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A Novel and Simple Method for Elements Determination in Aerobiological Samples by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis

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Abstract The identification of heavy metals in the atmosphere has increased a strong and growing interest. Thereby, monitoring of elements in aerobiological samples could be a powerful tool for detection of environmental pollution. In this work, a simple and fast method for the determination of trace metals bound to aeroparticles such as pollen was optimized. A single-step procedure for the dissolution of aerobiological samples in nitric acid and further determination by inductively coupled plasma mass spectrometry (ICP-MS) with a high-efficiency sample introduction system was developed. The procedure involved low dilution and low detection limits with adequate precision and its direct introduction into the ICP-MS system. The novel method proposed was successfully applied to determine five elements in concentrations from 0.04 mg g^{-1} (U) to 14.1 mg g^{-1} (Mn) in aerobiological samples. Through this procedure, the most significant correlations between pollen of Cupressaceae, *Ulmus*, and Moraceae with Mn, and pollen of Moraceae with Pb were found. This methodology could be a very useful tool to assess air pollution. We are not only proposing a new strategy to analyze air samples particles but also giving new information of the elemental composition carried by pollen.

Keywords Pollen · Aerobiological samples · Air pollution · Heavy metals · ICP-MS

Highlights
- A novel and simple method for elements determination in aerobiological samples has been developed.
- We used ICP-MS to analyze aerobiological samples after acid dissolution.
- The procedure gives a fast, precise, accurate, and less expensive sample preparation in complex matrices.
- The method showed a positive association between the pollen contents and heavy metal concentration.
- The determination of heavy metals in aerobic particles is a powerful tool for detection of environmental pollution.

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1 Introduction

Metal and metalloid pollutants have been increased rapidly over the past century because of anthropogenic emissions into the environment (Hladun et al. 2016; Han et al. 2002). Furthermore, literature shows an increase in the number and severity of pollen allergies in urban areas (Gumowski et al. 2000; Grewling et al. 2016). It has been suggested that this problem is partly due to contaminants carried by pollen grains (Hladun and Trumble, 2015; Muradoglu et al. 2017).

Like any other plant cells, pollen grains contain many different types of proteins that are found in the cytoplasm and on the surface of exine and intine (Farkhondeh, 2009). These molecules are strategically sited in the pollen particle to participate in intercellular recognition reactions with the female stigma and they are the ones involved in allergic processes (Sedghy et al. 2018).

The effect of air pollutants on respiratory allergies depends on a combination of factors including components and concentrations of environmental pollutants, exposure duration, and the interaction between pollutants and pollen (Sedghy et al. 2018). In this sense, metals are natural components of the earth's crust. They play an important role in organisms, as they are a fundamental part of biochemical and physiological functions (Al-Khashman, 2004; Tahri et al. 2005). Some metals are essential trace elements for the maintenance of biochemical systems of living beings, for example, cobalt, copper, which are necessary in the metabolism of mammals (Miller et al. 2004). Depending on the levels detected, metals can be toxic, and some can even be carcinogenic (Boffetta and Nyberg, 2003). However, there are others originated by anthropogenic processes, such as industrial activities, agricultural, mining, livestock, or the vehicular traffic itself. Such processes should also be considered sources of heavy metals (Chapman et al. 2003; Chandra Sekhar et al. 2005; Ouyang et al. 2006). Due to the cumulative character of metals, these are not only found in the various environmental compartments (air, water, soil, flora, and fauna) but also detected in the human organism (Meindl et al. 2014; Bukowiecki et al. 2007). In the case of air pollution, the particles that contain metals may trigger a wide variety of potential adverse effects on health (Bocio et al. 2005). The growing need for mobility of modern society has transformed traffic into one of the main causes of air pollution by fossil fuels (Kovats et al. 2014).

Lead (Pb) is pollutant found in water, soil, and air. The free metal ion (Pb^{2+}) is the most toxic form of this pollutant. Lead has contaminated soils worldwide, and its occurrence in agricultural, rural, and urbanized areas has been studied extensively (Morón et al. 2012). Lead contributes to air pollution through motor traffic (automobile and airplane), as well as through mining and industrial sources (Van der Steen et al. 2015). On the other hand, the anthropogenic sources of manganese (Mn) include cement production plants, power plants, urban solid waste incineration, and the combustion of fossil fuels (Prieto Méndez et al. 2009).

Likewise, barium (Ba) is abundant in nature and represents approximately 0.04% of the earth's crust. Barium is widely used in the manufacture of alloys for the nickel-barium parts of the automobile ignition system and in the manufacture of glass, ceramics, and image tubes of television sets (Chen et al. 2009). The Ba concentrations are a good indicator of coal combustion emissions. The strong relationship of particulate matter mass with Ba, Mn, and Pb in rural location has pointed out the anthropogenic emissions as the primary source of the fine particles in atmosphere (Gonca Çakmaka et al., 2019).

The objective of this work is to offer a profiling tool to identify aerobiological particles according to trace elements composition (Mn, Co, Ba, Pb, and U) as potential contaminants in aerobiological samples obtained with a Hirst-type volumetric spore trap.

2 Materials and Methods

2.1 Instrumentation

2.1.1 Aerobiological Sampling

Airborne pollen was continuously sampled in 2018 from August 8–20 to September 8–19 using a 7-day Hirst-type volumetric spore trap (model Lanzoni VPPS 2010, Italy), corresponding to the beginning of spring in the southern hemisphere. The trap was placed on the terrace of the Faculty of Chemistry, Biochemistry and Pharmacy of the National University of San Luis, Argentina, approximately 18 m above ground level. Lanzoni trap samples 10 l of air per minute, simulating human breathing. The air impacts onto a Melinex tape coated with silicone oil (CAS 8009-03-8) is placed over a drum, which rotates once a day for a span of 7 days. This tape had been previously cut lengthwise (Thibaudon et al., 2015) to perform elemental determination in one-half tape and aerobiological analysis on the other half tape.

Every week the drum was replaced, and the two half of the sampled tape was cut into seven daily segments and one of the half tapes was mounted on glass slides with fuchsine-stained glycerol jelly. The sampling methodology common to aerobiological studies used here followed the Spanish Aerobiology Network Quality and Management Handbook (Galán et al. 2007). The daily number of pollen grains per cubic meter of air was

Table 1 Instrument settings and data acquisition parameters for ICP-MS

Instrument	Elan DRC-e (PerkinElmer Canada)
Sample uptake rate (μl min^{-1})	200
Sample introduction	Nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC3 system from ESI (Omaha, NE, USA)
RF power (W)	1200
Ar flow rates (mL min^{-1})	Nebulizer, 0.87
Interface	Conos de Ni (sampler y skimmer)
Standard mode	^{55}Mn, ^{59}Co, ^{138}Ba, ^{208}Pb and ^{238}U ^{8288}Sr,$^{111}C^{138}Ba$, ^{205}Tl, ^{208}Pb, ^{238}U,
Scanning mode	Peak hopping
Dwell time (ms)	50 in standard mode
Number of replicate	3

determined with an optical microscope (CX-21 LED OLYMPUS®, Tokio, Japón) at a magnification of 400×. Four full transverses were observed, to increase the sampled area and to avoid problems related with the homogeneity of the adhesive in the sample (Thibaudon et al. 2015). The pollen types Cupressaceae, Moraceae, and *Ulmus* were chosen for this study because they were the most representative in the air, with higher concentration during the study period and agree with the data recorded for the same period in previous years (Micheletti et al. 2012 and Moglia et al. 2011).

2.1.2 ICP-Ms

An inductively coupled plasma mass spectrometer (ICP-MS), PerkinElmer SCIEX, ELAN DRC-e (Thornhill, Canada), was used (Institute of Chemistry of San Luis INQUISAL, UNSL-CONICET). Air liquid (Córdoba, Argentina) supplied the argon gas with a minimum purity of 99.996%. An HF-resistant and high performance Teflon Nebulizer model PFA-ST was coupled to a quartz cyclonic spray chamber with internal baffle and drain line cooled with the PC3 system from ESI (Omaha, NE, USA) (Table 1). Tygon black/black 0.76-mm i.d. and 40-cm length peristaltic pump tubing was also used. The instrument conditions were auto lens mode on, peak hopping measure mode, dwell time of 50 ms, 15 sweeps/reading, 1 reading/replicate, and 3 replicates. Nickel sampler and skimmer cones were used. A microconcentric nebulizer was used to obtain a high sensitivity and to decrease the formation of double charged ions and oxides.

2.2 Reagents and Samples

The water employed was distilled and de-ionized, with a resistivity of 18.2 MΩ cm, produced by an Easypure RF system from Barnstead (Dubuque, IA, USA). Concentrated nitric acid (65%v/v) from Sigma Aldrich (Germany) was used. Multi-element calibration standard solution 1 containing 10 mg l^{-1} of, Al, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, Tl, U, V, and Zn in 5% HNO_3, Hg standard solution with 10 mg l^{-1} in 5% HNO3, and setup solution from PerkinElmer Pure Plus, Atomic Spectroscopy Standard (Norwalk, USA), were used.

For the external calibration against aqueous standards, the standard solutions were prepared in 1%v/v nitric acid. The concentrations of the analytes were 5.0, 10, 20, 40, 80, and 100 μg l^{-1}. As internal standard, Rh was added to all solutions, including the samples, to a 20-μg l^{-1} final concentration.

2.3 Analytical Procedure

As an alternative to the decomposition process, a novel method for the dissolution of aerobiological samples (Melinex tape with pollen samples) was optimized. The samples and a portion of Melinex tape without pollen samples (used as blank) were manually excised ("Aerobiological Sampling" section) then placed into 15-ml plastic tubes.

The observation of the obtained solutions enabled qualitative conclusions about the preferred conditions, which were 2 ml of concentrated nitric acid and 2 ml of hydrogen peroxide. The tubes were placed in a water bath heated to 90 °C until complete dissolution (2 h), obtaining transparent sample solutions. After that, solutions were brought to a final volume of 15 ml with ultrapure water and stored at 4 °C until analysis.

2.4 Statistical Analysis

Shapiro–Wilk's test has been performed to test data for normality. Since the data were not normally distributed, Spearman's non-parametric statistical test was used to determine the correlation between aerobiological particles and heavy metals. The data analyzed were carried out under statistical package for the *Minitab* program version 17.0 and the correlation has been presented by scatter plot, in which the strength of the relation has been expressed by the coefficient of determination (R^2).

3 Results

3.1 Microscopic Analysis of Pollen

Moraceae, Cupressaceae, and *Ulmus* were the most abundant pollen types in the atmosphere of San Luis during the studied period. The microscopic analysis determined that there is an increase in *Ulmus* and Cupressaceae pollen during mid-August, decreasing as spring approaches. The maximum concentration values of pollen were 166 pollen grains per cubic meter of air for Cupressaceae and 55 pollen grains per cubic meter of air for *Ulmus*. In September, the pollen of Moraceae increases, being extremely abundant in middle September, with a maximum concentration values of 800 pollen grains per cubic meter of air.

Table 2 Elemental profile of pollen treated with nitric acid. Comparison with microwave-assisted digestion approach

Analyte	Pollen with nitric acid (mg g^{-1})[a]	Pollen with microwave acid digestion (mg g^{-1})[a]
Mn	14.11 ± 0.61	14.60 ± 0.31
Co	1.35 ± 0.04	1.40 ± 0.01
Ba	5.81 ± 0.31	5.02 ± 0.52
Pb	2.20 ± 0.05	1.17 ± 0.03
U	0.04[b]	< LOD

Values expressed as mass of metal per mass of Melinex tape with the aerobiological samples

[a] Confidence intervals as 2 standard deviation ($n = 3$)

[b] Values between LOQ and LOD

Table 3 Figures of merit for determination of trace elements in pollen samples treated with nitric acid

Analyte	Mass (u.m.a.)	LOD[a] (mg g^{-1})	LOQ[b] (mg g^{-1})	RSD[C] (%)
Mn	55	0.14	1.52	1.9
Co	59	0.05	0.31	6.6
Ba	138	0.08	0.57	4.8
Pb	208	0.03	0.43	7.7
U	238	0.008	0.06	8.6

Values expressed as mass of metal per mass of aerobiological samples

[a] As three times the standard deviation of blank ($n = 10$)

[b] As ten times the standard deviation of the blank ($n = 10$)

[c] Relative standard deviation at 10 μg l^{-1} ($n = 3$)

3.2 Calibration Choice, Analytical Performance, and Application

A novel method for the dissolution of aerobiological samples was optimized. The accuracy of the results obtained with the optimized method and analyte addition calibration was assessed through comparison (*t* test, 95% confidence interval) with an independent sample treatment procedure such as acid digestion in microwave (Table 2).

Under the optimum conditions described above, the performance data of the system for elemental profile determination was established. Table 3 shows the figures of merit for the elemental determination in aerobiological samples treated with acid dissolution. The reproducibility of the method was evaluated with 2 ml of

Table 4 Application to aerobiological real samples taken in August and September

Analyte	Mass (u.m.a.)	Aerobiological sample, August 12 (mg g^{-1})	Aerobiological sample, September 19 (mg g^{-1})
Mn	55	15.74	3.83
Co	59	1.26	0.43
Ba	138	9.27	2.37
Pb	208	1.08	0.47
U	238	0.25	< LOD

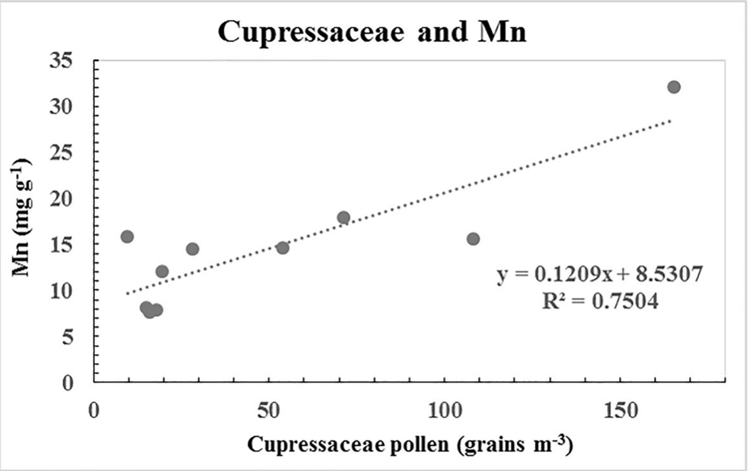

Fig. 1 Correlation between pollen grains of Cupressaceae and Mn, made under the experimental conditions optimized ($r = 0.86$, $p = 0.0015$)

blank sample solution (Melinex tape without samples) repeating this procedure 10 times. The relative standard deviation (RSD) was calculated (from 1.9% for Mn to 8.6% for U). The calibration graph using this method for elementary profile was linear with a correlation coefficient of 0.999 in all cases. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the amount of trace element required to yield a net peak equal to three and ten times the standard deviation of the background signal, respectively.

Validation of ICP-MS analysis was performed by using spike–recovery tests on polled data obtained from the analyzed aerobiological samples ("Aerobiological Sampling section"). The samples were treated with the recommended procedure and small amounts of standard multi-elemental solutions were added to three aliquots in ways to obtain low, medium, and high spike levels, plus an aliquot without addition of standard. In all cases, the recoveries were quantitative from 87% for ^{208}Pb to 118% for ^{55}Co. The proposed method ("Aerobiological Sampling section") for solubilization with nitric acid was applied to two pollen samples collected (12 August and 19 September), and the elemental content obtained is shown in Table 4.

3.3 Analysis of Pollen Versus Heavy Metals

The results of the heavy metal studies in aerobiological samples showed positive association between the pollen contents of Cupressaceae and Mn (Fig. 1), *Ulmus* and Mn (Fig. 2), Moraceae and Pb (Fig. 3), and Moraceae and Mn (Fig. 4). In August, the amount of *Ulmus* grains increased as well as the concentration of Mn (Fig. 5). In the same sense, when Cupressaceae increased, Mn also rose (Fig. 5). In September, there was an increase in

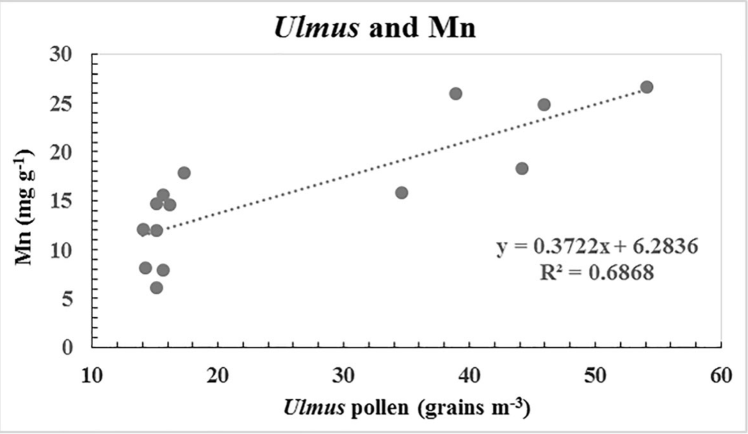

Fig. 2 Correlation between pollen grains of *Ulmus* and Mn, made under the experimental conditions optimized ($r = 0.83$, $p = 0.0011$)

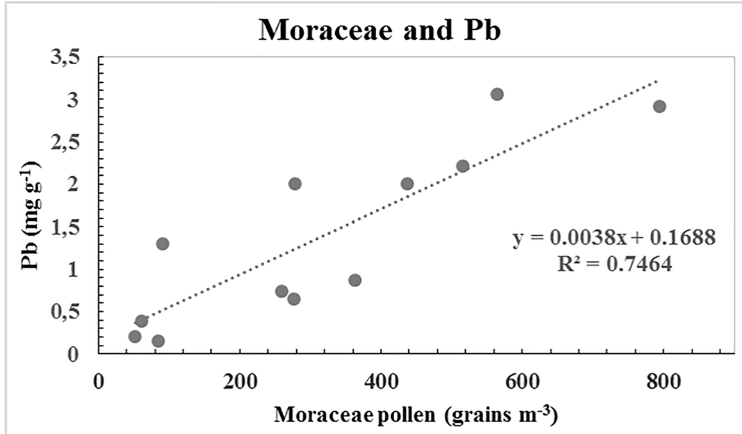

Fig. 3 Correlation between pollen grains of Moraceae and Pb, made under the experimental conditions optimized ($r = 0.78$, $p = 0.0026$)

Moraceae pollen, and jointly of the heavy metals Pb and Mn (Fig. 6).

4 Discussion

In this work, the analysis of heavy metals with ICP-MS in aerobiological samples obtained with a type Hirst spore trap has been reported for the first time. Kalbande et al. (2008) have reported the analysis of fresh pollen grain samples with the technique of ICP-OES for the elemental determination. In addition, Hladun and Trumble (2015) have registered in their work the accumulation of Cd, Cu, and Pb in vegetative and reproductive organs of plants.

In this work, positive correlations were found between pollen of Cupressaceae, *Ulmus*, and Moraceae with Mn, and pollen of Moraceae with Pb. This information could indicate the presence of metallic contaminants in pollen grains. Considering the previous analysis, *Ulmus* pollen showed a potential affinity for Mn with respect to Cupressaceae pollen. This could suggest a low affinity of this pollen to Mn, despite its high concentration, compared with *Ulmus* pollen. That could be related to the different surface characteristics of these pollen grains. The surface of the *Ulmus* grain is rugulate and 4–6 porate, while the surface of the Cupressaceae grain is psilate and inaperturate. This could be related on the greater transport of particles on the surface of *Ulmus* pollen compared with that of Cupressaceae (Belmonte et al., 1986).

Elemental content quantified in aerobiological samples studied could be due not only to pollen, but to also the carried in other particles, both biological and non-biological. For example, the behavior of Mn could be due to the presence of other aerobiological particles associated with this metal, such as *Ulmus* pollen, fungal spores, or other particles. Accordingly, future studies

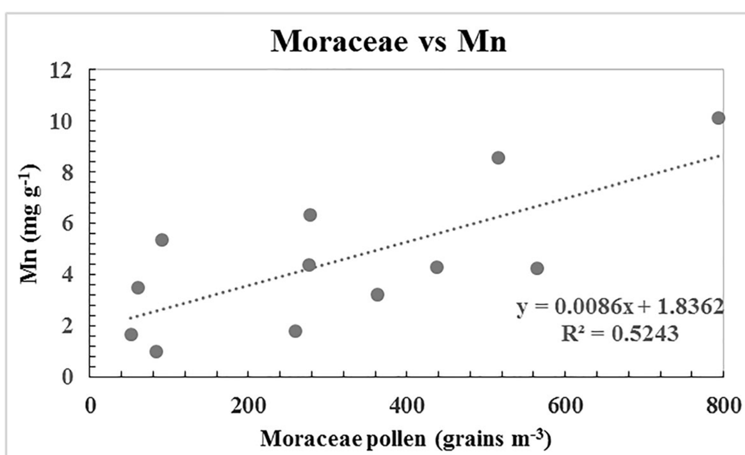

Fig. 4 Correlation between pollen grains of Moraceae and Mn, made under the experimental conditions optimized ($r = 0.72$, $p = 0.0082$)

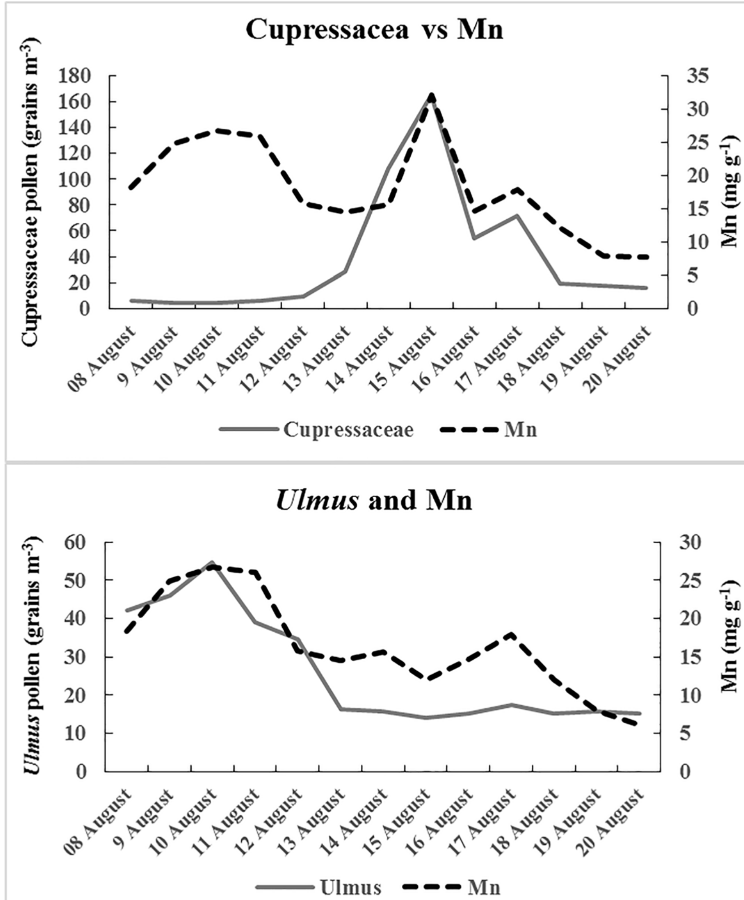

Fig. 5 Scatter plot. Increase in *Ulmus* pollen grains related to the increase in Mn concentration and increase in Cupressaceae pollen grains related to the increase in Mn concentration

should to consider studying elemental content in other types of aerosols trapped in the Melinex tape. In addition, it would be important to compare elemental content of fresh pollen grain samples with that of aerobiological samples collected simultaneously. In this sense, aerobiological particles can cause allergy symptoms, but when they are associated with contaminants present on the surface of pollen, their allergenicity power increases (Sedghy et al. 2018). Aeroparticles may be acting as carriers of pollutants from one area to another. In this way, the quality and composition of particles attached to pollen grains may reflect a measure of the quality of local environment (Gomes et al. 2019).

Furthermore, considering the pollen threshold category criteria, established by the Spanish Aerobiology Network Quality and Management Handbook (Belmonte et al. 2000; Galán et al. 2007), the pollen concentration of Cupressaceae, *Ulmus*, and Moraceae was found between the moderate and very high categories. The maximum records (pollen grains per cubic meter of air) of the pollen types studied in this work were obtained in the Aerobiology laboratory (UNSL) during 2011 and corresponded to exotic taxa of Cupressaceae with 988 grains of pollen m3 of air, Moraceae with 1946 grains of pollen per m3 of air and Ulmus with 82 pollen grains m3 of air, characteristic of the urban afforestation of the city of San Luis (Moglia et al. 2011). However, in 2010, the type of dominant pollen was that of Cupressaceae (Micheletti et al. 2012); this difference can be attributed to profuse afforestation with *Morus alba* "fruitless" cultivars, which emits large amounts of pollen (Cariñanos and Casares-Porcel, 2011). Regarding the presence of heavy metals in the city of San Luis, Bernasconi et al. (2000), working with *Usnea densirostra* as a bioindicator, concluded that there would be no seasonal variations in the concentration of heavy metals and that their levels could be related to vehicular traffic in the urban area.

The proposed methodology constitutes a novel and simple method to obtain information on the contamination in the air with heavy metals, from aerobiological samples obtained with Hirst-type sensors.

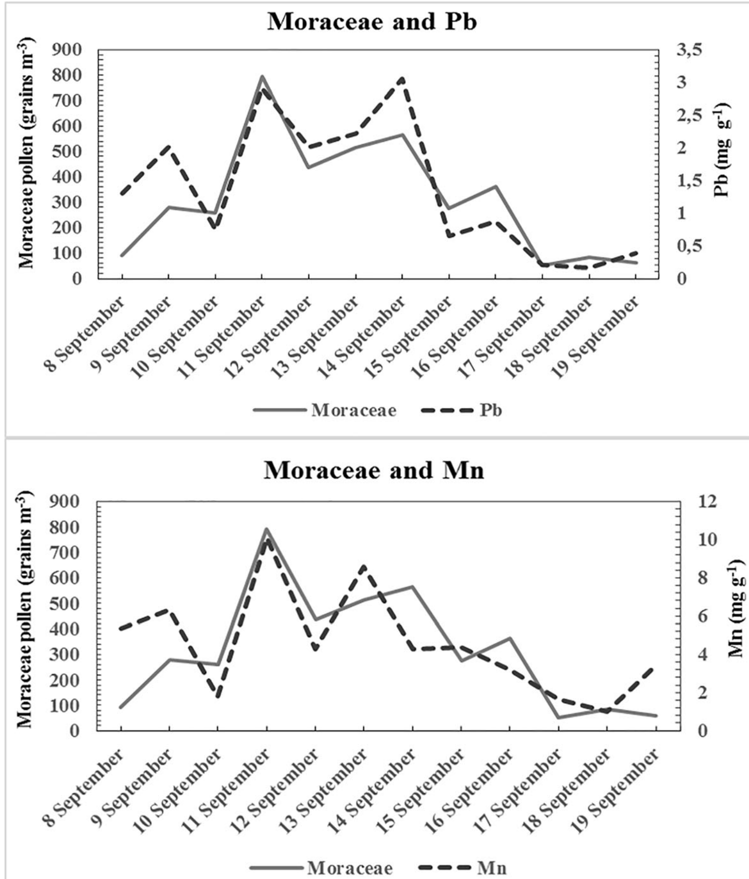

Fig. 6 Scatter plot. Increase in Moraceae pollen grains related to the increase in the concentration of Pb and Mn

5 Conclusions

In this study, ICP-MS was used to analyze aerobiological samples after acid dissolution. We conclude that the ICP-MS procedure gives a fast, precise, accurate, and less expensive sample preparation in complex matrices compared with other types of digestion, and allows high sample throughput. Furthermore, this entire procedure to identify the elemental profile of aerobiological samples makes the method an attractive approach for routine analysis. We are not only proposing a new strategy to analyze air samples but also giving new information of the local elemental composition of pollen in the period and conditions corresponding to this study.

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