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Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens

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"Capsule": *The transplanted lichen* Usnea amblyoclada *was used to determine relative air quality in Córdoba, Argentina.*

Abstract

The objective of the present study was to test the concentrations of some elements in the transplanted lichen *Usnea amblyoclada* transplanted in Córdoba, Argentina, and to investigate the relative air quality of the area as indicated by a Pollution Index. Analyses of Cu, Co, Pb, Fe, Ni, Mn, S and Zn in addition to analyses of physiological parameters were performed after the transplantation period. No significant differences were observed among the sampling stations for the physiological parameters, except the dry weight/fresh weight ratio. The concentration of most elements was similar to or lower than those found in non-polluted and even polluted areas. The significant correlation found between Cu, Pb and Zn with the content of hydroperoxy conjugated dienes suggests an important oxidative effect probably caused by these ions. The distribution patterns of the elements were quite similar, with maximum values around a cement plant and the metallurgical industries. The Pollution Index distribution pattern does not coincide with the elements distribution, due to the fact that the index values probably reflected the emissions of gaseous phytotoxic pollutants.

Keywords: *Usnea amblyoclada*; Lichens; Biomonitoring; Air quality

1. Introduction

Heavy metals have been the object of many studies since they are persistent and belong to the most widely dispersed industrial pollution. Several aims have been pursued such as the impact of heavy metal pollution on natural or urban ecosystems.

Lichens were recognized as potential indicators of air pollution as early as the 1860s in Europe (Hawksworth and Rose, 1976). Since then, lichens have played prominent roles in air pollution studies throughout the world. They have been described as one of the best biomonitors in the assessment of airborne heavy metal pollution because they can accumulate and tolerate metal level far above their expected physiological needs (Garty, 1988) due to their relatively large surface area and slow growth rate. Epiphytic lichens have a great advantage over other plants in that there is no possibility of uptake of metals from the soil, and their morphology do not change with season. Therefore, they have been widely used as bioindicators of heavy metals in national or regional surveys (Freitas et al., 1999; Garty, 2000; Nimis et al., 1993).

In Argentina, the great industrial expansion, the increasing number of vehicles and the large concentration of people in major cities, together with an unfortunate lack of environmental policies, are forecasting serious environmental problems. However, unlike the situation present in other countries of the region, in Argentina the information about atmospheric pollutant levels is very scarce and the measurement of atmospheric pollutants has only recently started in a few urban centers (Klumpp et al., 2000). Those measurements comprised gaseous pollutants and particulate material (PM_{10}, minor to 10 μ), but at the time this study was done there was no systematic information about the atmospheric heavy metals levels in our country.

The purpose of the present study was to investigate the content of some heavy metals in transplanted thalli of

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Usnea amblyoclada (Müll. Arg.) Zahlbr., to evaluate the relative air quality of the area. Even if concentrations in the air cannot be extrapolated from concentrations in the lichens, these organisms seem to offer a most valuable tool to detect possible emitting sources, to delimit "high risk areas", and to study the deposition patterns of various elements. Thus, for the first time in this region we were able to obtain information about the relative quality of the air with reference to different heavy metals in some rural, suburban and urban sites around Córdoba City, Argentina.

2. Materials and methods

2.1. Sampling area

The study area measures 20,000 km^2 and includes the city of Córdoba (1.4 million inhabitants) and its surroundings. There are two major car-manufacturing factories located to the southeast and southwest of the town, and many small car-part factories around them. To the north there is another industrial area where many small and medium sized industries, predominantly metallurgical, are located. To the west there is an important cement plant. Its highest chimney is 35.5 m tall and 4.0 m in diameter. This plant uses fossil fuel and 14% of secondary fuels such as waste oil, waste tyres, oil contaminated soils, polymeric plastics, tars, solvents, mixtures and emulsions of hydrocarbons and water, waste paints, latex, photo products, etc. Heavy metals are brought into the cement kiln with the raw materials, fossil and waste fuels and are emitted as gaseous pollutants: Pb, and Zn; or with the particulate at all stages of the cement production: Cd, Hg, Ti, Be, As, Ni, Se, Fe, Sb, Pb, Cr, V and Zn. Another important source of heavy metal pollution could be the city of Córdoba, where higher than normal seasonal average values of NO_x, CO and PM_{10} (Olcese and Toselli, 1998) have been measured. The climate is sub-humid, with an average annual precipitation of 790 mm, concentrated principally in summer. Mean annual temperature is 17.4°C and prevailing winds originate in the northeast and southeast. Natural vegetation belongs to the Espinal Phytogeographical Province (Cabrera, 1971) which consists of low thorny woodlands.

In the chosen area 29 sampling points were selected taking into account the location of the most important fixed emission sources and the distribution of mobile sources. They were located at least 300 m from main roads and at least 100 m from any road or house. Due to the loss of some lichen bags, only 24 bags were collected from the study area (Fig. 1).

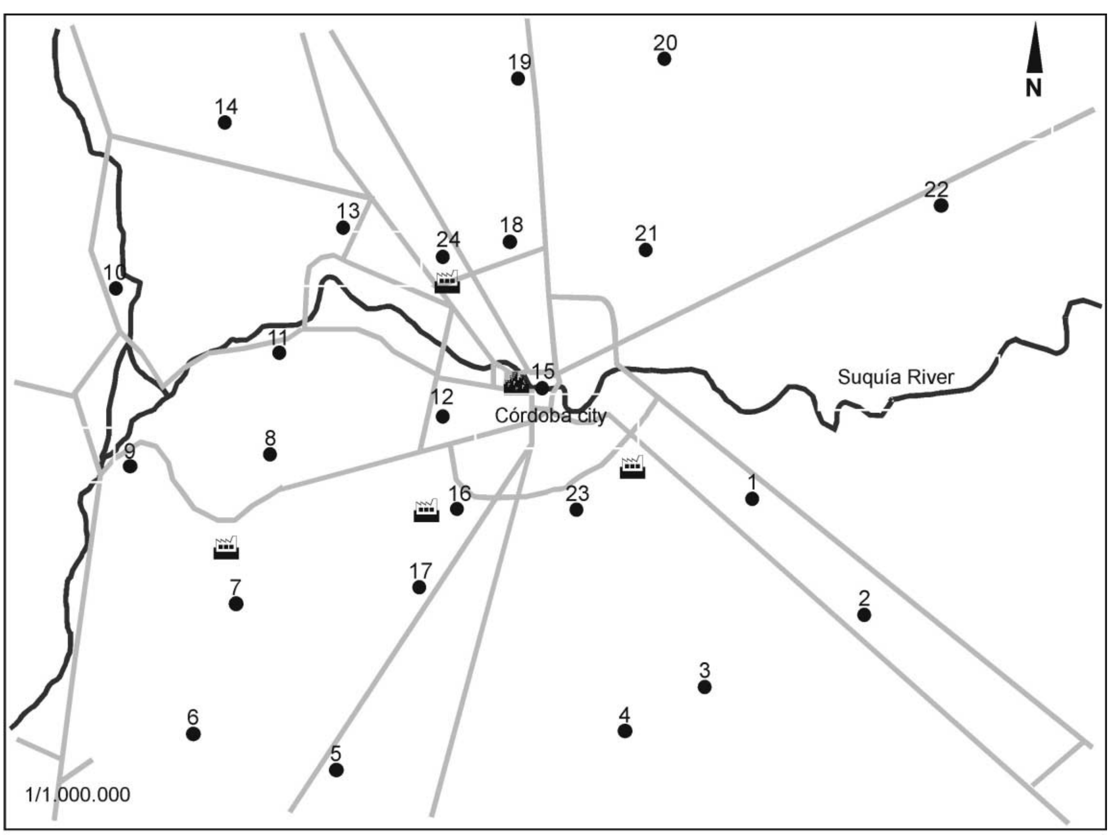

Fig. 1. Sampling stations and location of the main emission sources in the lichen survey made in Córdoba, Argentina, 1997 (Industrial areas; urban areas).

2.2. Sampling and sample preparation

The species selected for sampling is the foliose lichen *Usnea amblyoclada* (Müll. Arg.) Zahlbr. This species was chosen because of its wide distribution in the Córdoba province, it can be easily separated from the rock substrate and it had already been used in biomonitoring studies in the area (Carreras et al., 1998; Carreras and Pignata, 2001).

Thalli of *U. amblyoclada* with a rock substrate were collected from an area with a low pollution level: Los Gigantes, 70 km west of the city of Córdoba. The basal parts of lichen thalli with adhering pieces of rock were detached and samples were stored in the laboratory at room temperature until exposure. They were not washed in order to avoid the leaching of soluble substances.

Lichen-bags were prepared by weighing out 6.0 g fresh weight, and packing it loosely in a fine nylon net. During the period May–August 1997, three lichen bags (each sample consisting of several thalli) were tied on a nylon rope on three different posts at a height of 3 m above the ground and exposed for 3 months at each monitoring station.

In the laboratory both the exposed samples and baseline material were air-dried and homogenized in a mortar with a pestle. After that they were freeze-dried in polythene bags. Three sub-samples of the homogenized material were taken from each lichen bag using plastic spatulas to obtain a mean arithmetic value ± standard deviation for each chemical determination.

2.3. Physiological determinations

The procedure followed for the quantification of chlorophyll a (Chl a), chlorophyll b (Chl b), phaeophytin a (Phaeo a), phaeophytin b (Phaeo b), carotenoids (Carot), hydroperoxy conjugated dienes (HPCD), malondialdehyde (MDA), dry weight/fresh weight ratio (DW/FW) and other ratios, are explained with detail in Carreras et al. (1998) and Carreras and Pignata (2001). Some of these parameters were used to calculate the Pollution Index (PI) cited for this species in Carreras et al. (1998). The HPCD content was replaced by the content of MDA in this index, due to the fact that the latter has more incidences in the data variability, according to the results of the principal component analysis made with physiological variables.

2.4. Heavy metal determination

An accurately weighed portion of each sample (1 g DW) was placed in a porcelain crucible and ashed at 500°C for 45 min. Ashes were digested using a 5:1 mixture of HCl (18%) and HNO_3 (conc.) and heated until boiling. The solid residue was centrifuged off and diluted to 50 ml volume with ultrapurified water. Analyses for Cu, Co, Pb, Fe, Ni, Mn and Zn 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 check on the analytical method, the certified reference material IAEA/V-10 Hay Powder was analyzed every 10 samples.

2.5. Sulfur determination

Five milliliters of Mg $(NO_3)_2$ saturated solution were added to 0.5 g of lichen and dried in an electric heater. Subsequently, the sample was heated in an oven for 30 min at 500°C. The ashes were then suspended in HCl 6M, filtered, and the resulting solution boiled for 3 min. The solution was brought to 50 ml with distilled water.

The amount of SO_4^{2-} in the solution was determined by the acidic suspension method with barium chloride (González and Pignata, 1994) which subsequently allowed for the calculation of sulfur content in each sample. The concentration was expressed in mg of total sulfur g^{-1} dry weight.

2.6. Statistical analysis

Physiological parameters and the content of some elements were submitted to a variance analysis among the sampling stations and were used to calculate Pearson's coefficients of correlation between them.

Besides, the concentration of metals and sulfur in lichens from each sampling station was used to construct a series of distribution maps by a mapping program. The differences among zones determined by the mapping procedure were also checked by variance analysis.

3. Results and discussion

The mean concentration of HPCD, MDA, DW/FW, Chl b/Chl a and Phaeo a/Chl a in fresh and transplanted lichens are presented in Table 1. No significant differences were observed between the fresh and the transplanted lichen, except for the DW/FW ratio and HPCD ($P = 0.000$ and 0.02, respectively). No significant differences were observed among the sampling stations for any of these parameters, but the DW/FW ratio.

Regarding the elements measured in fresh and transplanted lichens, Fe, Mn, Pb, S and Zn were higher in transplanted lichens than in fresh thalli. Meanwhile, the values measured in fresh thalli for Ni were similar to (and in site four even higher than) the values measured in transplanted lichens. A remarkable heterogeneity in the amounts of Fe accumulated by lichens was found among the sampling sites. The concentrations of other

Table 1
Mean values ± standard deviation of physiological parameters quantified in fresh and transplanted *Usnea amblyoclada* to 24 stations in Córdoba, Argentina

St.	*n*	HPCD mmol g^{-1} DW	MDA μmol g^{-1} DW	DW/FW	Chl b/Chl a	Phaeo a/Chl a
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Fresh	3	0.127 ± 0.019	0.124 ± 0.006	0.916 ± 0.002	0.725 ± 0.183	2.097 ± 0.182
1	3	0.157 ± 0.010	0.125 ± 0.022	0.896 ± 0.007	0.633 ± 0.267	1.735 ± 0.137
2	3	0.167 ± 0.037	0.126 ± 0.014	0.894 ± 0.019	0.549 ± 0.059	2.197 ± 0.395
3	2	0.150 ± 0.055	0.126 ± 0.005	0.909 ± 0.002	0.651 ± 0.173	2.025 ± 0.053
4	3	0.199 ± 0.005	0.128 ± 0.023	0.922 ± 0.008	0.573 ± 0.120	2.001 ± 0.088
5	3	0.123 ± 0.010	0.126 ± 0.014	0.927 ± 0.023	0.580 ± 0.031	1.952 ± 0.344
6	2	0.153 ± 0.004	0.123 ± 0.038	0.868 ± 0.005	0.591 ± 0.043	2.016 ± 0.245
7	2	0.128 ± 0.025	0.119 ± 0.016	0.918 ± 0.002	0.457 ± 0.141	1.970 ± 0.247
8	2	0.227 ± 0.025	0.130 ± 0.021	0.911 ± 0.001	0.553 ± 0.188	2.100 ± 0.385
9	3	0.164 ± 0.035	0.146 ± 0.002	0.884 ± 0.021	0.640 ± 0.107	1.937 ± 0.155
10	2	0.146 ± 0.011	0.121 ± 0.003	0.881 ± 0.008	0.611 ± 0.072	1.954 ± 0.060
11	2	0.165 ± 0.035	0.132 ± 0.018	0.925 ± 0.006	0.799 ± 0.185	1.840 ± 0.212
12	2	0.175 ± 0.040	0.140 ± 0.002	0.908 ± 0.000	0.631 ± 0.249	2.187 ± 0.184
13	3	0.167 ± 0.055	0.132 ± 0.014	0.911 ± 0.009	0.654 ± 0.413	1.983 ± 0.078
14	3	0.159 ± 0.021	0.144 ± 0.009	0.854 ± 0.000	0.498 ± 0.105	1.666 ± 0.132
15	3	0.151 ± 0.018	0.133 ± 0.018	0.914 ± 0.008	0.626 ± 0.236	1.872 ± 0.129
16	3	0.190 ± 0.080	0.131 ± 0.019	0.944 ± 0.002	0.538 ± 0.166	1.921 ± 0.251
17	2	0.218 ± 0.057	0.136 ± 0.003	0.927 ± 0.023	0.812 ± 0.059	2.018 ± 0.227
18	2	0.182 ± 0.055	0.135 ± 0.015	0.929 ± 0.008	0.694 ± 0.141	2.059 ± 0.044
19	2	0.216 ± 0.001	0.128 ± 0.004	0.946 ± 0.003	0.722 ± 0.041	2.093 ± 0.127
20	2	0.228 ± 0.006	0.131 ± 0.015	0.935 ± 0.007	0.710 ± 0.137	2.130 ± 0.239
21	2	0.151 ± 0.031	0.119 ± 0.011	0.933 ± 0.003	0.786 ± 0.201	2.053 ± 0.354
22	3	0.175 ± 0.011	0.135 ± 0.003	0.945 ± 0.001	0.476 ± 0.106	1.903 ± 0.092
23	3	0153 ± 0.019	0.130 ± 0.003	0.934 ± 0.011	0.672 ± 0.064	2.102 ± 0.171
24	2	0.148 ± 0.028	0.116 ± 0.015	0.945 ± 0.012	0.282 ± 0.215	2.142 ± 0.051
ANOVA		ns	ns	*	ns	ns

Results of the variance analysis among sampling points (*$P<0.05$) are also presented. HPCD, hydroperoxy conjugated dienes; MDA, malondialdehyde; DW/FW, dry weight/fresh weight ratio; Chl a, chlorophyll a; Chl b, chlorophyll b; Phaeo a, phaeophytin a.

metals were more homogeneous and high levels were observed in relation to emission sources. The analysis of variance among sampling stations showed significant differences for Co, Mn, Ni and Fe (data not shown).

Table 2 shows the mean concentration of the elements measured in this study and data obtained from other authors in different lichen species. Even if baseline levels are still low, metal values in transplanted lichens were similar or lower than those reported in polluted or non-polluted areas, probably due to the short exposure time. However, Cu levels were higher than the ones cited by other authors even for in situ studies. Fe, Ni, Pb and Zn levels were lower than the ones found by Garty et al. (1996) using transplants of *Ramalina* during a 45-day exposure period in Israel. The use of the coefficient of variation (CV) has been suggested for distinguishing between metals incorporated in lichen thalli as particulate or small, well-dispersed particles (Garty and Ammann, 1987). In the present study we analyzed the CV for each metal detected in *U. amblyoclada* and we compared it with data from other authors (Table 2). In the study area, the highest CV was for Ni (0.59), which suggests that this metal occurs partly or mainly in a particulate form. The lowest CV found for S, Zn and Pb (0.29 for all of them) suggests that they are dispersed as small particles and that many stations have similar pollution levels. These values are in perfect agreement with the CV reported by Garty and Ammann (1987) for metals in the thalli of *Pseudevernia furfuracea*, and those reported by Bargagli et al. (1987) for metals accumulated in *Parmelia caperata*.

Analysis of correlation between the content of some elements and physiological parameters is presented in Table 3. A significant correlation was found between Cu, Pb and Zn with the content of HPCD in transplanted lichens. This result suggests an important oxidative effect, probably caused by these ions, as peroxidation of unsaturated fatty acids is most probably found only during high oxidation pressure, as cited by Heath and Castillo (1988). A positive correlation was also observed between Pb and Chl b, Phaeo b and the ratio Chl b/Chl a, which would indicate a relationship between the degradation of chlorophyll a and the presence of this ion. An inverse relationship between Pb content and chlorophyll integrity was already found by Garty et al. (1985). The correlation between Co, Mn and Zn with the content of Phaeo b as well as Fe with the content of Phaeo a and b, reveals a direct

Table 2
Mean metal concentrations (μg g^{-1} DW) ± standard deviation (S.D.), coefficients of variation (CV) and methodology employed with lichens (*) corresponding to: (A) present study; (B) Bargagli et al., 1987; (C) Halonen et al., 1993; (D) Sloof, 1995; (E) Garty et al., 1996

	A 3 months *		B in-situ		C in-situ	D 1–12 months		E 46 days	
	Mean±S.D.	CV	Mean±S.D.	CV	Mean±S.E.	Mean±S.D.	CV	Mean±S.D.	CV
Co	0.16±0.06	0.36				1.90±0.25	0.13		
Cu	15.5±5.69	0.37	8.10±2.60	0.32	10.1±0.50			7.5±1.4	0.19
Fe	186±86.0	0.46			2.12±0.21			462±106	0.23
Mn	48.2±16.2	0.34			61.6±2.30			54.0±7.0	0.13
Ni	4.30±2.49	0.59	3.50±2.00	0.57	4.10±0.40			7.90±3.1	0.39
Pb	2.58±0.75	0.29	23.5±8.20	0.35	22.7±4.0			15.4±0.5	0.03
S	1.71±0.51	0.29			2.50±0.14				
Zn	29.0±8.36	0.29	48.5±11.3	0.23	88.3±3.2	166±36.0	0.21	87±14	0.16

Table 3
Correlations among heavy metal contents[a] and physiological variables quantified in the lichen *Usnea amblyoclada*

	Cu	Pb	Co	Fe	Mn	Zn
Chl b	0.23	*0.48*	0.33	0.23	0.33	0.35
Phaeo a	0.33	0.39	0.32	*0.46*	0.19	0.28
Phaeo b	0.30	*0.50*	*0.50*	*0.45*	*0.53*	*0.58*
HPCD	*0.43*	*0.46*	0.40	0.13	0.30	*0.45*
Chl b/ Chl a	0.13	*0.41*	0.28	0.04	0.34	0.34

[a] Only data that correlated significantly are shown.
Italic coefficients indicate that the correlation was significantly different from zero ($P<0.05$). Chl a, chlorophyll a; Chl b, chlorophyll b; Phaeo a, phaeophytin a; Phaeo b, phaeophytin b; HPCD, hydroperoxy conjugated dienes.

relationship between these ions and the degradation of chlorophyll to the corresponding phaeophytin.

The distribution maps of the elements are shown in Figs. 2–9. The mean concentration of the zones determined by the krigging method of the mapping procedure was checked by analysis of variance and resulted to be significantly different at $P<0.05$ for each element.

Cobalt shows the highest concentrations in the surroundings of the cement plant (Fig. 2). High levels were also observed to the north in relation to the industrial zone, and to the southeast and southwest in relation to the two major car-manufacturing factories.

Cu ions tend to bind with N and S containing molecules and are extremely toxic to lichens; they may be also detrimental in combination with sulfur dioxide (Nieboer and Richardson, 1981). In the survey area, the geographical distribution of Cu is mainly dominated by the cement plant emissions (Fig. 3). High values were also found near the industrial zone to the north and southwest. The high levels observed to the east could be related to the presence of agriculture farms where Cu-containing pesticides are usually used.

Fe is mainly associated with the coarse atmospheric particles; if associated with other sources, it is generally deposited in the neighborhood of the emission sources (Berg et al., 1995). In the study area, the content of Fe in transplanted lichens could be explained mainly by soil particle input. However, higher levels were also observed by the influence of the already mentioned cement plant and the car industries to the southeast that may contribute with some iron-containing particles (Fig. 4).

The distribution pattern of Mn is very similar to that of Co and shows high levels around the industrial areas (Fig. 5). The high concentrations detected in the eastern part may be derived also from the upper soil layer, blown off by wind.

It is well known that high Ni concentrations are indicative of the use of fossil fuels or the presence of power plants or metallurgical industries (Garty, 1993). The regional distribution of Ni in transplanted *U. amblyoclada* is largely explained by the oil-powered industries located to the north and to a lesser extent by the cement plant, because of the combustion of both oil and secondary fuels (Fig. 6). Another possible Ni source is the automobile exhaust (Garty, 1988), but this origin was not evident in our study.

Lichens are very efficient accumulators of Pb, through aerosols, particulate metal fallout or acid rain. Pb is bound to insoluble anionic sites, accumulated extracellularly and concentrated in the medulla. Once bound, it is not easily removed by rain or winds. Despite the introduction of unleaded fuel, Pb is still the most abundant heavy metal in urban environments (Monaci et al., 1997). The main source of Pb in rural areas or isolated industrial sites is any process involving combustion or ore reduction. Fig. 7 shows the distribution map of lead measured in *U. amblyoclada* in the study area. Its pattern makes two main contaminated areas evident: the surroundings of the cement plant, where Pb comes mainly from the secondary fuels used, and the industrial zone to the north.

The assessment of S content in lichens provides a good estimation of the atmospheric SO_2 concentration in rural, suburban and urban sites (Garty et al., 1988).

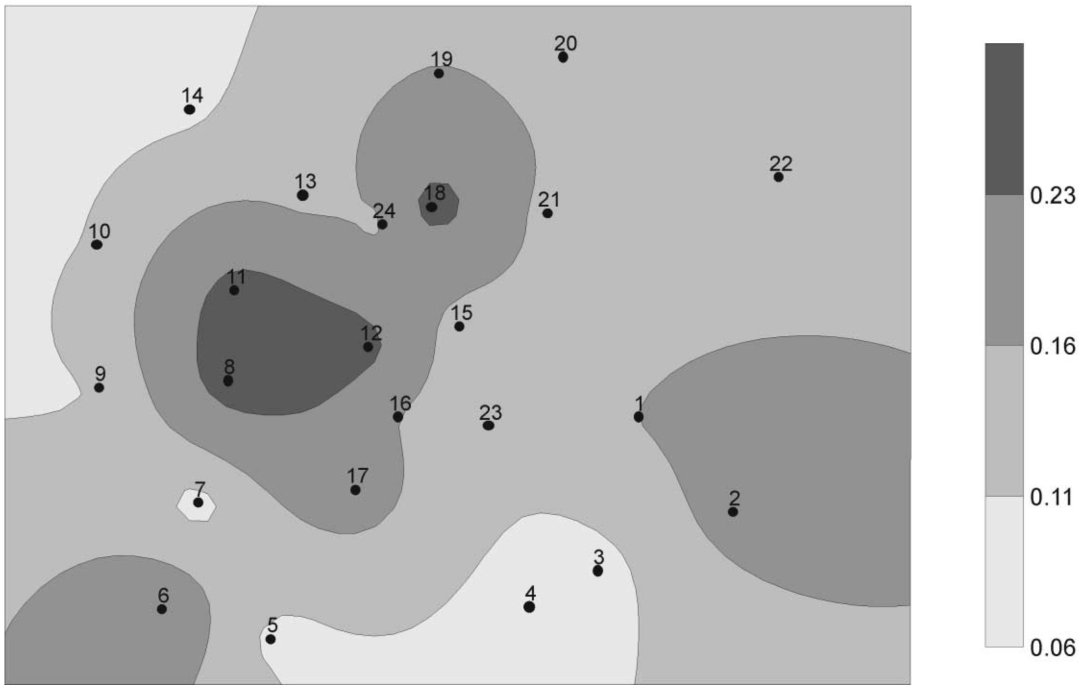

Fig. 2. Lichen content of cobalt (μg g^{-1} DW) in the survey area.

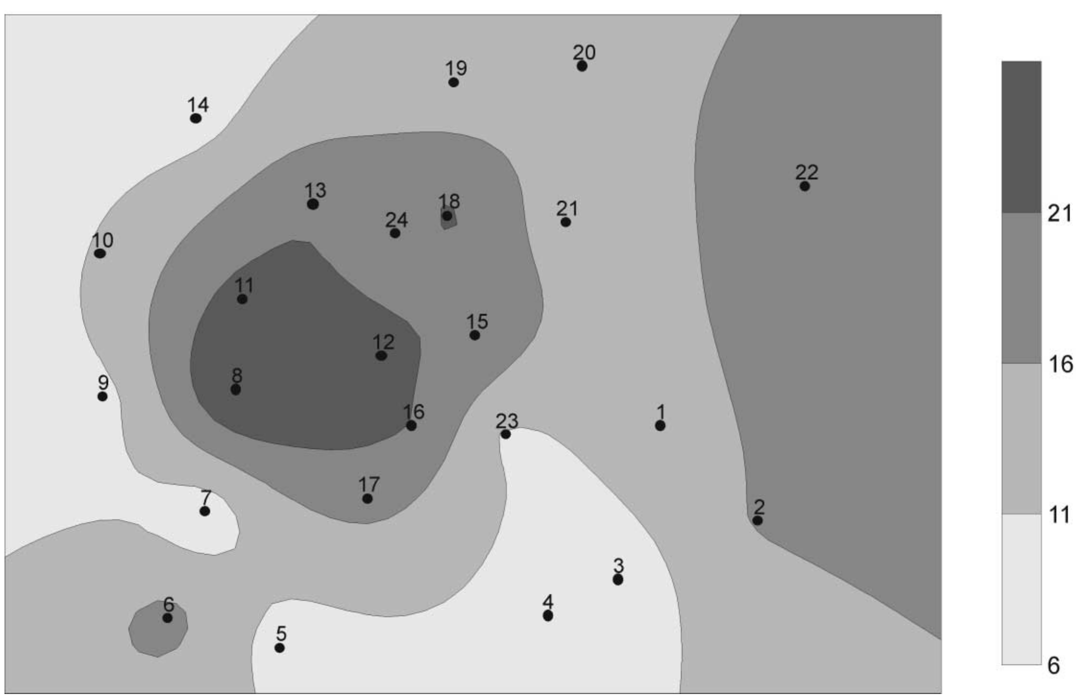

Fig. 3. Lichen content of copper (μg g^{-1} DW) in the survey area.

Sulfur dioxide is a by-product of coal or fuel oil combustion, many industrial processes and vehicle exhausts. The high concentrations of sulfur measured in lichens transplanted to the far surroundings of the industrial areas, points toward the transport of sulfur-containing particles derived from the combustion of heavy fuel oil (Fig. 8). No sulfur levels were detected in lichens associated with emissions from the cement plant.

Zn particles may derive from industrial sources, whereas the abrasion of tires of motor vehicles may be a second source of emission (Beckwith et al., 1985; Garty et al., 1988). The levels of Zn measured in the survey

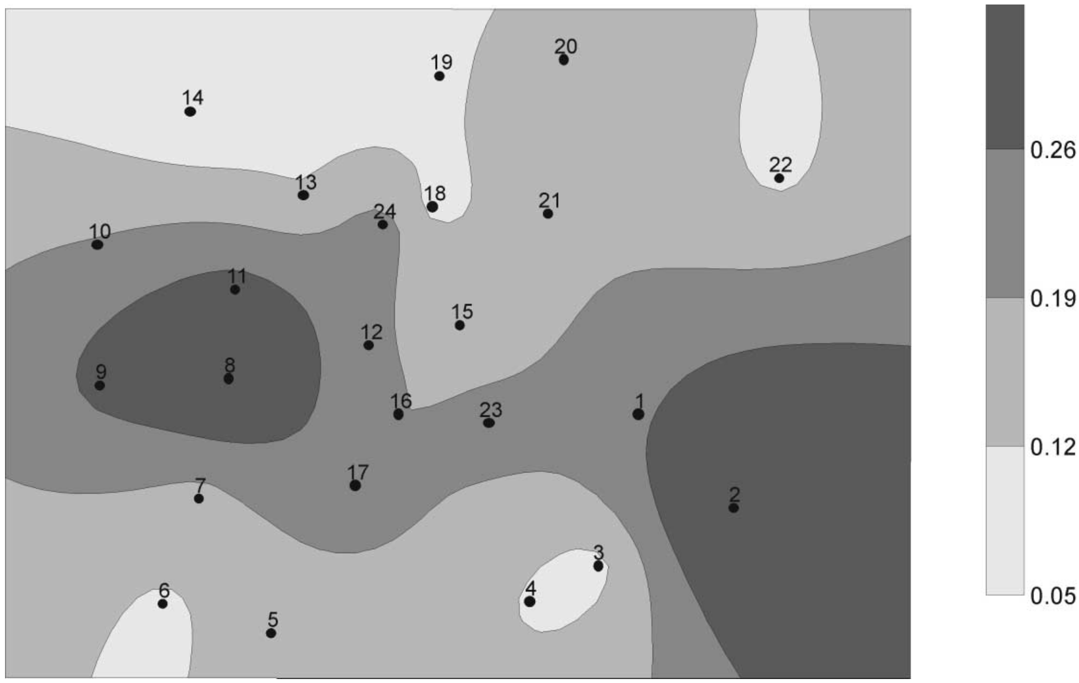

Fig. 4. Lichen content of iron (mg g^{-1} DW) in the survey area.

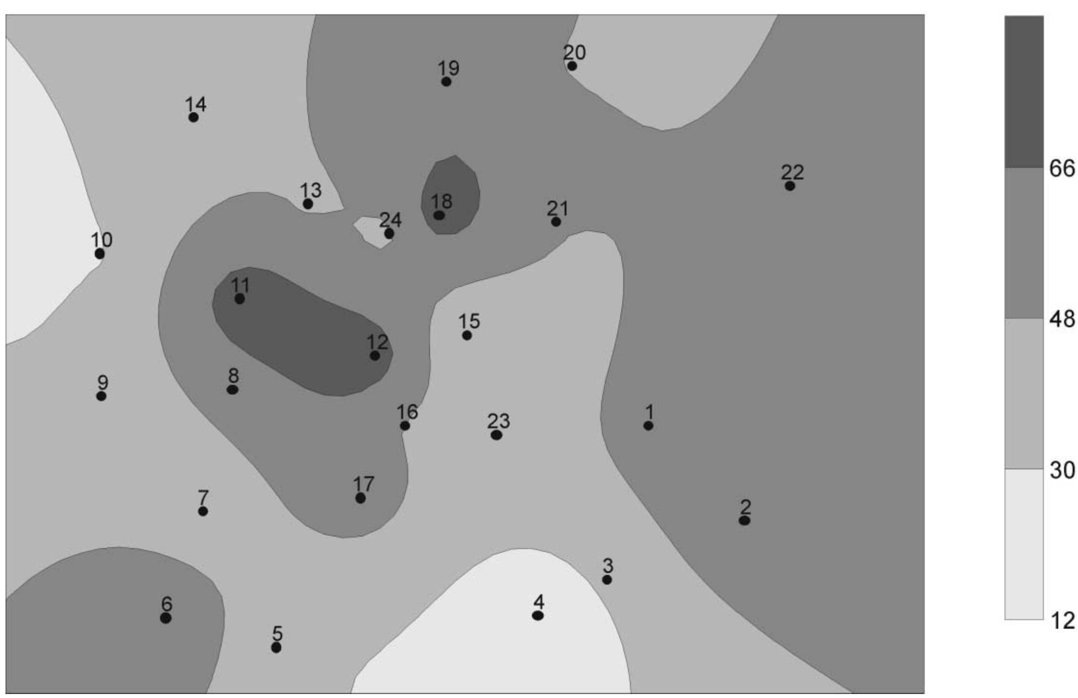

Fig. 5. Lichen content of manganese (μg g^{-1} DW) in the survey area.

area are associated mainly with the emission sources already mentioned: the cement plant, the metal-working industries and the car factories located to the southeast and southwest (Fig. 9). Another possible source could be linked to the use of Zn compounds as foliar nutrients for agriculture, as suggested by Garty (1988).

Fig. 10 shows the PI distribution pattern in the survey area. This index can be used to compare different sampling sites in terms of physiological damage on the bioindicator. Taking into account this pattern, it seems that lichens are more damaged by other gaseous pollutants than by the elements measured in this study. The

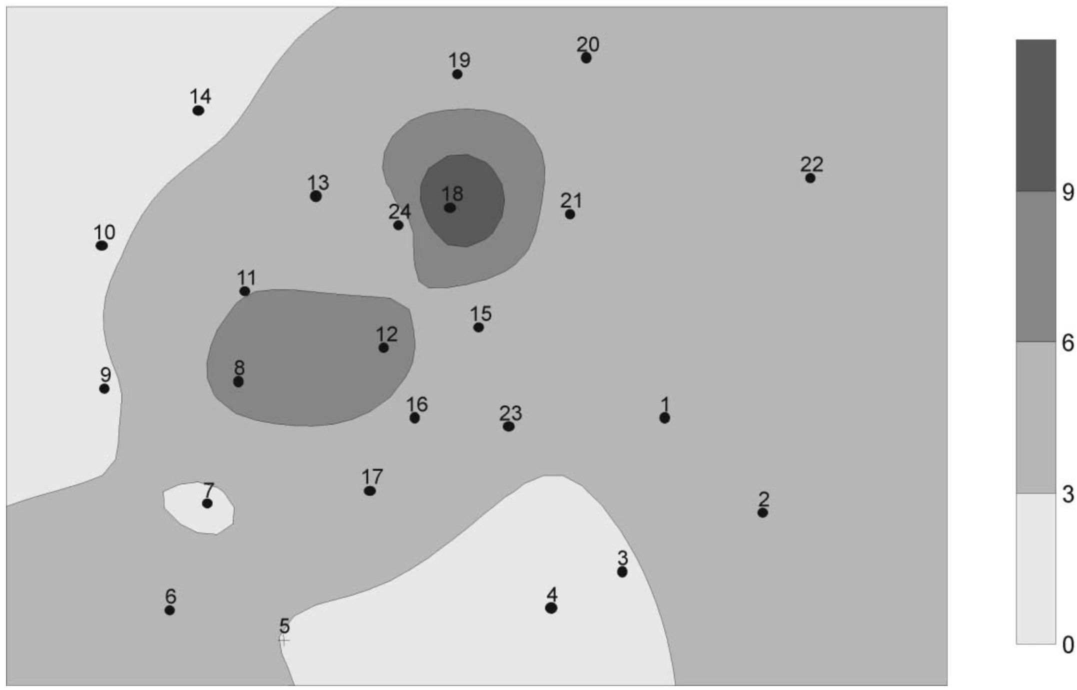

Fig. 6. Lichen content of nickel (μg g^{-1} DW) in the survey area.

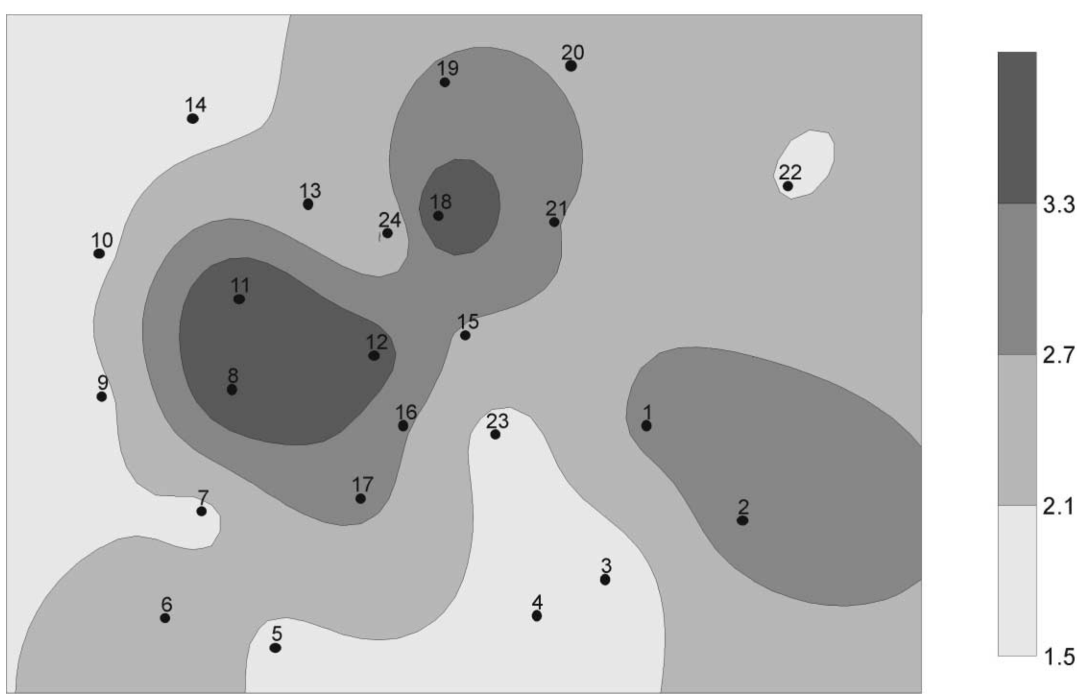

Fig. 7. Lichen content of lead (μg g^{-1} DW) in the survey area.

lack of a coincidence between the concentration of the elements measured in *U. amblyoclada* thalli and PI values, is probably due to the fact that PI values reflect environmental levels of phytotoxic gaseous pollutants such as SO_2 and NO_x rather than those of elements that are mainly associated with airborne particulates.

The lichen survey shown gives, in relative terms, a general idea of the state of the atmosphere on the pollutants Co, Cu, Fe, Ni, Zn, Pb, S and Mn in an area from Córdoba, Argentina. Although it is still hard to point out the exact sources of elements that reach the lichen thalli in the study area, their distribution patterns

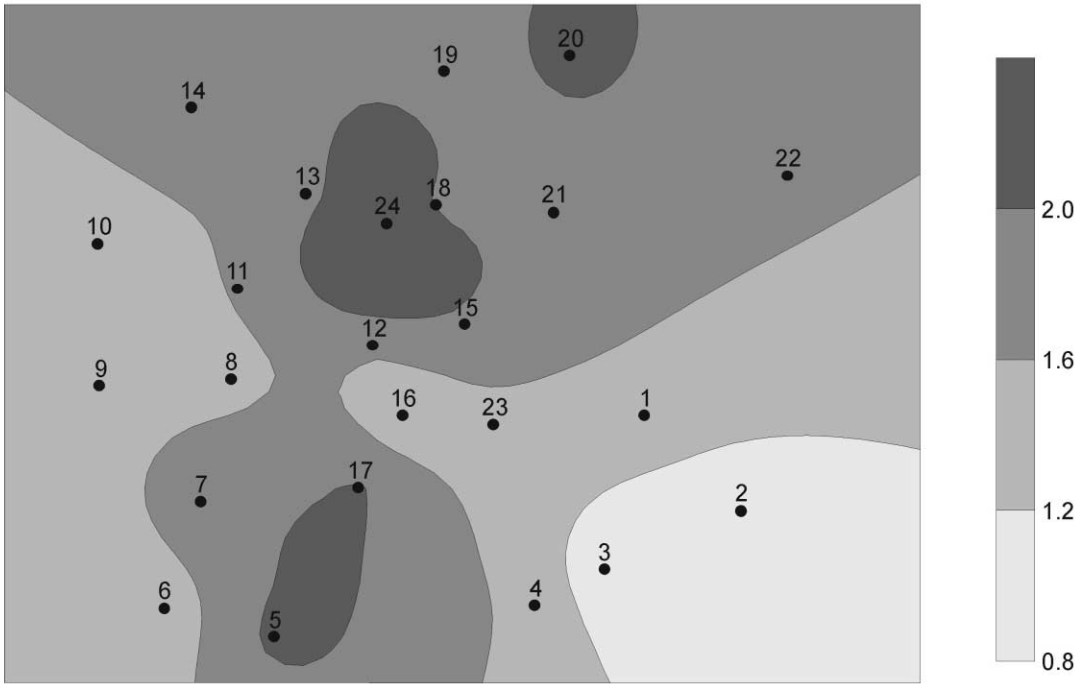

Fig. 8. Lichen content of sulfur (mg g^{-1} DW) in the survey area.

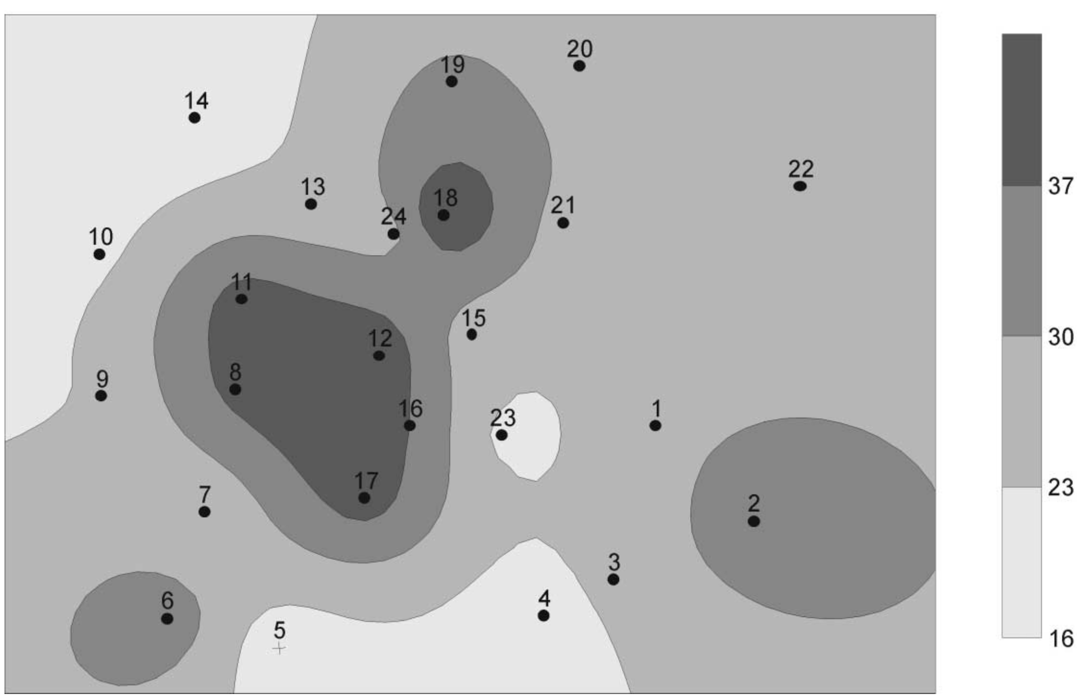

Fig. 9. Lichen content of zinc (μg g^{-1} DW) in the survey area.

helps to elucidate their origin. The main results can be summarized as follows:

1. When compared with other countries, this region of Argentina has very low content of the elements measured in this study.
2. Although the content of the elements measured is still low, their distribution pattern could indicate the areas where the highest pollution levels are most probable.
3. Most studies implementing the technique of lichen transplantation to monitor air pollutants were

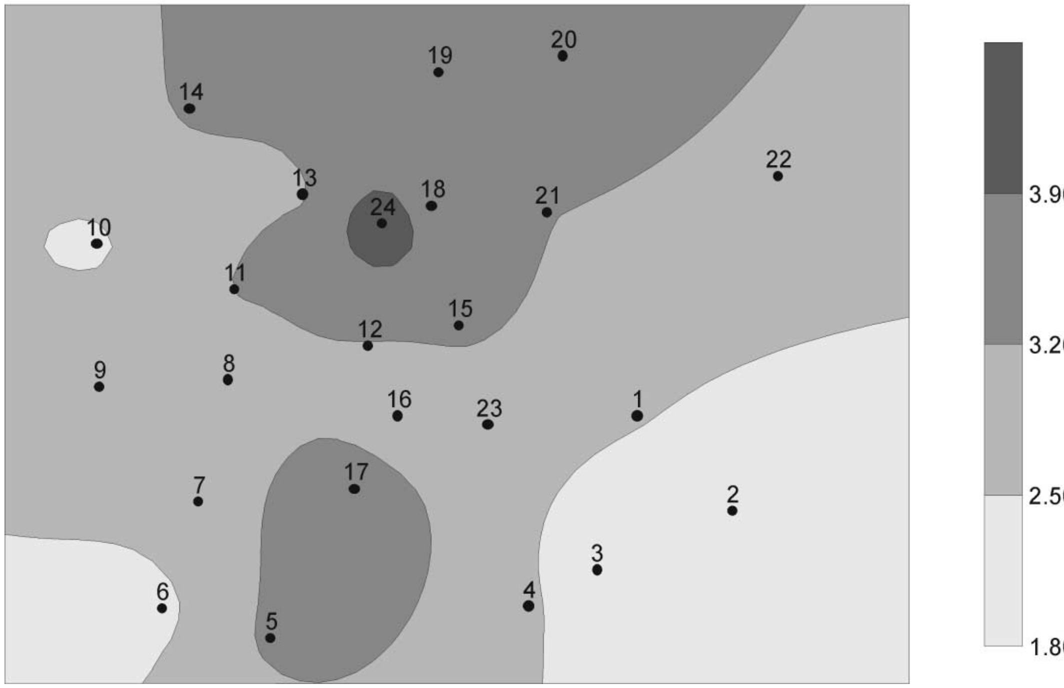

Fig. 10. Pollution Index (PI) distribution in the survey area.

carried out for rather long periods. Our study revealed that *U. amblyoclada* provides important information about the anthropogenic activity in a relatively short period of time.

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