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Assessment of the relationship between total suspended particles and the response of two biological indicators transplanted to an urban area in central Argentina

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ABSTRACT

Samples of the vascular plant *Tradescantia pallida* and the lichen *Usnea amblyoclada* were exposed from October 2004 to April 2005 in three sites with different local sources of air pollution in Córdoba city, Argentina. Simultaneous determinations of the ambient levels of total suspended particles were made for each site. Young inflorescenses of *T. pallida* were collected in November, February and April and the frequency of micronuclei was determined on early tetrads of pollen mother cells. Physiological parameters and the elemental composition of lichen thalli were measured from samples exposed and replaced every month. Significant differences among sampling sites were observed in the frequency of micronuclei measured in *T. pallida* as well as in many physiological parameters and elements accumulated in lichen thalli. The mass of particulate material as well as the concentration of Ca, Mn, Cu, Zn and Sr was significantly different in different sampling sites, too. These results suggest that *in situ* biomonitoring using both higher plants and lichens may be of use to characterize air pollution in areas devoid of instrumental monitoring techniques or where it is necessary to explore the distribution of air contaminants at a microscale.

1. Introduction

Biological indicators, which show an integrated response to air pollution and other environmental factors, can be used as a complementary system to monitor the effects of air pollutants and to provide reliable indications on the quality and characteristics of the environment. In this context, a number of approaches may be followed and a number of techniques may be applied according to specific needs (Nali et al., 2007). Lichens are the most widely used bioindicators of air pollution because they are largely dependant on the atmosphere for their nutrient supply, reflecting the gaseous, dissolved or particulate elements and compounds present in the air (Garty, 2001; Nimis and Purvis, 2002; Basile et al., 2008; Bergamaschi et al., 2007). Lichens are particularly useful to monitor air particulate matter because they absorb constituents from wet or dry precipitation more or less constantly during their entire life cycle and excretion is negligible, thus providing a measure of integrated exposure over a long period of time (Costa et al., 2002). Even though some fractions of particulate material can be located intracellularly, most elements are accumulated on the thallus surface or bound to the cell wall, binding sites extracellularly, readily interchangeable, thus reflecting recent environmental inputs (Bergamaschi et al., 2007).

On the other hand, monitoring genotoxins in the environment has become an important objective of public health due to the high risk of human exposure (Falistocco et al., 2000; Kim et al., 2003; Klumpp et al., 2006). For this reason, plant tests have been widely used for *in situ* monitoring of genotoxic pollutants in environmental media. The *Tradescantia* micronuclei (MCN) test, based on the formation of micronuclei resulting from chromosome breakage in the meiotic pollen mother cells, is one of the most used and validated plant bioassays because it is quick, simple and inexpensive (Falistocco et al., 2000; Klumpp et al., 2006). Positive results of this test were obtained in studies investigating traffic emissions and urban air (Guimaraes et al., 2000; Klumpp et al., 2006), air pollutants released by the rubber industry (Monarca et al., 2001), gas stove emissions (Monarca et al., 1998) and incinerators (Fomin and Hafner, 1998). It was also demonstrated that the MCN test is

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able to detect effects of individual genotoxic carcinogens formed as combustion products (Rodrigues et al., 1997).

Since air-monitoring networks in Argentina were not established until the 1990s and were then only implemented in a few cities, the use of biomonitors has represented an important contribution to the assessment of air quality in this country. Thus, many different surveys have been performed using trees, lichens or vascular epiphytic species (Carreras et al., 1998; González et al., 1998; Carreras and Pignata, 2001; Pignata et al., 2002; González et al., 2003).

The aim of the present work is to assess the relationship between the constituents of suspended particles and the response of *Tradescantia pallida* and *Usnea amblyoclada* in three different sampling areas in the city of Córdoba.

2. Materials and methods

2.1. Study area and sampling sites

Córdoba City, capital of Córdoba Province, Argentina, is at 31°24' South latitude and 64°11' West longitude, 440 m above sea level. The climate is sub-humid, with a mean annual precipitation of 790 mm concentrated mainly in summer; a mean annual temperature of 17.4 °C and prevailing winds are from NE and SE. Natural vegetation belongs to the Spinal Phytogeographical Province (Cabrera, 1976) which consists of low thorny woodlands. Córdoba is one of the most polluted cities in the country. Because of the concentration of industrial and commercial activities in a relatively small area, severe environmental degradation has taken place with high concentrations of mainly primary pollutants, released by mobile sources (Olcese and Toselli, 1997).

Three sites with different environmental characteristics in the urban area of Córdoba were selected. One of them corresponded to the city center (C) and is characterized by main avenues that concentrate traffic activity -cars, taxis, as well as public transportation vehicles. This area is topographically depressed which, together with the prevailing high buildings, hinders pollutant dispersion. There are no industries. Another sampling site was located 3 km from the first one in the university campus (UC) along a side road with heavy traffic of gasoline and diesel powered vehicles, buses and trucks. The third site is located 15 km from the other ones in a residential area (R) and is characterized by plenty of green areas with abundant vegetation and very low traffic intensity.

2.2. U. amblyoclada

Thalli of the lichen *U. amblyoclada* (Müll. Arg.) Zahlbr. were collected from Los Gigantes, an area 70 km west of Córdoba city, which has a very low level of pollution. The basal parts of lichen thalli were detached with the adhering pieces of rock substrate and were stored for two days in the laboratory at room temperature until exposure. These thalli corresponded to the baseline material.

Lichen-bags were prepared with 6.0 g fresh material loosely packed in a fine nylon net (1 × 1.5 mm), so each bag (20 × 20 cm) included several thalli. Three of these bags were tied on a nylon rope on different posts 3 m above ground at each sampling site and exposed for one month. This procedure was repeated every month from October 2004 to April 2005. After collection, lichen samples as well as baseline material were air-dried, homogenized in a mortar with a pestle and then freeze-dried in polyethylene bags. From each lichen bag, three sub-samples of the homogenized material were taken to obtain a mean arithmetic value ± standard deviation for each physiological determination.

Procedure followed for the quantification of physiological determinations: chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Carot), hydroperoxy conjugated dienes (HPCD), malondialdehyde (MDA), electrical conductivity (EC), dry weight/fresh weight ratio (DW/FW) and the ratio chlorophyll b/chlorophyll a (Chl b/Chl a), are explained in detail in Carreras et al. (1998) and Carreras and Pignata (2001). All determinations were expressed on a fresh weight basis. Some of these parameters were used to calculate the Pollution Index (PI) cited for this species in Carreras et al. (1998).

The multielemental composition of lichen thalli was determined by Total Reflection X-Ray Fluorescence (TXRF) using Synchrotron Radiation. From each sample, 2 g of dry material was ground and reduced to ashes at 500 °C for 4 h. The ashes were digested with HCl (18%): HNO_3 (3:1) at 25 ± 2 °C and the solid residues were then separated by centrifugation. Finally, the volume was adjusted to 25 mL with Milli-Q water and 10 ppm of a Ge solution was added as an internal standard. Aliquots of 5 μL were taken from this solution and dried on an acrylic support. For quality control, samples of Hay Powder IAEA-V-10 standard reference material were prepared in the same way as described for the samples. The system was calibrated with known concentrations of standard solutions of metals and Ge as an internal standard. Samples were irradiated for 200 s using the total-reflection technique, at an X-ray fluorescence beamline of the National Synchrotron Light Laboratory, Campinas, Brazil. For the excitation, a polychromatic beam approximately 2 mm wide and 1 mm high was used. A Si detector with a resolution of 140 eV at 5.9 keV was used for the X-ray detection.

Contamination factors (CFs) were calculated dividing the concentration of each element in the biomonitor by the concentration of the element in the baseline material (Conti and Cechetti, 2001). In order to minimize the effect of soil deposition, and considering the fact that Fe is one of the main elements present in the soil of the study area (Gaiero et al., 2003), the concentration of each element was normalized with respect to Fe according to the formula presented in Wannaz et al. (2006). Thus, the values of CF indicate the enrichment of a certain element with respect to the soil of the sampling site as well as its increase compared to the initial material before the exposure.

2.3. T. pallida

Samples of *T. pallida* Rose. Hunt. cv. *purpurea* Boom were cultivated in pots (20 cm in diameter) using the same lot of commercial soil. During the exposure period (October 2004–April 2005) 10 pots were distributed at each sampling site. In the city center plants were located 10 m from the main avenue at 3 m above ground. At the university campus plants were located approximately 150 m from roads in the internal garden of the School of Engineering, held over wood platforms at 1 m above ground. In the residential area plants were located in the back garden over wood platforms at 1 m above ground. All pots were kept in open air without shade and were watered twice a week. Every week, plants were closely inspected to keep them clean and free of pests. No pesticides were applied to or around the plants during the experiment.

Young inflorescences were collected from each sampling site during all the study period. The inflorescences were fixed overnight in 1:3 glacial acetic acid–ethanol solutions and then the buds were stored in 70% ethanol. Inflorescences were dissected and young anthers squashed in a solution of acetocarmine stain on a microslide. Only preparations containing early tetrads were considered. Examination was done at a magnification of 400×, and 300 tetrads were examined per slide. Micronuclei frequencies were calculated by dividing the total number of MCN by the total number of tetrads scored and expressed in terms of MCN/100 tetrads. The counting of micronuclei was done on coded slides and the code was revealed only after completing the entire experiment.

2.4. Total suspended particles

Total suspended particles (TSP, 24-h mean) were collected in the October 2004–April 2005 period during working days, at least once a week. Samples were taken during 24 h on pre-conditioned and pre-weighed glass-fiber filters using medium volume samplers ($12\ m^3\ h^{-1}$ flow rate). XRay Fluorescence with Sincrotron Radiation was also used for the determination of Br, Ca, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, Rb, S, Sr and Zn in TSP. A more detailed description of the methodology employed is presented in Carreras et al. (2006).

2.5. Statistical analysis

Physiological parameters measured in lichens, the elemental content measured in both lichens and filters, and the MCN percentages were submitted to a variance analysis (ANOVA) among sampling sites. A post-hoc comparison was run by the Least Significant Difference (LSD) method whenever the ANOVA indicated substantial effects ($P < 0.05$). The level of significance was set at 5%. Spearman correlation coefficients were also calculated between the elemental content measured in lichens and filters. Besides, *T. pallida* and *U. amblyoclada* data were subjected to principal component analysis (PCA) with Varimax rotation and cluster analysis (CA) to identify the main biomarkers associated to each sampling site and to investigate the common variability of the measured elements.

3. Results and discussion

The physiological parameters and concentrations of elements measured in lichen thalli at each sampling site are presented in Table 1, along with the results of ANOVA. A significant increase was observed in the DW/FW ratio in samples exposed at both urban sampling sites, which can be related to a higher evaporation rate due to the presence of pollutants in urban environments that may cause loss of integrity of cell membranes and alter the hydric status of thalli. Previous investigations showed that the water content may be altered in thalli transplanted to heavily polluted environments (Levin and Pignata, 1995; Cañas et al., 1997). The integrity of cell membranes in *U. amblyoclada* was checked by changes in the EC of the water in which intact thalli were immersed. The EC value of samples exposed in the city center was the highest and the value of samples exposed at the UC was the second highest, suggesting a higher damage to cell membranes in samples exposed at urban sites, with significant contribution from vehicular emissions. This result agrees with the higher concentration of suspended particles found in the glass-fiber filters collected at these sites. The concentration of chlorophyll a and chlorophyll b were also higher in samples exposed in the city center, though they were not significantly different from samples exposed in the residential area. A similar response was already observed in the same lichen species and it was attributed to a fertilizing influence of pollutants (Von Arb and Brunold, 1989; Carreras and Pignata, 2001). On the other hand, the fact that the ratio Chl b/Chl a also increases in samples exposed in the city center, indicates that atmospheric pollutants can also promote the degradation of chlorophyll a. The fact that chlorophyll a is more sensitive to pollutants than chlorophyll b has already been observed in some other lichen species (Rao and Le Blanc, 1966; Gries, 1996). Therefore, it can be suggested that atmospheric pollutants are probably responsible for both pigment degradation and increase at the same time. The highest value of the Pollution Index corresponded also to samples exposed in the city center, which reinforces the usefulness of this index to resume the effects of air pollutants in a single parameter.

Regarding the accumulation of elements on lichen thalli, a significantly higher content of Zn was observed in thalli exposed in the city center, which may be related to the blending of metal-based additives into the oil in order to improve its performance (Lim et al., 2007). High levels of Zn have been reported for other areas in which the dispersion of traffic emission contaminants is influenced by topographical characteristics and meteorological conditions (Viard et al., 2004). On the other hand, lichens exposed in the R area had statistically significant higher content of Co and high content of other soil derived particles, like Fe and Rb, too.

Table 1
Mean values (±SD) of physiological parameters and concentration of elements measured in thalli of the lichen *U. amblyoclada* transplanted at the city center (C), the university campus (UC) and a residential area (R) in Córdoba city. Results of ANOVA and LSD test: values in each horizontal line followed by the same letter do not differ significantly.

	Baseline material		C		UC		R		ANOVA *p*-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
DW/FW	0.92 b	0.007	0.94 a	0.01	0.94 a	0.01	0.93 ab	0.01	0.0002
EC $\mu S\ m^{-1}\ mL\ g^{-1}$	12.4 c	9.79	28.9 a	4.02	16.7 b	6.89	12.21 c	1.29	<0.0001
Chl a $mg\ g^{-1}$	0.09 b	0.016	0.14 a	0.05	0.09 b	0.04	0.12 a	0.03	<0.0001
Chl b $mg\ g^{-1}$	0.04	0.02	0.17	0.12	0.10	0.09	0.12	0.01	ns
Carot $mg\ g^{-1}$	0.07	0.01	0.05	0.02	0.05	0.01	0.04	0.02	ns
MDA $\mu mol\ g^{-1}$	0.12	0.01	0.11	0.01	0.11	0.02	0.11	0.01	ns
HPCD $mmol\ g^{-1}$	0.13	0.02	0.06	0.04	0.09	0.07	0.08	0.07	ns
Chl b/Chl a	0.44 b	0.16	1.21 a	0.35	1.11 a	0.45	0.98 a	0.34	0.008
PI	0.97 b	0.17	1.27 a	0.23	0.99 b	0.16	0.98 b	0.15	0.035
Br $mg\ g^{-1}$	5.90	4.00	4.04	2.83	5.58	5.18	6.67	4.72	ns
Ca $mg\ g^{-1}$	9209	4042	9232	2432	9235	2331	10 047	2965	ns
Co $mg\ g^{-1}$	0.68 ab	0.31	0.73 ab	0.14	0.66 b	0.14	0.86 a	0.26	0.028
Cr $mg\ g^{-1}$	2.39	0.91	4.2	2.03	1.72	0.54	1.7	0.52	ns
Cu $mg\ g^{-1}$	4.48	1.45	5.17	1.42	4.5	0.98	4.74	1	ns
Fe $mg\ g^{-1}$	1162	477.3	1229	284.3	1170	348.9	1408	407.2	ns
K $mg\ g^{-1}$	1781	374.3	1647	174.6	1651	303.0	1510	186.5	ns
Mn $mg\ g^{-1}$	40.99 a	8.59	36.82 ab	2.44	37.67 a	5.77	32.99 b	2.55	0.025
Ni $mg\ g^{-1}$	4.29	7.11	1.56	0.78	2.72	2.37	1.19	0.67	ns
Pb $mg\ g^{-1}$	2.98	0.89	2.60	0.46	3.17	0.78	3.47	1.37	ns
Rb $mg\ g^{-1}$	5.51	1.78	4.18	0.81	4.66	1.10	5	1	ns
S $mg\ g^{-1}$	123.67	56.24	163.43	72.87	144.54	61	145.72	70.11	ns
Sr $mg\ g^{-1}$	17.57	5.71	14.8	3.12	17.59	4.46	18.65	2.87	ns
Zn $mg\ g^{-1}$	11.24 c	4.38	14.73 a	2.67	12.15 b	1.98	13.65 ab	3.27	0.035

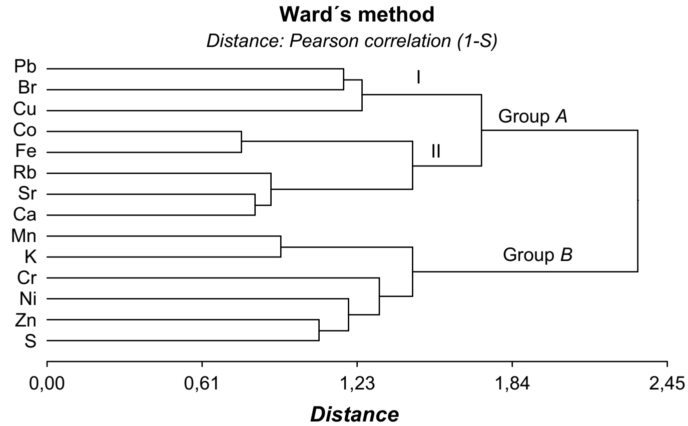

Fig. 1. Results of hierarchical cluster analysis (dendrogram) of the elemental composition in the lichen *Usnea amblyoclada* ($n = 32$).

In order to discriminate the groups of elements as tracers of natural or anthropogenic sources, an explorative hierarchical cluster analysis was performed with data from composition of TSP accumulated on lichen thalli (Fig. 1). The results allowed the elements to be divided into two main groups and two subgroups. The first group (*A*) is constituted by Pb, Br and Cu (subgroup I) and Co, Fe, Rb, Sr and Ca (subgroup II). The second group (*B*) is constituted by Mn and K (closely associated), Cr, Ni, Zn and S. The results suggest that the elements in subgroup II have a natural origin, in agreement with the results obtained by Gaiero et al. (2003) for atmospheric particulate matter collected in Argentina. These authors reported that together with Al, the source of Fe in eolic dust samples is mainly from natural weathered materials. On the contrary, elements from the subgroup I and group B include elements with an anthropogenic origin. Pb, Zn and Cu can be identified as tracers of anthropogenic pollution as Manta et al. (2002) showed in Italian soils. Moreover, Carreras and Pignata (2001) have previously reported high levels of Ni and Cu in transplanted *U. amblyoclada* samples in an industrial area of Córdoba.

Regarding the CF values, it was observed that the samples exposed in the city center were enriched in elements of anthropogenic origin like Cr, Cu and Zn (Table 2). In agreement, Viard et al. (2004) informed high levels of Zn, Pb and Cd in an area near a highway in France. These authors suggest that it is possible that these metals are enriched in urban soils and so become dispersed as particulate atmospheric matter downtown.

On the other hand, at the UC a higher enrichment was observed in Sr and Pb which can also be attributed to emissions derived from traffic (Caussy et al., 2003; Thorpe and Harrison, 2008). In the residential area (R), the values of CF of most anthropogenic elements (S, Ni, Cu, Zn) were lower than the values calculated for the other two sampling sites, suggesting that these samples were less affected by anthropogenic emissions. This finding is also in agreement with the lower concentrations of elements measured in the particulate matter collected at the residential sampling site.

With the aim of studying the association between the accumulation of elements and the damage observed on lichens, Spearman correlation coefficients were calculated between the CF of each element and the physiological parameters measured on lichen thalli (Table 3). The concentration of pigments as well as the ratio Chl b/Chl a was positively correlated with the CFs for Cr and Zn which are mainly associated with anthropogenic emissions, as indicated in the cluster analysis. Besides, it was observed that the values of EC correlated with Ni content; the DW/FW ratio with Mn and Rb content and HPCD with K, Mn and Pb. Among these metals Rb is the only one that could be associated with soil particles as indicated by the cluster analysis (Fig. 2). Finally, the PI correlated positively with the values of CFs for S and Cr, which are tracers of vehicle emissions, as stated by Lim et al. (2007). Calvelo and Liberatore (2004) have also found increased levels of Br, Cr, Pb, Sb, V and Zn in lichens from the south of Argentina and associated this enrichment with pollutants released by traffic. The analysis performed indicates that when the concentration of elements was accounted for the “soil effect” in the calculation of the CFs, significant positive correlations were found between these elements and the parameters indicative of lichen damage, suggesting that even though soil particles are providing part of these metals, their origin could also be anthropogenic.

Table 2
Contamination Factor (CFs) for elements measured in thalli of the lichen *U. amblyoclada* exposed at the city center (C), the university campus (UC) and a residential area (R) in Cordoba city.

Element	C	UC	R
S	1.015	1.006	0.849
K	0.822	0.876	0.708
Ca	0.955	1.006	0.926
Cr	1.637	0.634	0.655
Mn	0.839	0.891	0.7
Fe	1	1	1
Co	0.957	0.947	0.977
Ni	0.408	0.609	0.241
Cu	1.09	1.001	0.88
Zn	1.05	0.921	0.889
Br	0.732	0.931	1.028
Rb	0.711	0.834	0.762
Sr	0.828	1.055	0.817
Pb	0.826	1.063	0.933

Table 3
Rank correlation coefficients between the contamination factors (CFs) and the parameters[a] indicative of lichen damage in *U. amblyoclada*.

	DW/FW	EC	Chl a	HPCD	Chl b/Chl a	PI
S	0.11	0.26	−0.12	0.08	−0.02	0.43*
K	0.25	0.28	0.05	0.3*	0.19	0.22
Cr	0.02	0.21	0.33*	−0.16	0.25*	0.39*
Mn	0.19	0.19	−0.09	0.34*	0.09	0.2
Ni	0.1	0.48*	−0.28	0.1	−0.2	0.21
Zn	0.04	0.36	0.11	0.17	0.34*	0.28
Rb	0.3*	−0.28	−0.28	0.01	−0.1	0.06
Pb	0.02	−0.05	−0.1	0.36*	−0.09	−0.13

[a] Only parameters that showed significant correlations are presented.

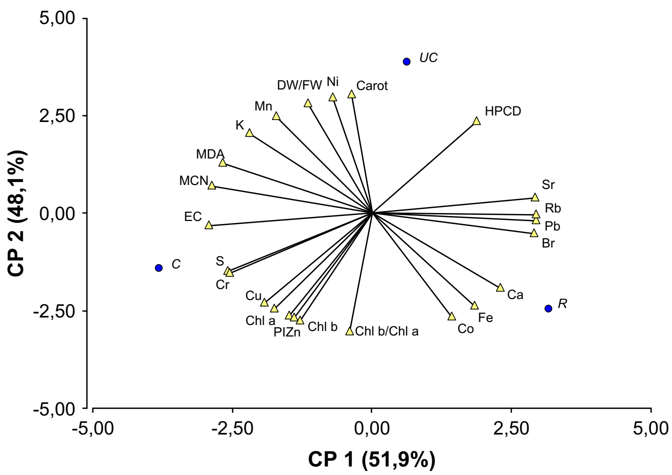

Fig. 2. Variable scores based on the first two principal components of PCA, showing physiological parameters (Carot, Chl a, Chl b,Chl b/Chl a, EC, MDA, HPCD DW/FW and PI), and elements accumulated in the lichen *Usnea amblyoclada* and micronuclei (MCN) measured in *Tradescantia pallida*, with sampling sites used as classification criterion (city center (C), university campus (UC) and residential area (R)).

3.1. *T. pallida*

Regarding the assessment of genotoxic air pollutants in the study area, a significant difference in MCN frequency was observed among the sampling sites (Table 4). Post-hoc analysis revealed that the residential area exhibited lower MCN frequency than the university and the town center sampling sites, which suggests that MCN frequency can be related with the high traffic load of these areas. According to IARC (1983), polycyclic aromatic hydrocarbons (PAHs) are the main cause of the genotoxic activity of the urban air, together with some metals and toxins (Schnelle-Kreis et al., 2005). These compounds are emitted into the air especially as a result of combustion of fossil fuels and occur in the air both in gas and particulate phases (Kamens et al., 1995); however, that particle-phase PAHs have higher mutagenicity than those in the gas phase. Besides, it has already been reported that many PAHs and their derivatives show strong mutagenicity and carcinogenicity (Nardini and Clonfero, 1992; Černá et al., 2000), by forming adducts with DNA, which lead to enhanced mitotic disturbances and cell death (Calderón Segura et al., 2004).

The results obtained are also in good agreement with the fact that in both urban areas (center and University campus) the mass of TSP was higher than in the residential area, which suggests a possible effect of genotoxic compounds that might be bound to suspended particles. Similar results have been already observed by Guimaraes et al. (2000), who reported that gradients of air pollution usually found in the urban scenario are associated with corresponding changes in the frequency of micronuclei in the same plant system.

Table 4
Descriptive statistics and ANOVA results for MCN frequencies measured in *T. pallida* plants at the city center (C), the university campus (UC) and a residential area (R) in Cordoba city.

Sites	*n*	Mean	SD	Minimum	Maximum
C	41	3.575 a	1.426	1.330	8.330
UC	74	2.749 b	1.201	0.330	5.670
R	31	1.689 c	1.179	0.00	4.670
ANOVA (*p*-value)	$<$0.001				

3.2. Total suspended particles

Data on mass and composition of TSP were already presented by Carreras et al. (2006). These authors showed that the particulate collected in the city center (C) has significant higher levels of Ca and Mn, which may have originated in the soil dust and Cu, Zn and Sr which can be tracers of vehicle emissions. In agreement with these findings, Lim et al. (2007) assert that the widespread use of metal-based oil additives (Zn and Mg based), anti-wear agents (Zn based) and detergents (Ca and Mg based) in fuels and lubricating oils increase the concentration of elements emitted from vehicular exhausts. Although differences were not significant, the composition of the particulate collected at the university campus (UC) reveals both, higher levels of elements like Co and Fe which may be attributed to soil, and high content of S, Rb, and Pb which are tracer of vehicular activity. Lower concentrations of all elements were measured in the particulate from the residential area (R).

3.3. Multivariate analysis

The results are presented in Fig. 2. It was observed that pigment content, the ratio Chl b/Chl a, MDA, EC, PI values as well as S, Zn, Cr and Cu accumulated on lichen thalli were higher on samples exposed in the city center with a clear association between them, which suggests that vehicular emissions are probably the cause of the damage observed on lichen thalli. The ratio DW/FW, MCN frequency and the content of HPCD, carotenoids and Ni were mainly associated to the UC sampling site. The residential area is characterized only by a higher content of Co, Fe, Ca, Br, Rb, Pb and Sr on thalli, which suggests that they are not harmful to lichens. Although most of them have a natural origin, a small anthropogenic contribution of Pb in this sampling area cannot be ruled out. As widely known, the main emission source of Pb into the earth's atmosphere was the combustion of gasoline. Therefore, despite its phasing-out and the observed decreasing trend in the atmosphere, Pb remains a significant urban air pollutant, particularly, in least developed countries.

4. Conclusions

The present study provides information about the simultaneous use of two different species, a lichen and a vascular plant, commonly found in our urban and natural environments as passive biomonitors. The MCN test performed with *T. pallida* seems to be sensitive enough to detect the levels of genotoxic air pollutants present in the city of Córdoba, since significant differences were measured between the sampling sites studied. Likewise, chlorophyll degradation and membrane damage found in lichens were also different between sampling sites. The information obtained by this two biomonitoring systems was related to the levels of particulate material retrieved from each sampling area, indicating that the sampling sites with high level of suspended particles had also high genotoxic risk. The results obtained suggest that both species, *T. pallida* and *U. amblyoclada*, can be used as monitoring systems and are especially suited for developing countries due to their flexibility, low cost and efficiency to distinguish high risk areas with high biological adverse effects.

Moreover, the fact that the elemental content measured in *U. amblyoclada* reflects the environmental characteristics of the sampling sites, confirms that this species can be used successfully to assess air quality.

The results of the present study are important in the context of some epidemiological studies focusing on chronic effects of air pollution on human health (Dockery et al., 1993; Pope et al., 2002). Since it was observed that variations of ambient levels of particles are associated with significant changes in lichen biomarkers and micronuclei rate, several injuries in humans could also be expected. Future research is needed to confirm this hypothesis.

Studies like the present one encourage the application of plant bioassays for air pollution monitoring. Not only because they allow the assessment of synergistic, antagonistic or additive effects of chemicals in complex pollutant mixtures, but also because they are suitable tools for the study of the mutagenic and carcinogenic potential of urban air pollution *in situ*, which is a topic far less well known.

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