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Relationship between trace elemental composition in *Fraxinus pennsylvanica* bark and the incidence of some respiratory diseases in Córdoba, Argentina

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Abstract: In order to evaluate the use of *Fraxinus pennsylvanica* as a biomonitor of trace elements and to investigate their relationship with the incidence of some respiratory diseases in children, bark samples of *F. pennsylvanica* were collected at ten sampling sites near healthcare centres in Córdoba city, Argentina, and analysed by Total Reflection X-ray Fluorescence with synchrotron radiation. Factor analysis was used to identify element emission sources. The first factor was associated with industrial activities, the second might represent soil sources and the third factor was related to vehicular traffic emissions. Pearson's correlation analysis between these factors and the incidence of respiratory diseases showed that factor 1 correlated with allergic rush, factor 2 with allergic rhinitis and factor 3 with respiratory infections. The results suggest that bark samples of *F. pennsylvanica* can be use as an efficient biomonitor to assess the human exposure to trace elements.

Keywords: *Fraxinus pennsylvanica*; bark; factor analysis; correlation; respiratory diseases.

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1 Introduction

The emission of heavy metals is one of the most serious environmental problems, with the content of these elements tending to increase particularly in urban environments where the combustion of fossil fuels and automobile exhaust emissions are primary sources of the atmospheric metallic burden.

Different methods have been applied to monitor heavy metal emissions. For many years, biomonitoring has been used to estimate airborne contamination and its changes over long periods of time (Al-Shayeb et al., 1995; Freitas et al., 1997; El-Hasan et al., 2002). For this purpose, different types of perennial plant matrices have been utilised, such as lichens, mosses and tree barks (De Bruin and Hackeniz, 1986; Walkenhorst et al., 1993; Bargagli, 1998; Asta et al., 2002; Garrec and Van Haluwyn, 2002; Catinon et al., 2009). The most frequently used tree species are *Pinus nigra* or *Pinus sylvestris* (Huhn

et al., 1995; Schulz et al., 1999; Haapala and Kikuchi, 2000; Narewski et al., 2000; Harju et al., 2002; Saarela et al., 2005), olive trees (Pacheco et al., 2001, Pacheco et al., 2002; Freitas et al., 2003; Pacheco and Freitas, 2004; Pacheco et al., 2004), *Fagus silvatica* (Bellis et al., 2004), *Betula sp*. (Herman, 1992) and *Populus nigra* (Berlizov et al., 2007). The main advantages of using trees are the availability and the simplicity of species identification, sampling and treatment. Moreover, they remain in a fixed position over a considerable period of time, thus facilitating the analysis of trends occurring over large time intervals. Tree bark, due to its porosity and potential for retention of aerosol particles, is considered to be a particularly promising indicator in air pollution monitoring.

Several organic or mineral atmospheric pollutants have been suspected of playing an important part in human respiratory diseases. Therefore, the quantification and mapping of atmospheric contaminants have become priority issues (Catinon et al., 2008).

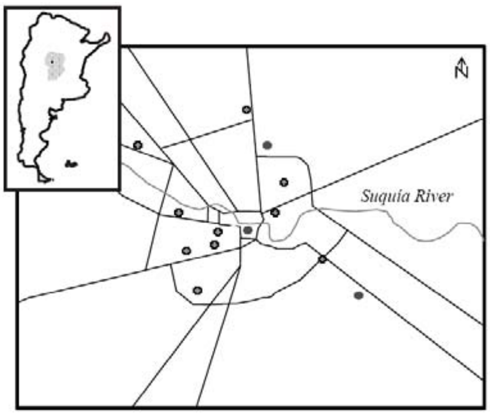
The aim of the present work was to explore the association between respiratory diseases in children and the elemental composition levels measured in tree bark of *Fraxinus pennsylvanica* in the city of Córdoba, Argentina. This city is the second biggest city in Argentina. Owing to its topographic characteristics, severe pollution episodes are frequently observed, especially during wintertime. In addition, recent studies have shown a relationship among the levels of heavy metal atmospheric deposition, their accumulation in biomonitors and the percentage of respiratory diseases (Carreras et al., 2009).

2 Materials and methods

2.1 Sampling sites

Samples of *F. pennsylvanica* bark were collected near health centres at ten sites in Córdoba, Argentina, during March and April 2008 (Figure 1).

Figure 1 Location of ten clinics (⊕) in the city of Córdosba, Argentina



2.2 Sampling methods

At each site, between six and eight trees of *F. pennsylvanica* were selected with the same trunk dimensions, located within 400 m of the health centres. Flakes of the deadened bark layer of about 4–5 mm thickness with maximum dimensions of approximately 1×3 cm^2

were cut with a stainless steel knife at 1.5 m above ground level. At each sampling site three samples were collected, which were then analysed independently, making a total of 30 samples.

2.3 Elemental analysis of the bark samples

Bark samples were put in a Petri capsule and dried in an oven at 50 ± 2°C for 72 h. Masses of about 2 g of dry material were reduced to ashes at 500°C for 4 h. Then, the ash was dissolved with HNO_3 and filtered. Finally, the volume was adjusted to 10 ml with Milli-Q water, and 10 ppm of a Ga solution was added as an internal standard. Aliquots of 5 μL were taken from each solution and dried on an acrylic support. The blank preparation followed the same procedure. Solutions with known elements at different concentrations were prepared in the same way to determine a calibration curve. The elemental composition of tree barks was determined using Total Reflection X-Ray Fluorescence with Synchrotron Radiation (SR-TXRF), with a polychromatic beam approximately 5 mm wide and 0.1 mm high being used for excitation. For X-ray detection, a Si(Li) detector was used with an energy resolution of 165 eV at 5.9 keV.

2.4 Health data

The city of Córdoba has two systems of medical care – public and private. For the present study, we decided to use the public system. In addition, only data from children under aged 6 years were analysed since they are not affected by work-related pollutants that may act as confounding factors. The number of children that attended the human health centres during the period January–December 2005 was obtained from the database records of the municipal health services. The population under study comprised a total number of 4679 children – 53.02% male and 46.98% female. The percentage of some respiratory diseases (asthma, acute bronchitis, respiratory infections, laryngitis, allergic rhinitis and allergic rush) in children was determined with respect to the total number of children that attended each sanitary district (Table 1).

Table 1 Percentage of diseased children aged under six, calculated on the basis of the total number of people treated at clinics situated in study area, for each of the respiratory diseases selected

Diseases	*Mean*	*SD*
Asthma	1.050	0.760
Acute bronchitis	2.190	1.750
Respiratory infections	0.246	0.702
Laryngitis	0.307	0.239
Allergic rhinitis	0.041	0.065
Allergic rush	0.093	0.099

2.5 Data analysis

All the elements measured were selected for factor analysis performed using SPSS version 10.0. The factors were generated using Principal Component Analysis (PCA) and then rotated with Varimax rotation. The number of samples used for analysis was 30 (n = 30) that had more cases than variables. By examining factors with eigenvalues larger than one, a total of three factors could be chosen that accounted for approximately

70% of the total variance, indicating that the results were statistically consistent. Also, Pearson's coefficients of correlation were calculated to study the relationships between the respiratory diseases and the elemental composition levels measured on the tree bark of *F. pennsylvanica*. A mapping procedure was used to evaluate the distribution of each of the factors found. A Kriging contour plot derivation was performed using Surfer 5.00.

3 Results and discussion

3.1 Metal content in bark of F. pennsylvanica

Table 2 shows the mean and standard deviation of the elements quantified in the ten sampling site barks of *F. pennsylvanica*.

The quantified values of Ca, Fe, Co, Br, Rb, Sr and Ba in *F. pennsylvanica* are within the range of values found in the Netherlands for tree species that included oak, poplar, elm and willow (Kuik and Wolterbeek, 1994). However, Zn values (mean = 39.5 ppm) and Pb (mean = 6.59 ppm) in *F. pennsylvanica* are below the range found by these authors (Zn range: 59–780 ppm; Pb range: 100–1000 ppm). Nevertheless, they are similar to the values observed by Perelman et al. (2006) in bark of *F. pennsylvanica* in downtown Buenos Aires (mean Zn = 29.17 ppm; mean Pb = 6.38 ppm), suggesting that the emission level for these elements in these two cities in Argentina could be similar.

Respect to the coefficient of variation in Table 2 shows high coefficient of variation values for Fe, Cu, Mo and Pb; this can be explained by the presence of emission point sources (industries) of these pollutants concentrated mainly in the southeast of the study area.

Table 2 Mean, standard deviation (SD) and coefficient of variation (CV) of 14 elements in the bark of *F. pennsylvanica* (milligrams per kilogram)

Elements n = 30	*Mean*	*SD*	*CV*
K	6230	3780	0.606
Ca	34400	10600	0.309
V	3.20	1.31	0.410
Mn	91.2	37.3	0.409
Fe	1570	1040	0.665
Co	0.638	0.149	0.234
Cu	44.3	28.9	0.654
Zn	39.5	13.7	0.347
Br	7.21	2.79	0.388
Rb	4.37	1.38	0.315
Sr	125	37	0.299
Mo	1.91	0.90	0.472
Ba	173	66	0.383
Pb	6.59	4.95	0.752

3.2 Factor analysis

The results of factor analysis used to identify possible element emission sources are presented in Table 3. The first factor (Ca, V, Br, Sr, Mo and Pb) is possibly associated with

industrial emissions, since a variety of industrial plants are located in the suburban areas surrounding the city of Córdoba, including major automobile factories, auto-part industries, agro-industries and food processing companies (Carreras et al., 2006). Similarly, previous studies in the city of Córdoba had found an enrichment of V, Co, Ni, Cu, Br and Pb in different biomonitors due to industrial emissions (Pignata et al., 2007; Wannaz et al., 2008). The second factor might represent soil contribution (K, Mn, Fe, Co, Rb and Ba), with K content in the bark possibly related to the physiological condition of the tree. Contents of Fe and Co have been associated with soil in some previous works in Córdoba using biomonitors (Pignata et al., 2002; Pignata et al., 2007). Ba, on the other hand, has been identified as a marker of vehicular traffic (Monaci et al., 2000), which shows that this factor may reflect not only the influence of soil particles but also the contribution of vehicular traffic. However, the small size of the study area surveyed in the present work makes difficult to discriminate both sources of pollutants. Thus, although factor analysis showed a first factor with predominantly industrial elements and a second factor with some elements from soil and traffic, the enrichment of some elements may have arisen from various contributions. For example, Fe in bark samples of *F. pennsylvanica* might have been enriched by both industrial and natural sources. In the third component was observed Zn, which has been shown to originate from vehicular traffic in a study analysing the atmospheric deposition of the city of Córdoba (Wannaz and Pignata, 2006), as similarly reported by Garty (1993) in Israel. Both these investigations were carried out at sampling sites near urban areas with scarce industrial development. In another study, Christensen and Guinn (1979) suggested that the particles emitted by automobile tyres are the main source of Zn in urban areas. However, Ward (1989) found that Zn was one of the elements that increased together with the density of traffic and proposed that the emission of this element is not only originated by the tyres but also from lubrication oils and brakes.

Table 3 Eigenvectors obtained in factor analysis of the elements measured in the bark of *F. pennsylvanica*

	Component		
	CP 1	*CP 2*	*CP 3*
Ca	0.960	–0.054	0.119
V	0.892	–0.121	–0.007
Br	0.786	0.175	–0.098
Sr	0.840	–0.103	–0.353
Mo	0.795	–0.229	–0.374
Pb	0.573	0.281	–0.206
K	–0.531	0.614	–0.436
Mn	0.025	0.856	–0.205
Fe	–0.001	0.726	0.414
Co	0.399	0.812	0.174
Rb	–0.128	0.585	–0.501
Ba	0.091	0.637	0.247
Zn	0.430	0.141	0.762
Cu	–0.413	–0.163	0.068
Acum. variance (%)	34	58	70
Eigenvalues	4.8	3.3	1.7

3.3 Mapping

Figures 2–4 show the geographical distribution maps of the three factors obtained by factor analysis. The highest values of the first component (Figure 2) are present in the central and southern study areas, demonstrating the contribution that industrial activity has on this component due to the large number of industries located in the south and southeast of the city. Figures 3 and 4 show the distribution maps of Components 2 and 3, respectively, showing that the highest values of these two components are found in the northwest of the city.

Figure 2 Distribution map of the first factor in the study area, Córdoba

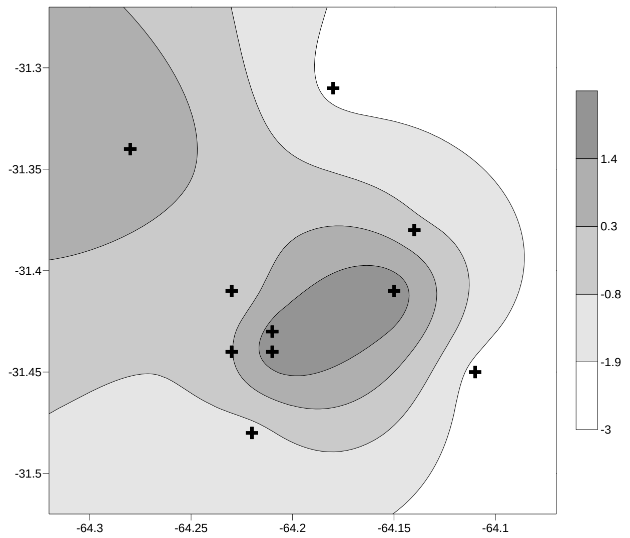


Figure 3 Distribution map of the second factor in the study area, Córdoba

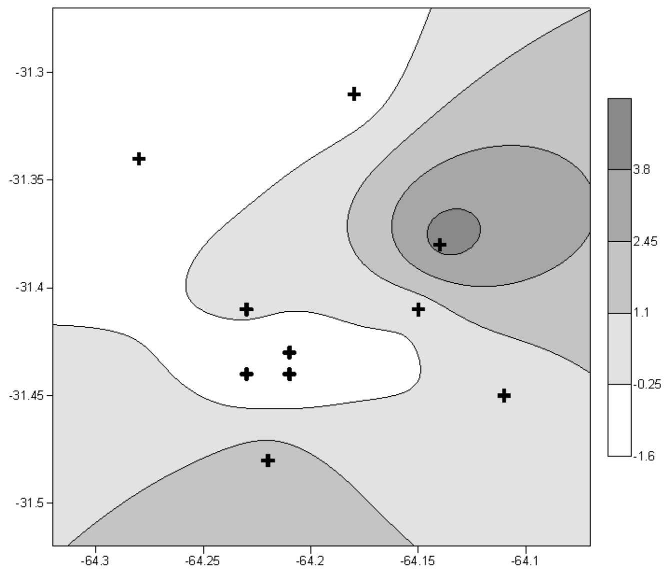
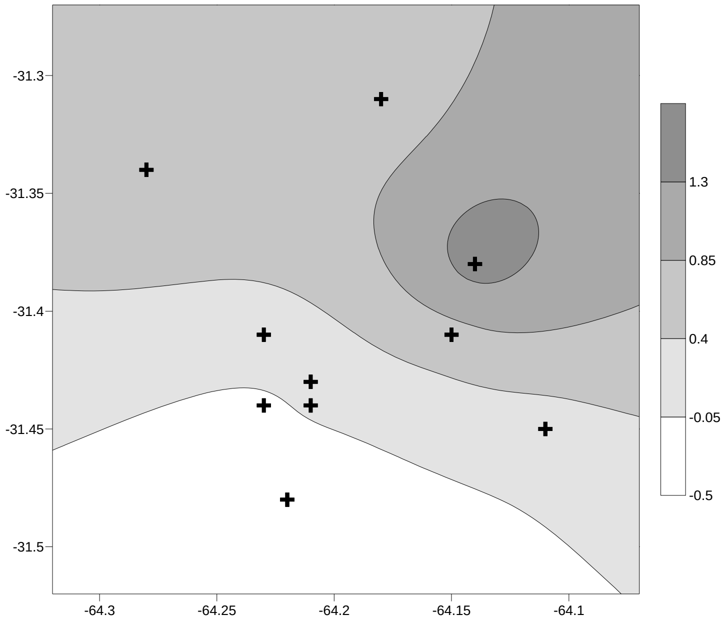


Figure 4 Distribution map of the third factor in the study area, Córdoba



Some of the metals contained in the second component can be attributed to soil (Mn, Fe and Rb) and another as Ba could be related to vehicular traffic emissions. The main contributor to Component 3 is Zn, which is possibly related, as mentioned earlier, to vehicular traffic emissions. This could indicate that emission sources exist for these two components, which may be explained by the high presence of traffic. These results support those found with factor analysis.

3.4 Correlation analysis between metal content in bark and frequency of diseases

Pearson's correlation analysis was performed on the factors previously obtained and the percentages of respiratory diseases (Table 4). Factor 1, an indicator of industrial activity, was correlated with allergic rush. Factor 2, which presented some elements from soil and others from traffic, was correlated with increased allergic rhinitis. Vehicular traffic, represented by Factor 3, was correlated with increased respiratory infections. Although some of the metals measured in the present study are essential to human biological function, such as Mn and Cu, they can be toxic when ingested or inhaled at elevated concentrations. It has been demonstrated that an increased copper content can lead to coronary diseases, arteriosclerosis and damage to the central nervous system (Goyer, 1997). Other metals, such as Pb, are toxic and have been shown to have a negative impact on neurological development of children (Wasserman et al., 2004).

Table 4 Correlation analysis between the percentage of diseased children under six-year old and the eigenvectors obtained in the PCA of the elements measured in the bark of *F. pennsylvanica*

	CP 1	*CP 2*	*CP 3*
Asthma			
Acute bronchitis			
Respiratory infections			0.49
Laryngitis			
Allergic rhinitis		0.54	
Allergic rush	0.48		

4 Conclusions

The results of the present study show that *F. pennsylvanica* can be used as a biomonitor of accumulation, thus permitting zones with different pollution levels to be identified of these elements. In addition, the observed correlation between these elements and the percentage of respiratory diseases shows that *F. pennsylvanica* can be useful when assessing human exposure.

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