



Use of epidermal characters as bioindicators of environmental pollution

Uso de caracteres epidérmicos como bioindicadores en contaminación ambiental

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ABSTRACT

The present study explores the use of micromorphological leaf epidermal characters of *Ficus benjamina* (evergreen) and *Fraxinus pennsylvanica* (deciduous) as indicators of pollution in the Metropolitan Area of Buenos Aires. Mature leaves of both species were sampled along an urban-periurban gradient, with a rural area as control for epidermal characters. Parameters examined from abaxial and upper epidermis were: (1) length and width of stomata, (2) number of stomata per area unit, (3) length and width of epidermal cells, (4) number of epidermal cells per area unit. Data were analysed with basic statistical and multivariate techniques. Principal Component Analysis (PCA) clearly grouped foliar samples according to three zones: urban, periurban and rural areas with different pollution values. No correlations were observed between epidermal character variations and climate variables, air pollutants or C, N, P and metals concentration in soil. Only one positive correlation ($p=0.006$) was found between stomata length in leaves from *Ficus* and iron concentration in soil.

RESUMEN

En el presente trabajo se explora el uso de los caracteres micromorfológicos de epidermis de hoja de *Ficus benjamina* (especie perenne) y *Fraxinus pennsylvanica* (especie caduca) como indicadores de contaminación en el área metropolitana de Buenos Aires. A lo largo de un gradiente urbano-periurbano, con un área rural como control, se colectaron hojas de ambas especies. Se examinaron parámetros de epidermis abaxial y adaxial: largo y ancho de estomas, número de estomas por unidad de área, largo y ancho de células epidérmicas, número de células epidérmicas por unidad de área. Los datos fueron analizados con técnicas básicas de estadística y análisis multivariado. El análisis de componentes principales (PCA) claramente agrupó las muestras foliares en tres zonas: urbana, periurbana y rural, con diferentes valores de contaminación. No se observaron correlaciones entre las variaciones de los caracteres epidérmicos y las variables clima, contaminación ambiental o C, N, P y concentración de metales en el suelo. Se encontró

una única correlación positiva ($p=0.006$) entre el largo de estomas en hojas de *Ficus sp.* y la concentración de Fe en el suelo.

Key words: bioindicators, urban-periurban gradient, seasonal variation, areal variations

Palabras clave: bioindicador, gradiente urbano-periurbano, variación estacional, variación areal

INTRODUCTION

Environmental pollution is increasingly higher due to human activities and results in the accumulation of heavy metals that cause various types of problems to living beings (Rai et al., 2005). Man's daily activities cause pollution of different kinds and degrees; indeed, air, soil and water get contaminated as a result of industrial and agricultural activities, transport, etc.

In big cities, air pollution levels are ground of concern. Monitoring programs and search for metals in environmental samples have become widely established as direct and indirect measurement methods. Biological monitoring constitutes an economic alternative for atmospheric pollution studies (Bellis et al., 2003; Castro & Faggi 2008; De Temmerman et al., 2004; Dion et al., 1993; Jasan et al., 2004; Panichev & McCrindle, 2004; Pignata et al., 2002; Walkenhorst et al., 1993). Plants have been observed to be far more sensitive to pollution than animals and man and are therefore used as indicators (Rani et al., 2006). Studies have recorded changes in plants due to all kinds of environmental pollutants, and most of these works refer to physiological alterations (Kabata-Pendias & Pendias, 2001; Linster, 1991; Ochiai, 1987; Patra & Sharma, 2000). Among passive monitor's assays, the multielement analysis of tree bark from

urban trees (Castro et al., 2008; Faggi et al., 2008; Jasan et al., 2004; Perelman et al., 2006; Steubing et al., 2002) has been recently introduced to detect and measure the elements of air pollution retained in tree bark.

The degree of impact in plants depends on pollutants concentration, location of entry into plant and species under consideration. Different species may present varying sensitivity/tolerance levels to different contaminating agents (Srivastava, 1999). Alderdice (1967) set two categories of toxic effects: acute toxicity (due to high pollution levels during short periods of time, usually lethal) and chronic toxicity (due to low pollution levels over long periods of time, lethal or sublethal).

Toxic effects produced by pollutants on soil organisms and plants have usually been studied in laboratories under controlled conditions, but field studies have been scarce (Patra & Sharma, 2000). Stomatal and epidermal cell size, lower frequency, thickening of cell wall, epicuticular wax deposition alterations and chlorosis are among the structural modifications in leaves deriving from pollution (Mhatre & Chaphekar, 1985; Mohapatra et al., 1991; Rao & Dubey 1991; Setia et al., 1994; Srivastava, 1999). In Brazil, Sant'Anna-Santos et al., (2008) worked with *Genipa americana* L. (Rubiaceae) in order to characterize injuries on leaf structure and micromorphology,

and to evaluate its degree of susceptibility to simulated acid rain. They found necrotic interveinal spots on leaf blade, with plasmolized guard cells and cuticle rupture. Their results highlighted the relevance of anatomical data for precocious diagnosis of injury and to determine species sensitivity to acid rain. Working with *Celosia cristata* samples subjected to gaseous and particulated pollution deriving from heavy traffic in Indian routes, Srivastava (1999) observed that general plant growth was affected with severe distortions in foliar epidermal characters. Pointing out the importance of cuticle and epidermal features in the determination of tolerance/sensitivity of each species to environmental pollutants.

Taking into account these characteristics, some authors consider foliar epidermis as a bioindicator of environmental quality (Masuch et al., 1992; Alves et al., 2008; Balasooriya et al., 2009). A multi-element analysis was carried out in the metropolitan area of Buenos Aires, using *Fraxinus pennsylvanica* tree bark as a bioindicator of air pollution (Castro et al, 2008; Perelman et al., 2006). Results confirmed the existence of a distribution pattern along an urban-periurban gradient (central, residential, periurban). Simultaneously, basic properties and heavy metal levels were analysed in soil from the basis of the same trees (López et al., 2006). Results were then compared with those from studies performed in the city of Mendoza (Faggi et al., 2008). Later, Fujiwara et al. (2006) correlated the concentration of elements in particulate matter (PM) from street dust with the concentration of pollutants retained in tree bark and with the concentration of pollutants in air in the same sites. They

concluded that tree barks are more sensitive as passive bioindicators than street dust.

Our objective was to evaluate the possibility of using epidermis as a pollution indicator in trees from Buenos Aires metropolitan urban and periurban areas, given the ease and low cost of the technique. To recognise areas of different pollution levels and the effect produced by the same level of pollutants over a short time period (deciduous species) and over a long time period (evergreen species).

MATERIALS AND METHODS

Selection of species

A deciduous and an evergreen tree were selected, namely *Fraxinus pennsylvanica* Marshall, and *Ficus benjamina* L, respectively. These species were selected given their high frequency along the streets of the metropolitan area of Buenos Aires, and because of the different life span of their leaves. If morphological or epidermal modifications were observed, their life span could allow us to determine whether they responded to a stimulus over a short period of time (stationary) or to the cumulative action of pollutants over long periods of time. Perelman et al. (2006) conducted sampling for tree bark at the same time as that reported for soil by López (2006).

Sample sites

Leaf samples were collected from urban and periurban areas characterized by different contamination levels. Three different sites belong to the urban area: (Ur 1) Constitución, affected by heavy car traffic and a railway terminal, (Ur 2) Flores,

a residential neighbourhood affected by car traffic, and (Ur3) downtown, with dense traffic incidence. A public park affected by the international airport and its access highway (**Figure 1**) conform the periurban area Ezeiza (Per). Chascomús (Buenos Aires Province), a rural environment (Rur), was selected as the “non-contaminated zone” where leaves from the same species under study were used as “blank samples”, to provide a baseline to test foliar anatomic characters.

Collection of Samples

Ten to twenty leaves were taken from the external part of the canopy, nearly 2 m high. Samples were taken from 10 trees

per site every season, at the same time as sampling for tree bark (Perelman et al., 2006) and soil for pollution studies (López et al., 2006) which documented air pollution and field conditions.

Specimens analysed

A total of 18 *Ficus* specimens (8 from spring and 10 from autumn), and of 16 *Fraxinus* (16 from Spring and 13 from autumn) were analysed. Samples were kept in open paper bags.

Preparation of leaves

In *Fraxinus*, a compound odd pinnate leaf species, the bigger foliole from the apical pair was selected. Epidermal peels

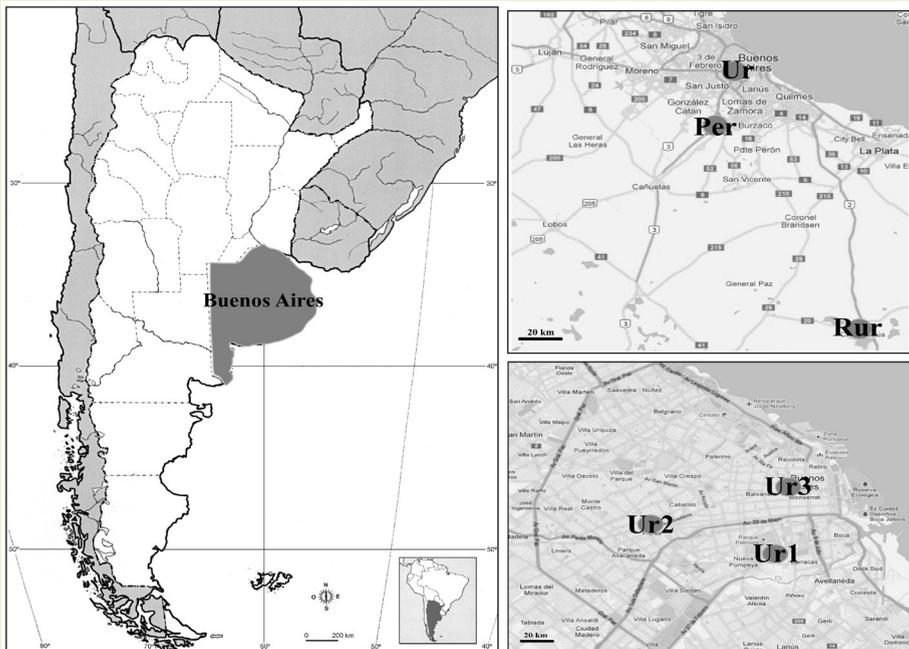


Figure 1. Location of sites sampled. References: (Ur) urban area: (Ur1) Constitución, (Ur2) Flores, (Ur3) downtown; (Per) periurban area; (Rur) rural area.

Figura 1. Ubicación de las zonas de muestreo. Referencias: (Ur) área urbana: (Ur1) Constitución, (Ur2) Flores, (Ur3) centro; (Per) área periurbana; (Rur) área rural.

from this foliole and from *Ficus* leaves were examined under optical microscopy (MO) and scanning electronic microscopy (SEM). Traditional preparation techniques were utilized (Arriaga, 1986; D'Ambroggio de Argüeso, 1986).

Anatomical data registration

The macroscopic characteristics analysed were leaf colour, stains on leaves, leaf/foliole contour, apex, nervation, chlorosis and necrosis. The microscopic characteristics analysed in upper and abaxial epidermis were: epidermal cells length and width (CLUE, CWUE, CLAE, CWAE); stomata cells length and width (SLUE, SWUE, SLAE, SWAE); stomata number per area unit (considering units of $19.110 \mu\text{m}^2$) (SNUE, SNAE); epidermic cell number per transect (CNUE, CNAE, considering number of complete cells placed on 10 transects $155 \mu\text{m}$ long, randomised take on each slide).

Climate variables considered and concentration of elements in soil

In order to evaluate the eventual incidence of climate conditions on the variations of the epidermis characteristics considered, information was requested from the Servicio Meteorológico Nacional (National Meteorology Service) on temperature, effective heliophany and precipitation corresponding to the time when samples were taken.

The concentration of C, N, P and metals present as soil cations (López et al., 2006), and metals retained in barks (Perelman et al., 2006) deriving from air pollution in the same locations were contrasted to evaluate their influence on the anatomical variations registered.

Data analysis

Data from observations were recorded on a matrix, which was then statistically analysed in order to find interspecific, zonal and/or seasonal differences and similarities. Mean and standard deviation values were recorded on the matrix.

PCA (principal component analysis) was used as ordination method to describe the morphological variability among samples. Data were transformed by the Pearson moment-product correlation coefficient on a similarity matrix used to obtain the principal components (Sneath & Sokal, 1973; James & McCulloch, 1990). Invariant characters were previously removed from the analysis. Each individual was considered an operational taxonomic unit (OTU).

This ordination method was used to identify the main groups according to locality of collection. PCA was repeated several times to detect similar groups in each locality. Hence, once a group of OTUs related to a given locality was recognised, it was then separated and re-analysed alone. This procedure was repeated until well-differentiated groups of OTUs were determined. To describe the variability among groups, the characters that contributed most to the variability of the first five PCA components ($r > 0.45$) were analysed, their axes being selected according to group representation.

Discriminant analysis (DA) (Sneath & Sokal, 1973; Affifi & Clark, 1984) was applied in order to verify the consistency of groups resulting from PCA, on the basis of correlated characters. The empirical method (Affifi & Clark, 1984) was used to estimate goodness of fit of the classification. Based on Mahalanobis distances of each specimen from the different centroids of each group, the respective

subsequent classification probabilities were computed. Thus, the proportion of individuals correctly classified for each group was calculated. Numerical analyses were carried out using NTSYS-pc program, version 2.02h (Rohlf, 1992).

Mean and standard deviations of quantitative characters, and the mode of discrete variables were calculated to describe differences among the most similar groups of species. One-way analysis of variance (for continuous characters; ANOVA) and non-parametric Kruskal-Wallis one-way analysis (for discrete characters) were performed in order to independently analyse morphological differences among groups. The goodness of fit of a normal distribution was assessed with the Kolmogorov–Smirnov one-sample test for all continuous characters. Variance homogeneity amongst groups was verified by Bartlett’s test.

Correlations were also carried out according to anatomical characters which presented variability in each species between either localities or times of collection and environmental variables

(weather conditions and amount of chemicals). Results were compared with those obtained from measurements of soil contamination, metal retention in cortex and PM, from the same locations (Lopez et al., 2006; Perelman et al., 2006). Correlations were performed considering the characteristics and concentrations of cations, both independently as well as each character contrasted against all metals together, should there be any synergistic effect. STATISTICA 99 Edition (Stat Soft, Inc., 1995) was used for these analyses.

RESULTS

Principal Component Analysis (PCA) clearly grouped foliar samples according to three zones: urban (Ur), periurban (Per) and rural (Rur), for each specie in each period (**Figure 2**). Discriminant Analysis (DA) corroborated PCA’s classification. DA showed a clear discriminate pattern taking into account that 100% of specimens from each site were correctly classified into the groups established.

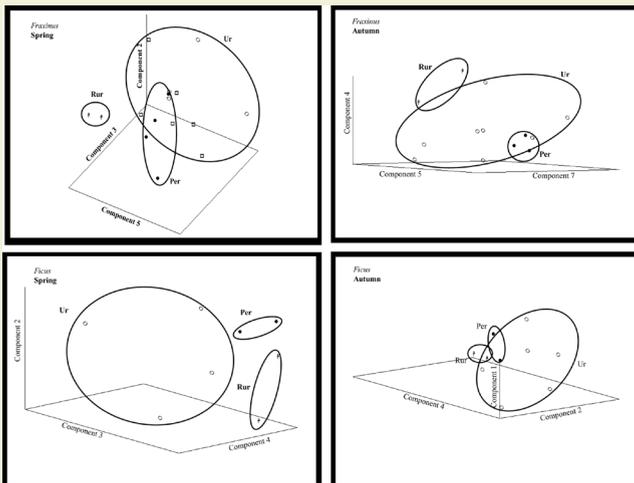


Figure 2. Epidermal characters analysed with multivariate techniques (PCA) References: Ur (urban area), Per (periurban area), Rur (rural area).

Figura 2. Gráfico de de caracteres epidérmicos analizados con técnicas de analisis multivariados (PCA). Referencias: Ur (area Urbana), Per (area periurbana), Rur (area rural)

Fraxinus

In autumn, *Fraxinus* samples presented significant differences between rural and periurban species regarding length of abaxial epidermal stomata, while urban stomata were the longest. Samples from the urban area presented meaningful differences in stomata width of the abaxial epidermis with respect to samples from the other areas. Indeed, rural stomata were narrower than urban stomata, but wider than periurban stomata. Upper epidermal cells from the rural area were narrower than those from urban and periurban areas. Abaxial epidermal cells from the periurban zone showed meaningful differences in width with respect to the other two zones. They were narrower than those from the urban and rural areas (Table 1).

Fraxinus spring samples showed meaningful differences in length and width of abaxial epidermal stomata, in abaxial epidermis cell width, and in upper epidermis cell length. Rural and also urban and periurban areas presented meaningful differences regarding length and width of abaxial epidermal stomata. Rural stomata from upper epidermal cells were the shortest (Table 1). A comparison of *Fraxinus* characters between seasons per area revealed meaningful differences in urban and periurban samples, but not in rural samples.

In the latter, stomata in abaxial epidermis were shorter in autumn than in spring samples, and epidermal cell number was greater in autumn than in spring in both epidermis (Table 1). In periurban samples, meaningful differences correspond to stomatal number, length and width and cell number, all from the abaxial epidermis. In autumn, stomata and cell number from this epidermis

Table 1. *Fraxinus* characters (significant differences between areas * $p < 0.05$, ** $p < 0.001$ and between periods in the same area (# $p < 0.05$ in all cases)

Tabla 1. Caracteres de *Fraxinus* (diferencias significativas entre áreas * $p < 0.05$, ** $p < 0.001$ y entre períodos en la misma área (# $p < 0.05$ en todos los casos)

	Autumn			Spring		
	Urban area	Periurban area	Rural area	Urban area	Periurban area	Rural area
SLAE	2.35 ± 0.17 * #	1.93 ± 0.05 #	2.25 ± 0.01 *	2.58 ± 0.22 * #	2.37 ± 0.09 * ** #	2.20 ± 0.12 **
SWAE	1.81 ± 0.27 * **	1.24 ± 0.05 **	1.40 ± 0.07 *	1.70 ± 0.13 *	1.46 ± 0.17 * **	1.43 ± 0.04 **
SNAE	12 *	17 * #	12 *	13	9 #	12
CWAE	1.56 ± 0.33 **	1.32 ± 0.05 *	1.54 ± 0.01 * **	1.39 ± 0.26 *	1.31 ± 0.22	1.89 ± 0.12 *
CWUE	1.53 ± 0.30	1.61 ± 0.25 *	1.32 ± 0.06 *			
CLUE	2.07 ± 0.64	3.24 ± 0.53	3.20 ± 0.72	3.23 ± 0.53 * **	3.20 ± 0.72 **	2.82 ± 0.85 *
CNAE	11 #	10 #	10	8 #	8 #	8
CNUE	11 #	8	11	9 #	10	8

were greater, while in spring, stomata in this epidermis were longer and wider (Table 1).

Ficus

In autumn leaves we found meaningful differences between samples from rural and urban areas regarding stomata width and cell length from abaxial epidermis. Both characters in samples from urban sites were bigger. We also found meaningful differences between samples from rural and periurban sites regarding the number of abaxial epidermal cells. Periurban specimens showed greater number of cells (Table 2).

In spring leaves we only found meaningful differences regarding the number of stomata cells from abaxial epidermis in *Ficus* specimens from urban and periurban areas. These cells were more numerous in periurban areas. Regarding cell length from the abaxial epidermis, we found meaningful differences between samples from rural and periurban areas. Cells from periurban areas were longer (Table 2).

When we compared *Ficus* samples from spring and autumn, only specimens from the periurban area presented meaningful differences just in length and width of upper epidermal cells: autumn samples were shorter and wider than those from Spring (Table 2).

Correlation with climate variables

No correlations were found in any of the two seasons, between variations of leaf characters measured in the two species studied, and the climate variations considered (mean temperature, mean precipitation and heliophany).

Table 2. *Ficus* characters (* significant differences between areas and # between periods in the same area, $p < 0.05$ in all cases)
 Tabla 2. Caracteres de *Ficus* (*diferencias significativas entre áreas y # entre periodos en la misma área, $p < 0.05$ en todos los casos)

	Autumn			Spring		
	Urban area	Periurban area	Rural area	Urban area	Periurban area	Rural area
SWAE	2.20 ± 0.57 *	1.84 ± 0.18	1.95 ± 0.57 *	1.94 ± 0.12	2.07 ± 0.23	1.25 ± 0.40
SNAE	9	10	10	13 *	18 *	16
CNAE	9	13 *	10 *	11	12	9
CLAE	1.95 ± 0.41 *	2.20 ± 0.01 *	1.76 ± 0.14	1.96 ± 0.08	2.16 ± 0.15 *	1.82 ± 0.75 *
CLUE	2.24 ± 0.40	2.09 ± 0.10 #	1.61 ± 0.1	2.30 ± 0.42	2.65 ± 0.18 #	2.07 ± 0.64
CWUE	1.40 ± 0.36	1.84 ± 0.10 #	1.56 ± 0.08	1.13 ± 0.41	1.36 ± 0.08 #	1.26 ± 0.42

Correlation with elements from soil or retained in tree bark

We contrasted data of character variations measured in this study with data on C, N, P and metals concentrations in soil (López et al., 2006) or elements from air retained in *Fraxinus* bark (from Perelman et al., 2006). The results revealed only one positive correlation ($p=0.006$) between stomata length in *Ficus* leaves and soil Fe concentration.

DISCUSSION

In order to evaluate morphological alterations produced by different pollutants, Sharma & Tyree (1973) compared leaf size from several populations of *Liquidambar styraciflua* L. (Hamamelidaceae) and verified diminished foliar length in specimens living next to a primary source of pollution, especially of particulate matter. Mandre et al. (1995) worked in the vicinity of Kunda (Estonia) with *Pinus* leaves from an area near a source of alkaline dust (cement industry), and observed diminished culm in leaves and in total tree length as symptoms of pollution.

However, we observed no alterations in shape, size or colour, nor the presence of chlorosis or necrosis in leaves from any of the areas sampled.

Alves et al. (2008) performed studies in foliar anatomy of *Eugenia* (Myrtaceae) in Sao Paulo, a large Brazilian metropolis. They compared individuals from heavily contaminated areas (urban zone: Dos Bandeirantes Ave.) and individuals from less contaminated areas (rural zone: Jarinu) and found that epidermis in leaves from urban zones showed statistically meaningful differences in stomatic density (higher density) than that in leaves

from rural areas. Higher stomata density was also recorded by other authors in plants from urban zones subjected to air pollutants (Masuch et al., 1992; Päänkönen et al., 1995 y 1997).

Working on *Ligustrum lucidum* Aiton f. (Oleaceae) in Córdoba city (Argentina), Bruno et al. (2007) also found higher stomata frequency in plants due to high pollution, presumably as a compensation for the negative effect of contaminants on photosynthesis.

Balasoorya et al. (2009), in the surroundings of Gent (Belgium) used *Taraxacum officinalis* (Asteraceae) in order to assess the potential of anatomical leaf characteristics (specifically leaf area, stomata density and cell size, among others) as effective parameters to biomonitor urban habitat quality. They monitored 4 different types of lands: U (urban), UB (urban green and forests), H (harbour and industrial areas) and P (pasture and grass lands), and found higher stomata density and bigger stomata in areas with better air quality.

Rani et al (2006) studied the effect of railway engine emission on the epidermal characteristics of some field plants growing along the railway track of Hapur Junction (India) affected by the emission of gases from diesel engines. Testing *Calotropis procera*, *Croton bonplandianum* and *Cannabis sativa*, they found that all three followed the same change pattern in leaf morphology due to environmental stress. Stomata and epidermal cells frequency declined in plants growing in close vicinity of railway tracks. This decline was greater regarding stomata frequency than regarding epidermal cells, and resulted in a meaningful fall in stomata index, accompanied by increased size

of epidermal cells. Salgare and Thorat (1990) presented similar results on some trees from Andheri (West) Bombay.

Perhaps lower stomata frequency and smaller size allowed less gaseous exchange between environment and plant, thus protecting the latter from toxic inhalation. Since differentiation of stomata mother cells require the division of epidermal cells, decrease in stomata frequency should normally be accompanied by increased size of epidermal cells (Sant'Anna-Santos et al., 2008).

We also observed differences in length and frequency of stomata and epidermal cells between samples from polluted and non-polluted areas. Although in our study those differences were statistically not meaningful, they would indicate a clear tendency for highly polluted areas to produce in spring and autumn longer and wider stomata in both epidermises of the two species studied. Stomata number in spring and autumn *Ficus* samples was lower in highly polluted areas than in less polluted ones. In *Fraxinus*, stomata number resulted higher in most polluted areas in spring and autumn.

After contrasting differences, no correlation was found in our work with pollutants retained in tree barks or soil from the same sites, except for *Ficus* (evergreen), where we found correlation between high concentration of iron in soil and stomata length.

Differences between our results and those from other authors (regarding the correlation between foliar morphology and pollutants) may be due to different pollution levels, not as high in our city as in the other big cities studied; different sensitivity level of our selected species, or else the period of time in which the species were subjected to pollution. In

the case of *Fraxinus* (deciduous species), the period of time during which leaves suffered pollution might not have been long enough to express alterations in epidermal characters.

Nevertheless, the differences found through PCA analysis clearly determined specific groups belonging to areas with different pollution values within the metropolitan area of the city of Buenos Aires

CONCLUSIONS

No differences in macroscopical characters but some in microscopical ones were detected between seasons or among areas in the species studied.

No correlation was observed between variations of the epidermal characters and the climate variables (mean temperature, mean precipitation and heliophany) or air pollutant values considered. Regarding the relation between variations in epidermal characters and soil concentration of heavy metals, a positive correlation was found ($p=0.006$) between stomata length of leaves in *Ficus* and iron concentration in soil.

Differences in PCA analysis clearly determine groups belonging to areas with different pollution values within the metropolitan area of Buenos Aires.

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