492 $\pm$ 0.000	2.8726 $\pm$ 2.722	0.854 $\pm$ 0.104
43	1.118 $\pm$ 0.237	0.335 $\pm$ 0.048	1.234 $\pm$ 0.179	0.303 $\pm$ 0.030	1.116 $\pm$ 0.085	3.432 $\pm$ 4.854	1.216 $\pm$ 0.660	3.4036 $\pm$ 1.408	0.876 $\pm$ 0.028
50	2.006 $\pm$ 0.175	0.804 $\pm$ 0.166	2.442 $\pm$ 0.256	0.406 $\pm$ 0.116	1.224 $\pm$ 0.177	4.654 $\pm$ 0.811	1.025 $\pm$ 0.334	6.2956 $\pm$ 0.346	0.915 $\pm$ 0.028
52	1.337 $\pm$ 0.065	0.471 $\pm$ 0.100	1.443 $\pm$ 0.067	0.353 $\pm$ 0.075	1.080 $\pm$ 0.012	5.167 $\pm$ 0.462	0.717 $\pm$ 0.035	3.1216 $\pm$ 0.606	0.890 $\pm$ 0.010
56	2.034 $\pm$ 0.075	0.544 $\pm$ 0.007	2.142 $\pm$ 0.100	0.268 $\pm$ 0.011	1.054 $\pm$ 0.037	6.044 $\pm$ 0.000	1.180 $\pm$ 0.138	4.7866 $\pm$ 2.754	0.864 $\pm$ 0.004
57	1.776 $\pm$ 0.024	0.585 $\pm$ 0.025	1.867 $\pm$ 0.060	0.329 $\pm$ 0.010	1.051 $\pm$ 0.037	5.834 $\pm$ 0.787	0.752 $\pm$ 0.107	2.7896 $\pm$ 0.335	0.902 $\pm$ 0.032
58	1.360 $\pm$ 0.235	0.283 $\pm$ 0.207	1.272 $\pm$ 0.146	0.228 $\pm$ 0.174	0.961 $\pm$ 0.234	4.014 $\pm$ 0.491	0.910 $\pm$ 0.283	3.4946 $\pm$ 0.106	0.885 $\pm$ 0.018
61	1.661 $\pm$ 0.080	0.413 $\pm$ 0.031	1.700 $\pm$ 0.135	0.248 $\pm$ 0.007	1.023 $\pm$ 0.032	4.475 $\pm$ 0.000	1.138 $\pm$ 0.089	3.3616 $\pm$ 0.574	0.883 $\pm$ 0.006
62	1.717 $\pm$ 0.043	0.555 $\pm$ 0.070	2.137 $\pm$ 0.077	0.324 $\pm$ 0.044	1.246 $\pm$ 0.073	7.988 $\pm$ 0.629	0.833 $\pm$ 0.070	3.8836 $\pm$ 0.291	0.886 $\pm$ 0.005
63	1.687 $\pm$ 0.083	0.586 $\pm$ 0.244	1.906 $\pm$ 0.134	0.352 $\pm$ 0.162	1.129 $\pm$ 0.135	4.412 $\pm$ 0.055	1.048 $\pm$ 0.260	3.4056 $\pm$ 0.579	0.897 $\pm$ 0.026
64	1.307 $\pm$ 0.011	0.452 $\pm$ 0.049	1.495 $\pm$ 0.208	0.346 $\pm$ 0.034	1.143 $\pm$ 0.149	5.802 $\pm$ 0.000	0.821 $\pm$ 0.088	7.0016 $\pm$ 0.285	0.864 $\pm$ 0.003
65	1.314 $\pm$ 0.309	0.565 $\pm$ 0.314	1.685 $\pm$ 0.446	0.411 $\pm$ 0.149	1.282 $\pm$ 0.119	4.141 $\pm$ 1.292	0.380 $\pm$ 0.186	4.3356 $\pm$ 0.590	0.867 $\pm$ 0.004

63	1.687 ± 0.083	0.586 ± 0.244	1.906 ± 0.134	0.352 ± 0.162	1.129 ± 0.135	4.412 ± 0.055	1.048 ± 0.200	3.4030 ± 0.577	0.877 ± 0.027
64	1.307 ± 0.011	0.452 ± 0.049	1.495 ± 0.208	0.346 ± 0.034	1.143 ± 0.149	5.802 ± 0.000	0.821 ± 0.088	7.0016 ± 0.285	0.864 ± 0.003
65	1.314 ± 0.309	0.565 ± 0.314	1.685 ± 0.446	0.411 ± 0.149	1.282 ± 0.119	4.141 ± 1.292	0.380 ± 0.186	4.3356 ± 0.590	0.867 ± 0.004

Table 1 continued

66	1.034 ± 0.140	0.402 ± 0.025	1.186 ± 0.146	0.392 ± 0.032	1.149 ± 0.024	6.491 ± 0.419	0.666 ± 0.111	3.0096 ± 0.679	0.915 ± 0.022
72	1.696 ± 0.050	0.622 ± 0.108	2.255 ± 0.293	0.368 ± 0.074	1.333 ± 0.212	5.570 ± 1.026	0.595 ± 0.627	3.9186 ± 0.311	0.887 ± 0.003
73	1.156 ± 0.030	0.356 ± 0.020	1.501 ± 0.183	0.308 ± 0.009	1.297 ± 0.125	5.543 ± 3.203	1.065 ± 0.072	5.0856 ± 0.841	0.895 ± 0.021
80	1.640 ± 0.234	0.531 ± 0.062	1.848 ± 0.199	0.324 ± 0.008	1.130 ± 0.056	5.488 ± 0.498	0.800 ± 0.274	4.8956 ± 0.425	0.913 ± 0.031
ANOVA	p < 0.001	p < 0.001	p < 0.001	p < .001	n.s.	p < 0.01	p < 0.01	p < 0.001	n.s.

**Table 2** Mean values ( $\pm$  S.D.) of heavy metals ( $\mu\text{g g}^{-1}$  DW) in *R. celastri* thalli corresponding to sampling sites and results of variance analysis between sites.

Sites	Cu	Zn	Pb	Co	Ni	Mn	Fe
3	2.499 $\pm$ 1.753	45.971 $\pm$ 4.417	0.833 $\pm$ 0.188	0.057 $\pm$ 0.010	2.518 $\pm$ 0.136	46.656 $\pm$ 1.473	1342.53 $\pm$ 311.49
4	16.505 $\pm$ 2.445	75.405 $\pm$ 26.395	1.241 $\pm$ 0.319	0.057 $\pm$ 0.028	2.222 $\pm$ 0.517	39.055 $\pm$ 3.145	1182.84 $\pm$ 201.21
8	5.591 $\pm$ 0.335	23.346 $\pm$ 1.989	1.104 $\pm$ 0.136	0.038 $\pm$ 0.001	1.922 $\pm$ 0.403	26.331 $\pm$ 1.782	1047.46 $\pm$ 106.56
11	5.685 $\pm$ 0.107	35.729 $\pm$ 9.146	0.781 $\pm$ 0.025	0.064 $\pm$ 0.006	2.374 $\pm$ 0.623	47.854 $\pm$ 6.315	1305.86 $\pm$ 680.42
12	4.592 $\pm$ 0.113	13.836 $\pm$ 1.563	1.127 $\pm$ 0.077	0.056 $\pm$ 0.003	2.048 $\pm$ 0.650	34.503 $\pm$ 0.540	557.82 $\pm$ 57.21
13	5.983 $\pm$ 0.591	11.394 $\pm$ 0.100	1.196 $\pm$ 0.047	0.052 $\pm$ 0.001	1.990 $\pm$ 0.007	33.075 $\pm$ 0.496	955.53 $\pm$ 67.05
16	5.966 $\pm$ 1.116	11.548 $\pm$ 0.960	1.009 $\pm$ 0.102	0.046 $\pm$ 0.004	1.888 $\pm$ 0.486	40.150 $\pm$ 0.425	1383.51 $\pm$ 103.07
18	4.492 $\pm$ 0.332	8.605 $\pm$ 2.382	0.928 $\pm$ 0.101	0.067 $\pm$ 0.002	2.875 $\pm$ 0.318	48.168 $\pm$ 3.933	1512.18 $\pm$ 314.85
19	4.237 $\pm$ 0.857	20.999 $\pm$ 3.392	0.914 $\pm$ 0.093	0.066 $\pm$ 0.005	2.949 $\pm$ 0.450	49.197 $\pm$ 4.151	1069.02 $\pm$ 70.30
20	3.630 $\pm$ 0.548	17.097 $\pm$ 4.689	0.795 $\pm$ 0.064	0.074 $\pm$ 0.004	3.206 $\pm$ 0.044	80.753 $\pm$ 7.091	2565.73 $\pm$ 358.42
23	4.684 $\pm$ 0.610	12.217 $\pm$ 3.501	1.105 $\pm$ 0.097	0.058 $\pm$ 0.003	1.851 $\pm$ 0.573	43.971 $\pm$ 2.199	1047.44 $\pm$ 226.06
32	13.585 $\pm$ 4.712	16.340 $\pm$ 8.835	1.125 $\pm$ 0.143	0.074 $\pm$ 0.038	2.818 $\pm$ 0.158	37.152 $\pm$ 1.742	876.05 $\pm$ 233.27
40	10.647 $\pm$ 8.855	12.920 $\pm$ 4.320	1.175 $\pm$ 0.241	0.080 $\pm$ 0.028	2.025 $\pm$ 0.219	44.043 $\pm$ 0.398	1205.88 $\pm$ 99.19
41	16.342 $\pm$ 1.977	12.100 $\pm$ 9.486	1.148 $\pm$ 0.240	0.081 $\pm$ 0.032	2.740 $\pm$ 0.244	52.323 $\pm$ 2.264	1184.68 $\pm$ 267.22
43	14.721 $\pm$ 4.971	12.376 $\pm$ 5.455	1.090 $\pm$ 0.107	0.074 $\pm$ 0.027	2.857 $\pm$ 0.191	63.252 $\pm$ 3.053	1833.25 $\pm$ 287.73
50	4.692 $\pm$ 0.361	3.883 $\pm$ 0.884	0.906 $\pm$ 0.089	0.072 $\pm$ 0.004	2.284 $\pm$ 0.379	72.329 $\pm$ 7.197	1784.07 $\pm$ 461.89
52	6.159 $\pm$ 1.782	4.954 $\pm$ 0.569	0.809 $\pm$ 0.034	0.068 $\pm$ 0.006	1.848 $\pm$ 0.561	59.349 $\pm$ 5.725	1981.90 $\pm$ 560.68
56	16.894 $\pm$ 2.579	10.685 $\pm$ 6.499	1.060 $\pm$ 0.165	0.075 $\pm$ 0.035	2.101 $\pm$ 0.460	78.372 $\pm$ 0.534	2193.07 $\pm$ 255.72
57	4.998 $\pm$ 1.206	10.571 $\pm$ 1.707	0.958 $\pm$ 0.100	0.072 $\pm$ 0.001	2.140 $\pm$ 0.136	55.697 $\pm$ 1.128	1168.04 $\pm$ 66.01
58	5.144 $\pm$ 0.269	19.443 $\pm$ 9.802	0.818 $\pm$ 0.045	0.063 $\pm$ 0.001	2.302 $\pm$ 0.596	51.701 $\pm$ 2.285	1185.62 $\pm$ 192.74
61	4.890 $\pm$ 1.406	28.540 $\pm$ 2.177	0.796 $\pm$ 0.038	0.056 $\pm$ 0.002	1.907 $\pm$ 0.613	49.469 $\pm$ 2.913	922.04 $\pm$ 147.67
62	6.017 $\pm$ 0.941	7.584 $\pm$ 4.157	0.868 $\pm$ 0.077	0.061 $\pm$ 0.009	1.867 $\pm$ 0.431	66.183 $\pm$ 2.966	1854.36 $\pm$ 133.92
63	3.843 $\pm$ 0.683	59.679 $\pm$ 10.218	0.860 $\pm$ 0.102	0.060 $\pm$ 0.004	1.633 $\pm$ 0.277	57.384 $\pm$ 3.078	1413.18 $\pm$ 203.61
64	11.209 $\pm$ 7.738	72.500 $\pm$ 6.015	0.993 $\pm$ 0.306	0.083 $\pm$ 0.035	2.496 $\pm$ 0.728	59.678 $\pm$ 4.235	1467.91 $\pm$ 191.94
65	11.134 $\pm$ 2.393	21.598 $\pm$ 11.619	1.066 $\pm$ 0.263	0.073 $\pm$ 0.029	2.279 $\pm$ 0.505	72.375 $\pm$ 3.265	1651.43 $\pm$ 242.07

Table 2 continued

66	3.307 ± 0.287	4.929 ± 0.560	0.803 ± 0.151	0.070 ± 0.006	1.888 ± 0.407	46.517 ± 1.926	911.52 ± 55.39
72	2.969 ± 0.610	43.269 ± 6.364	1.016 ± 0.192	0.065 ± 0.004	2.119 ± 0.376	62.856 ± 2.335	1188.56 ± 194.78
73	4.393 ± 1.393	12.768 ± 3.405	0.848 ± 0.130	0.065 ± 0.005	1.979 ± 0.272	75.893 ± 1.867	1251.15 ± 243.99
80	3.823 ± 0.139	11.236 ± 3.268	0.906 ± 0.139	0.073 ± 0.002	1.798 ± 0.299	56.386 ± 3.711	1165.60 ± 86.01
ANOVA	$p < 0.001$	$p < 0.001$	$p < 0.001$	n.s.	$p < 0.001$	$p < 0.001$	$p < 0.01$

### 3.1 Principal component analysis

In order to find the variables that best explain the total variability of data a principal component analysis was carried out. Table 3 shows the eigenvalues of four components for data on chemical variables.

**Table 3** Eigenvectors obtained in principal component analysis of chemical physiological parameters and heavy metals measured in *R. celastri*.

	Component			
	1	2	3	4
Phaeoph- <i>a</i> /Chl- <i>a</i>	-0.042	-0.150	0.036	<b>0.797</b>
Chl- <i>b</i> /Chl- <i>a</i>	0.135	-0.226	0.437	0.542
HPCD	<b>0.780</b>	-0.185	0.008	-0.432
MDA	0.331	0.031	-0.286	0.640
Sulfur	0.206	<b>0.725</b>	-0.053	-0.086
DW/FW	0.584	0.017	-0.422	0.275
PI	<b>0.835</b>	0.174	-0.196	0.311
Cu	-0.348	0.142	<b>0.805</b>	-0.123
Pb	-0.085	-0.240	<b>0.819</b>	0.202
Zn	-0.571	-0.135	-0.008	-0.042
Co	-0.095	0.679	0.330	-0.315
Ni	0.104	0.285	0.511	-0.378
Mn	-0.038	<b>0.926</b>	-0.184	-0.076
Fe	0.090	0.805	0.026	0.028
Accumulated variance (%)	23.04	45.08	56.38	66.72
Eigenvalues	3.22	3.08	1.58	1.44

The first component accounts for 23% of the variance. This component, greatly determined by PI and HPCD, confirms that the PI can be used as a biomarker of air quality. This result indicates that the variability of chemical responses is more significant than the variability of the elemental accumulation in this lichen species. Thus, the chemical parameters are more suitable to discriminate zones with different air qualities. The second principal component accounts for 22.03% of the total variance and is defined by Mn, Fe and sulfur. The third and fourth components explained 11.24% and 10.34% of the variance, respectively, and show Phaeoph-*a*/Chl-*a*, Cu and Pb as important variables.

This analysis allowed for the selection of parameters that can then be used to compare different sampling sites. The PI will be used as an indicator in terms of physiological damage on the bioindicator, while the S, Cu and Pb contents will be used as accumulation indicators.



### 3.2 Correlation analysis among chemical variables

Table 4 shows the Pearson's correlation between the quantified chemical variables in *R. celastri*. Significant positive correlations were found between those elements characteristic of soil contribution, Fe and Mn. Positive correlation was also found between Mn and Pb and between Mn and Co, which suggests that all these metals originate from soil particles blown by wind. The content of Co also correlates with Ni, an element released mainly by anthropogenic sources, so there may be an input of anthropogenic Co particles. This hypothesis is supported by the significant correlation between Co and Cu, which are emitted by metallurgical industries. The close correlation between Cu and Pb indicates that these metals have the same origin, which could be the combination of fuel oil. The content of sulfur in lichen thalli correlates positively with Co, Mn and Fe, suggesting that some sulfur particles have a common origin with those metals or that they are associated with them in short- or long-range transport. Finally, the concentration of Cu correlates negatively with PI values, which could be due to a protective effect of this action on the physiological processes of *R. celastri*, as was observed by Garty [14].

Individual pigments, Chl-*a*, Chl-*b* and phaeoph-*a* showed a positive correlation with sulfur. Chl-*a* showed a positive correlation with Co and Mn; Chl-*b* correlates with the content of Co and Phaeoph-*a* with Mn. A negative correlation was found between Phaeoph-*a*/Chl-*a* and the content of Co in lichens, while Chl-*b*/Chl-*a* correlates negatively with the content of Mn.

Membrane lipid peroxidation caused by atmospheric pollutants [28] was estimated by MDA and HPCD measurements. Both parameters showed positive correlations with the PI. MDA correlation with Cu was negative. HPCD reflected a positive correlation with the PI. These results suggest an important oxidative effect, because an increase in MDA or HPCD concentrations has been noted as an indicator of oxidative damage produced by air pollution in lichens [21, 29]. Moreover, the PI showed a positive correlation with S, which could be evidence that the damage is mainly caused by the presence of sulfur in the atmosphere, because the correlation with Cu was inverse.

### 3.3 Mapping

Figures 2–10 show the geographical distribution maps of the metal content, sulfur and PI values in *R. celastri*.

The distribution pattern of Fe is very similar to that of Mn (Figures 2 and 3), the highest levels being found in samples taken in the southeastern and central zones of the study area. According to the correlation analysis, both metals could have the same origin, so soil particles could be considered their main source, as mentioned by Steinnes *et al.* [30]. This fact coincides with the land use in these areas, since they represent agricultural areas with practices that favour soil erosion.

The distribution map of Zn (Figure 4) shows high content of this element in the southern and eastern zones, which reveals anthropogenic sources. The highest contents were found in the area with an important industrial activity. In the southern area, high values could be associated with the use of fungicides, such as zinc tetramethyl dithiocarbamate and Zn-Mn ethyl bisdithiocarbamate [31].

**Table 4** Pearson's correlation coefficient of chemical variables measured in *R. celastri*.

	Chl- <i>a</i>	Chl- <i>b</i>	Phaeoph- <i>a</i>	Chl- <i>a</i> / Chl- <i>b</i>	Phaeoph- <i>a</i> / Chl- <i>a</i>	HPCD	MDA	Sulfur	DW/ FW	PI	Cu	Pb	Zn	Co	Ni	Mn
Chl- <i>b</i>	0.76 <sup>c</sup>															
Phaeoph- <i>a</i>	0.88 <sup>c</sup>	0.80 <sup>c</sup>														
Chl- <i>b</i> /Chl- <i>a</i>	-0.40 <sup>a</sup>	0.28	-0.20													
Phaeoph- <i>a</i> / Chl- <i>a</i>	-0.31	-0.02	0.16	0.36												
HPCD	-0.07	-0.22	-0.19	-0.17	-0.21											
MDA	0.07	0.21	0.18	0.13	0.13	-0.13										
Sulfur	0.52 <sup>a</sup>	0.42 <sup>a</sup>	0.49 <sup>a</sup>	-0.27	-0.14	0.08	-0.05									
DW/FW	0.01	0.20	0.10	0.23	0.16	0.26	0.30	0.11								
PI	0.16	0.20	0.29	0.01	0.23	0.56 <sup>a</sup>	0.57 <sup>b</sup>	0.38 <sup>a</sup>	0.53 <sup>a</sup>							
Cu	-0.06	-0.07	-0.10	0.04	-0.08	-0.16	-0.35	-0.07	-0.57 <sup>b</sup>	-0.42 <sup>a</sup>						
Pb	-0.14	0.06	0.01	0.35	0.31	-0.06	-0.23	-0.18	-0.28	-0.12	0.63 <sup>b</sup>					
Zn	-0.18	-0.18	-0.17	0.03	0.04	-0.24	-0.27	0.06	-0.24	-0.37	0.15	0.07				
Co	0.48 <sup>a</sup>	0.40 <sup>a</sup>	0.28	-0.20	-0.54 <sup>a</sup>	-0.19	-0.05	0.49 <sup>a</sup>	-0.22	-0.15	0.41 <sup>a</sup>	-0.03	-0.08			
Ni	-0.02	0.15	-0.15	0.15	-0.33	0.08	-0.28	0.21	-0.20	-0.17	0.26	0.02	0.02	0.42 <sup>a</sup>		
Mn	0.54 <sup>a</sup>	0.33	0.46 <sup>a</sup>	-0.42 <sup>a</sup>	-0.23	-0.12	0.07	0.64 <sup>c</sup>	0.03	0.14	0.06	-0.41 <sup>a</sup>	-0.11	0.56 <sup>a</sup>	0.17	
Fe	0.34	0.28	0.33	-0.18	-0.06	0.04	0.03	0.44 <sup>a</sup>	0.02	0.19	0.17	-0.29	-0.10	0.35	0.28	0.7 <sup>c</sup>

<sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.001$ , <sup>c</sup>  $p < 0.000$ .

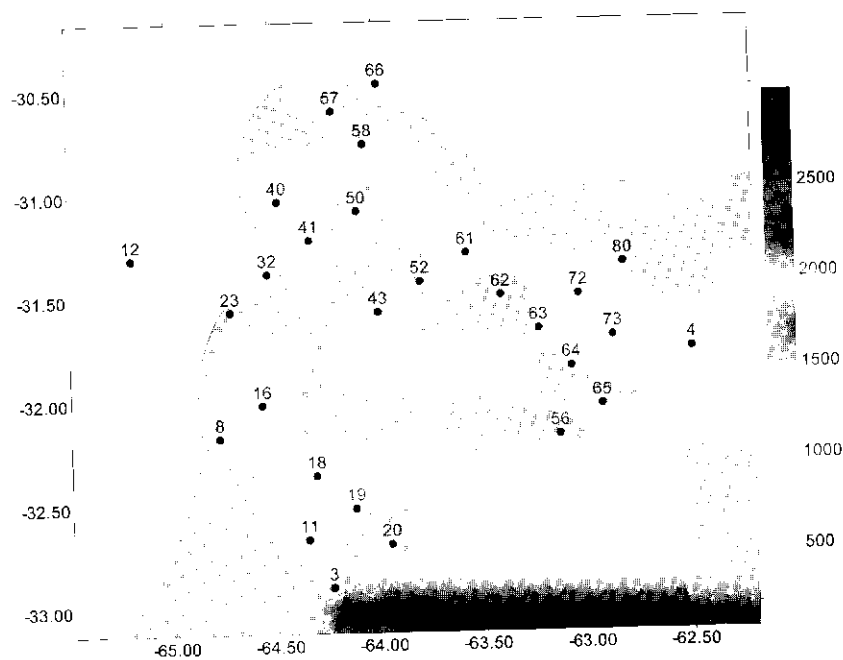


Figure 2 *R. celastri* content of iron ( $\mu\text{g g}^{-1}$  DW) in the survey area.

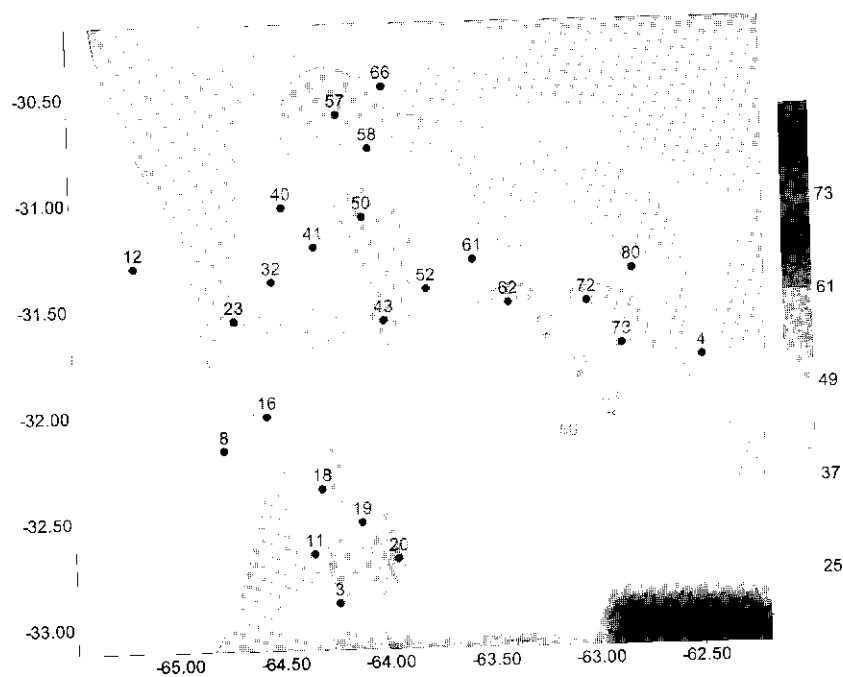


Figure 3 *R. celastri* content of manganese ( $\mu\text{g g}^{-1}$  DW) in the survey area.

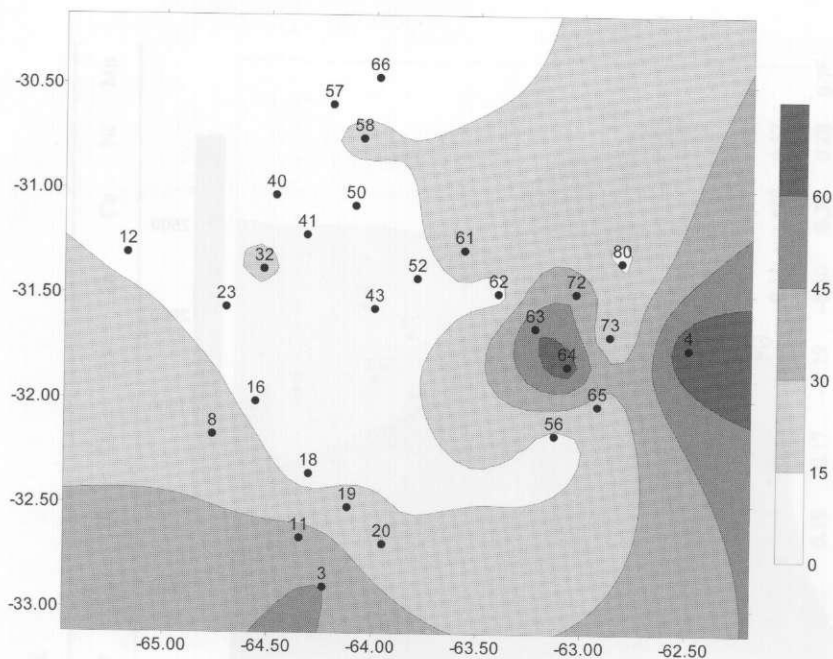


Figure 4 *R. celastri* content of zinc (µg g<sup>-1</sup> DW) in the survey area.

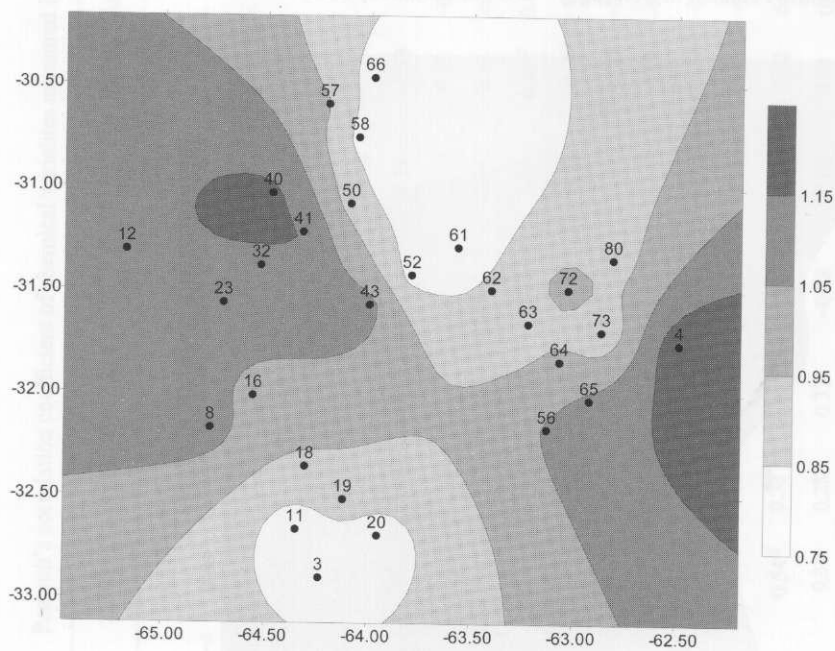


Figure 5 *R. celastri* content of lead (µg g<sup>-1</sup> DW) in the survey area.

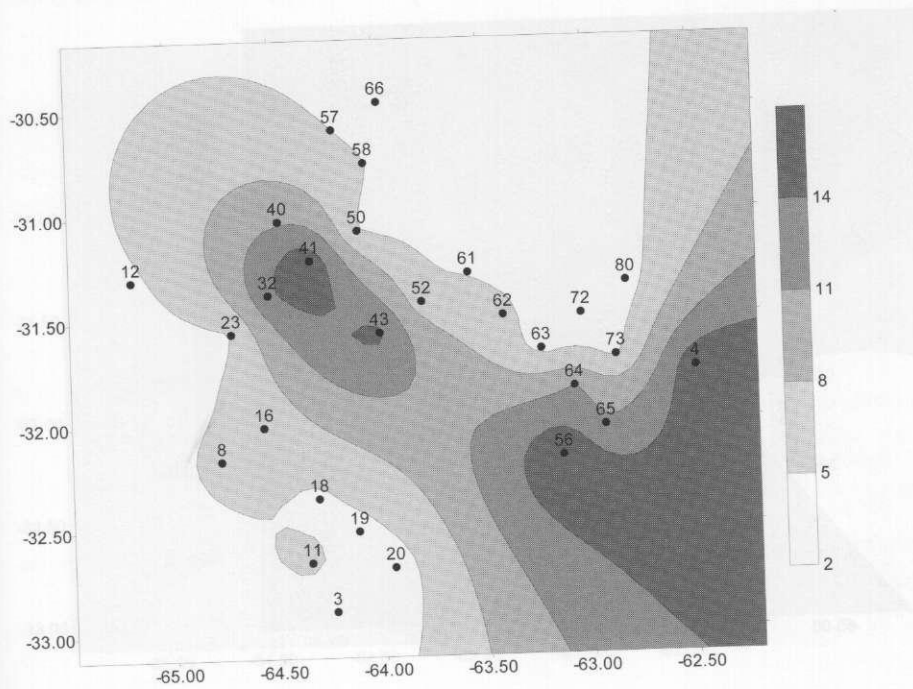


Figure 6 *R. celastri* content of copper ( $\mu\text{g g}^{-1}$  DW) in the survey area.

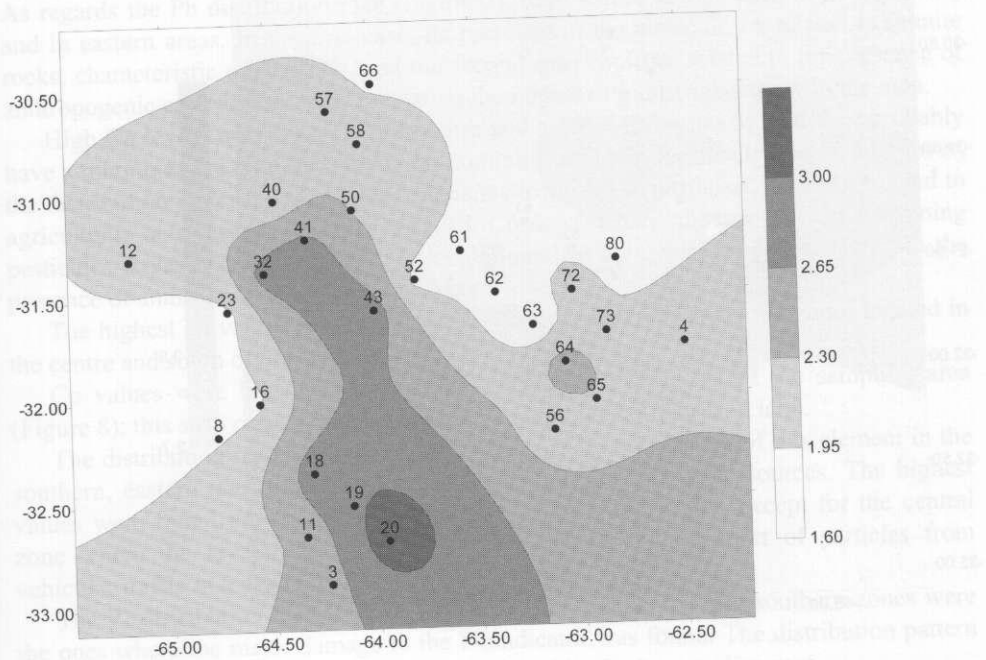
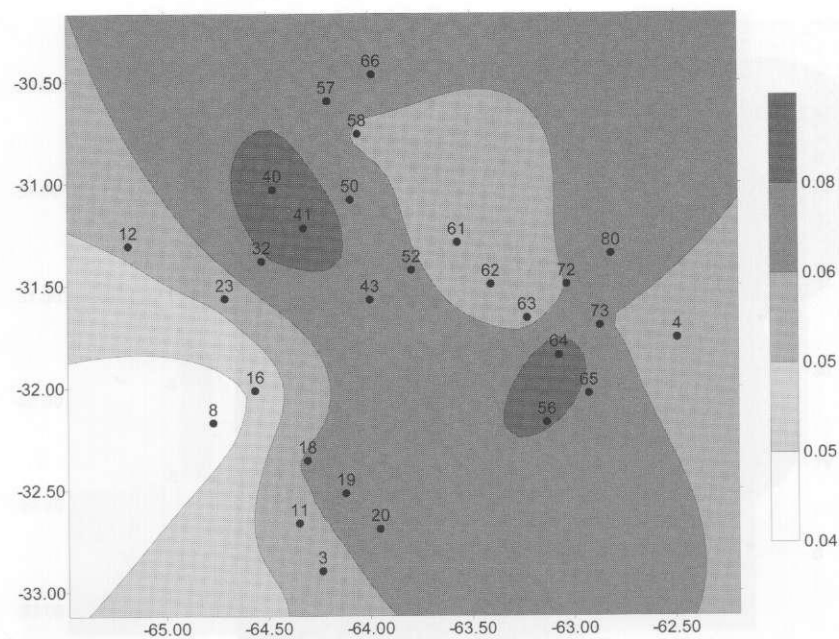
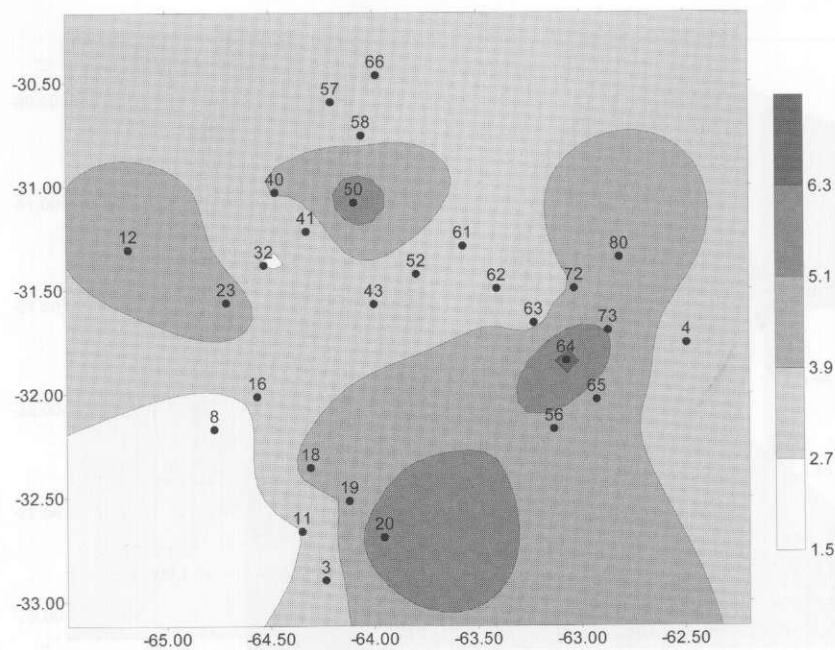


Figure 7 *R. celastri* content of nickel ( $\mu\text{g g}^{-1}$  DW) in the survey area.





**Figure 8** *R. celastri* content of cobalt ( $\mu\text{g g}^{-1}$  DW) in the survey area.



**Figure 9** *R. celastri* content of sulfur ( $\mu\text{g g}^{-1}$  DW) in the survey area.

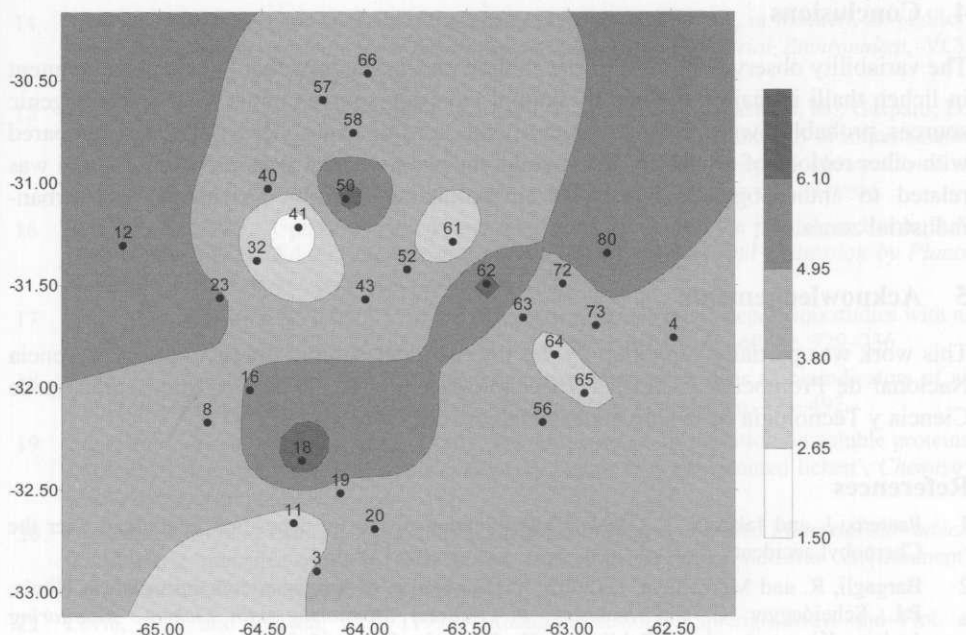


Figure 10 Pollution index (PI) distribution in the survey area.

As regards the Pb distribution map (Figure 5), high values can be seen in mountainous and in eastern areas. In the first case, its presence in the air could be related to granite rocks, characteristic of Córdoba, and the second case could be related to the presence of anthropogenic emission sources, indicating the impact of industrial activity in the area.

High Cu levels were found in the centre and southeast (Figure 6), and they probably have a mining origin with some particles coming from long-distance transport. Moreover, the distribution pattern, with a strong tendency towards the northeast, could be related to agricultural activities in the province of Córdoba where the use of Cu-containing pesticides is very common. The high levels found in the centre could be related to the presence of anthropogenic emission sources.

The highest Ni values (Figure 7) observed were related to industrial zones located in the centre and south of the study area.

Co values were higher from the northwest to the southeast of the sampling area (Figure 8); this strip corresponds mostly to farming and cattle-raising land.

The distribution map of sulfur (Figure 9) shows a high content of this element in the southern, eastern and central zones, which reveals anthropogenic sources. The highest values were found in areas with an important industrial activity, except for the central zone where the levels of sulfur could be related to the transport of particles from vehicular traffic in a nearby city.

The PI distribution map (Figure 10) shows that the central and southern zones were the ones where the major damage to the bioindicator was found. The distribution pattern shows the impact of the urban and industrial activities in the sampling area.

#### 4 Conclusions

The variability observed in most of the studied metals suggests that the elemental content in lichen thalli is mainly defined by natural emission sources rather than anthropogenic sources, probably owing to the low rate of emissions of atmospheric pollutants compared with other regions of the world. Meanwhile, the physiological response of *R. celastri* was related to anthropogenic sources of air pollutants, mainly agricultural and urban-industrial zones.

#### 5 Acknowledgements

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#### References

- 1 Paatero, J. and Jakkola, T. (1994) 'Determination of the Pu deposition in Finland after the Chernobyl accident', *Radiochemica Acta*, Vol. 64, pp. 139–144.
- 2 Bargagli, R. and Mikhailova, I. (2002) 'Accumulation of inorganic contaminants', in Nimis, P.L., Scheidegger, C. and Wolseley, P. (Editors) *Monitoring with Lichens: Monitoring Lichens*, Kluwer Academic Publishers, The Netherlands, pp. 65–84.
- 3 Nylander, W. (1866) 'Les lichens du Jardin du Luxembourg', *Bulletin Societe Botanique Francaise*, Vol. 13, pp. 364–372.
- 4 Galun, M. and Ronen, R. (1988) 'Interaction of lichens and pollutants', *CRC Handbook of Lichenology*, Vol. 3, pp. 55–72.
- 5 Nimis, P.L. (1990) 'Air quality indicators and indices: the use of plants as bioindicators of air pollution', Colombo, A.G. and Premazzi, G. (Editors), Ispra, JRC, Italy.
- 6 Loppi, S., Corsini, A., Chiti, F. and Bernardi, L. (1992) 'Preliminary data on the integrated use of lichens as indicators and monitors of atmospheric pollutants in central Italy', *Giornale Botanico Italiano*, Vol. 126, pp. 360–365.
- 7 Seaward, M.R.D. (1992) 'Large scale air pollution monitoring using lichens', *GeoJournal*, Vol. 28, p. 403.
- 8 Gries, C. (1996) 'Lichens as indicators of air pollution', in Nash, T.H. III (Editor) *Lichen Biology*, Cambridge University Press, Cambridge, pp. 240–254.
- 9 Loppi, S. (1996) 'Lichens as indicators of geothermal air pollution in central Italy', *Bryologist*, Vol. 99, pp. 41–48.
- 10 Ronen, R. and Galun, M. (1984) 'Pigment extraction from lichens with DMSO and estimation of chlorophyll degradation', *Environmental and Experimental Botany*, Vol. 24, pp. 239–245.
- 11 Garty, J., Kardish, N., Hagemeyer, J. and Ronen, R. (1988) 'Correlations between the concentration of adenosine triphosphate, chlorophyll degradation and the amounts of airborne heavy metals and sulfur in a transplanted lichen', *Archives of Environmental Contamination and Toxicology*, Vol. 17, pp. 601–611.
- 12 Balaguer, L. and Manrique, E. (1991) 'Interaction between sulfur dioxide and nitrate in some lichens', *Environmental and Experimental Botany*, Vol. 31, pp. 223–227.
- 13 Kardish, N., Ronen, R., Bubrik, P. and Garty, J. (1987) 'The influence of air pollution on the concentration of ATP and on chlorophyll degradation in the lichen *Ramalina duriaiei* (De Not.) Bagl', *New Phytology*, Vol. 106, pp. 697–706.

- 14 Garty, J. (1993) 'Lichens as biomonitors for heavy metal pollution', in Markert, B. (Editor) *Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment*, VCH Publishers, New York, pp. 193–263.
- 15 Nimis, P.L., Cicarelli, A., Lazzarin, G., Bargagli, R., Benedet, A., Castello, M., Gasparo, D., Lausi, D., Olivieri, S. and Tretiach, M. (1989) 'I licheni come bioindicatori di inquinamento atmosferico nell'area di Schio-Thiene-Breganze (VI)', in *Bolletino del Museo Civico di Storia Naturale di Verona*, Vol. 16, CO.GE.V. s.r.l., Verona, Ecothema s.r.l., Trieste.
- 16 Baker, D.A. (1983) 'Uptake of cations and their transport within the plants', in Robb, D.A. and Pierpoint, W.S. (Editors) *Metals and Micronutrients: Uptake and Utilization by Plants*, Academic Press, London, pp. 3–19.
- 17 Nash, T.H. and Gries, C. (1995) 'The use of lichens in atmospheric deposition studies with an emphasis on the Arctic', *The Science of the Total Environment*, Vol. 160, pp. 729–736.
- 18 Conti, M.E. and Cecchetti, G. (2001) 'Biological monitoring: lichens as bioindicators of air pollution assessment: a review', *Environmental Pollution*, Vol. 114, pp. 471–492.
- 19 González, C.M. and Pignata, M.L. (1994) 'The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulfur and heavy metals in a transplanted lichen', *Chemistry and Ecology*, Vol. 9, pp. 105–113.
- 20 González, C.M. and Pignata, M.L. (1997) 'Chemical response of lichen *Punctelia subrudecta* (Nyl.) Krog transplanted close to a power station in an urban-industrial environment', *Environmental Pollution*, Vol. 97, pp. 195–203.
- 21 Levin, A.G. and Pignata, M.L. (1995) '*Ramalina ecklonii* (Spreng.) Mey. and Flot. as bioindicator of atmospheric pollution in Argentina', *Canadian Journal of Botany*, Vol. 73(8), pp. 1196–1202.
- 22 Sloof, J.E. (1993) 'Environmental lichenology: biomonitoring trace-element air pollution', Thesis, Delft University of Technology, Delft, The Netherlands.
- 23 Pfeiffer, H.N. and Barclay-Estrup, P. (1992) 'The use of a single lichen species, *Hypogimnia physodes*, as an indicator of air quality in Northwestern Ontario', *Bryologist*, Vol. 95, pp. 3–41.
- 24 Winternans, J.F.G.M. and De Mots, A. (1965) 'Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol', *Biochimica et Biophysica Acta*, Vol. 169, pp. 448–453.
- 25 Heath, R.L. and Castillo, F.J. (1988) 'Membrane disturbances in response to air pollutants', in Shulte-Hostede, S., Darral, N.M.D., Blank, L.W. and Wellburn, A.R. (Editors) *Air Pollution and Plant Metabolism*, Elsevier Applied Science, London, pp. 55–75.
- 26 Kosugi, H., Jojima, T. and Kikugawa, K. (1989) 'Thiobarbituric acid-reactive substances from peroxidized lipids', *Lipids*, Vol. 24, pp. 873–881.
- 27 González, C.M., Casanovas, S.S. and Pignata, M.L. (1996) 'Biomonitoring of air pollutants from traffic and industries employing *Ramalina ecklonii* (Spreng.) Mey. and Flot.', *Environmental Pollution*, Vol. 91, pp. 269–277.
- 28 Sakaki, T., Kondo, N. and Sugahara, K. (1983) 'Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation', *Physiologia Plantarum*, Vol. 59, pp. 28–34.
- 29 Cañas, M.S., Orellana, L. and Pignata, M.L. (1997) 'Chemical response of lichens *Parmotrema austrosinense* and *P. conferendum* transplanted to urban and non-urban environments', *Annales Botanica Fennici*, Vol. 34, pp. 27–34.
- 30 Steinnes, E., Rambaek, J.P. and Hanssen, J.E. (1992) 'Large scale multi-element survey of atmospheric deposition using naturally growing moss as biomonitor', *Chemosphere*, Vol. 25, pp. 735–752.
- 31 Novo, R.J., Cragnolini, C.L., Cavallo, R.L. and Conces, M.Y. (1998) 'Fungicidas', Departamento de Protección Vegetal y Terapéutica Vegetal, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, pp. 8–48.