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Correlation study of air pollution and cardio-respiratory diseases through NAA of an atmospheric pollutant biomonitor

M. Saiki · J. O. Santos · E. R. Alves · F. A. Genezini · M. P. Marcelli · P. H. N. Saldiva

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Abstract In this study neutron activation analysis was applied to analyze lichen samples used as atmospheric pollutant biomonitors in order to verify if there is correlation between air pollution and its effects on the cardio respiratory system. *Canoparmelia texana* lichenized fungii species was chosen for passive biomonitoring of atmospheric pollutants. The population group selected for this study was adults over 45 years. Lichen samples collected in São Paulo city were cleaned, freeze-dried and ground for the analyses. Aliquots of samples were irradiated at the IEA-R1 nuclear research reactor for short and long periods along with synthetic element standards. The induced gamma activities of the samples and standards were measured using a gamma ray spectrometer with an HPGe detector and the concentrations of As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cs, Fe, Hf, K, Mg, Mn, Na, Rb, Sb, Sc, Se,Th, V, Zn and lanthanides were determined. For quality control of the results, certified reference materials were analyzed together. Mortality data for the population due to cardio-respiratory diseases were obtained from the database of the Secretariat of Health of the São Paulo Municipality for the years 2005–2009. Results obtained point to vehicular and industrial emissions as the origins of pollutants in São Paulo city. The statistical treatment of Pearson's correlation applied to the results of lichen element concentrations and mortality rates indicated significant positive correlation for the elements Co, Mn and Zn for adults.

Keywords Air pollution biomonitoring · Lichen · Cardio respiratory diseases · Correlation · Neutron activation analysis

M. Saiki (✉) · E. R. Alves · F. A. Genezini
Instituto de Pesquisas Energéticas e Nucleares, Av. Professor Lineu Prestes, No. 2242, São Paulo, SP CEP 05508-000, Brazil
e-mail: mitiko@ipen.br

J. O. Santos
Centro Federal de Educação Tecnológica de Sergipe, Av. Engenheiro Gentil Tavares da Mota, 1166, Aracaju, SE 49055-260, Brazil

M. P. Marcelli
Intituto de Botânica, Av. Miguel Stefano No. 3687, São Paulo, SP CEP 01064-970, Brazil

P. H. N. Saldiva
Faculdade de Medicina da USP, Av. Dr Arnaldo 455, São Paulo, SP CEP 01246-903, Brazil

Introduction

Throughout the world, urban and industrial developments have been responsible for the increase of atmospheric pollutant emissions. These emissions directly interfere with the health of the general urban population. Thus, health effects of air pollution have been subject to intense study in recent years.

Serious environmental and health problems have also been observed in São Paulo city, one of the largest and most populous cities in the world. The São Paulo Metropolitan Region (SPMR) is composed of 39 municipalities and has a population of about 20 million people in an area of about 8,051 km^2 [1]. The area suffers from severe environmental problems due to the atmospheric emissions of about 2,000 highly pollutant industries and from about 7.3 million motor vehicles according to the Environmental Protection Agency of the State of São Paulo (CETESB), the governmental agency for air quality control [2]. The car license plate rotation day is implemented in central area of São Paulo city to improve urban traffic and to reduce the level of pollution during the whole year, excepting weekend.

In order to evaluate the levels of the air pollutants, their origins and their effects on human health, atmospheric biomonitors such as lichens, mosses and some higher plants have been widely analyzed. These atmospheric biomonitors have proved their advantages over conventional air sampling techniques in air filters since they present easy sampling, low cost and the possibility of monitoring wide areas. Furthermore their high degree of element accumulation enables the determination of several elements with high precision and accuracy. Due to these advantages, the use of biomonitor may be implemented quite easily in areas devoid of conventional air pollution monitoring systems.

The analyses of atmospheric biomonitors have been also performed in order to investigate the association between the element concentrations obtained and health status of individuals of a given population. Wappelhorst et al. [3] found a connection for thalium content in mosses with the occurrence of cardiovascular diseases and for Ce, Fe, Ga and Ge levels with the incidence of respiratory diseases. Wolterbeek and Verburg [4] studied the correlation between moss metal concentrations and epidemiological data of health and mortality rates in the Netherlands. Sarmento et al. [5] presented results concerning the association between the concentrations of elements obtained through atmospheric biomonitoring using lichens and neoplasm mortality in the Portuguese population. Cislghi and Nimis [6] compared biodiversity maps of pollution-sensitive organism, lichens, with mortality maps of a large part of Northeastern Italy and the resulting map showed correlation between air pollution and the incidence of lung cancer.

Based on previous studies [3–6], in this study a systematic sampling of *Canoparmelia texana* lichenized fungi species was performed in São Paulo city and analyzed in order to investigate if there is an association between element concentrations found in these lichens and data on cardio-respiratory mortality rate.

Experimental

Study area

The study area is located in São Paulo city which is the most populous city in the Southern hemisphere. This city is located at the center of the heavily urbanized São Paulo metropolitan area (23.53°S 46.62°W, elevation of about 779 m above sea level) with an area of 1,522.9 km^2. The prevalent wind direction is from the South and Southeast which affects the dispersion of pollutants. The samples were collected in an area of 658.7 km^2 of following sub prefectures: Butantan, Pinheiros, Vila Marina, Sé, Lapa, Pirituba, Mooca, Santana and Parelheiros. Samples were also collected in reference (control) region located at the Intervales Park, Atlantic Forest, SP considered clean region. The geographic coordinates of sampling sites were recorded using a global position system (GPS). The map of São Paulo city with the sub prefectures where the lichens were collected is presented in Fig. 1.

Collection and preparation of the lichen samples

During the period December 2009–January 2010, *Canoparmelia texana* (Tuck.) Elix & Hale lichenized fungi samples were carefully collected from the bark of trees at a height of about 1.5 m from the ground and stored in paper bags. In this process a titanium knife was used. For the analyses, lichen samples were first cleaned by examining them under a stereomicroscope to remove foreign materials, and then immersed in purified water for about 3–5 min. The cleaned samples were freeze-dried and then ground to a powder using a vibratory micro mill.

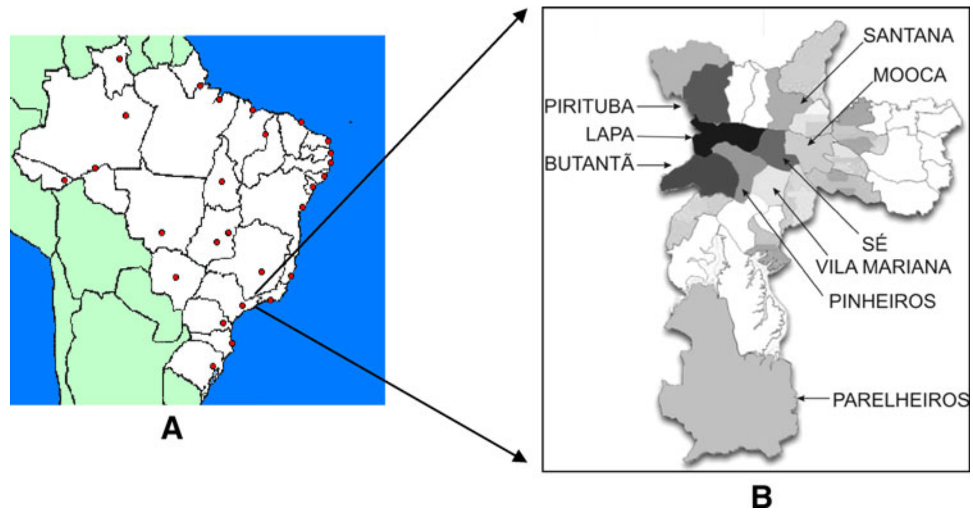
Preparation of synthetic element standards

Synthetic standards were prepared by pipetting 50 µL of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. All the pipettes and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator and then placed into clean polyethylene bags and sealed. In these standards the quantities of each element, in µg (in parentheses) were the following: As(1.5), Ba(200.2), Br(5.0), Ca(1000), Cd(10.0), Ce(4.0), Cl(500.0), Co(0.150), Cr(2.0), Cs(0.60), Fe(360), Hf(1.0), K(500.0), La(1.0), Mg(998), Mn(4.0), Na(200), Nd(2.0), Rb (10.0), Sb(0.6), Sc(0.10), Se(8.0), Th(1.0), V(24.0) and Zn(36.0).

Neutron activation analysis procedure

About 180 mg of the sample weighed in clean polyethylene bags were irradiated at the IEA-R1 nuclear research reactor with synthetic standards of elements. Short irradiations of 10 s under a thermal flux of about 6.6×10^{12} n cm^{-2} s^{-1} were carried out for the determination of Cl, K, Mg, Mn, Na and V. 16-h irradiations under a thermal neutron flux of about 5×10^{12} n cm^{-2} s^{-1} were performed for As, Ba, Br, Ca, Ce, Cd, Co, Cr, Cs, Fe, Hf, K, La, Na, Nd, Rb, Sb, Sc, Se, Th, and Zn. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GX1820 coupled to a Digital Spectrum Processor DSA100, both from Canberra. The resolution (FWHM) of

Fig. 1 Map of Brazil (**A**) and of São Paulo city (**B**) divided in sub prefectures (sub prefectures where the lichens were collected are indicated)



the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1,332 keV gamma ray of ^{60}Co. Counting times from 300 to 50,000 s were used, based on the half-lives or activities of the radioisotopes considered. Spectra were collected and processed using Canberra Genie 2000 Version 3.1 software. Every samples and standards were measured at least twice for different decay times. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by a comparative method. The short lived radionuclides used were ^{38}Cl, ^{42}K, ^{27}Mg, ^{56}Mn, ^{24}Na and ^{52}V. Long lived radionuclides were: ^{76}As, ^{131}Ba, ^{82}Br, ^{47}Ca, ^{141}Ce, ^{115}Cd, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{59}Fe, ^{182}Hf, ^{42}K, ^{140}La, ^{24}Na, ^{147}Nd, ^{233}Pa (Th), ^{86}Rb, ^{122}Sb, ^{46}Sc, ^{75}Se and ^{65}Zn. More than one sample were analysed in each sub prefecture and its mean concentration was considered as the value for this element in this area.

The quality control of the analytical results was evaluated by analyzing certified reference material, IAEA 336 Lichen provided by the International Atomic Energy Agency and CTA-VTL-2 Virginia Tobacco Leaves from the Institute of Nuclear Chemistry and Technology, Poland. These results indicated good accuracy and precision with relative standard deviations and relative errors lower than 12.6 %. The standardized difference or *Z*-score values [7] obtained for elements quantified in these references materials were $|Z\text{-score}| < 2$, indicating that our results are satisfactory and are within the ranges of certified data at the 95 % confidence level.

Population health data

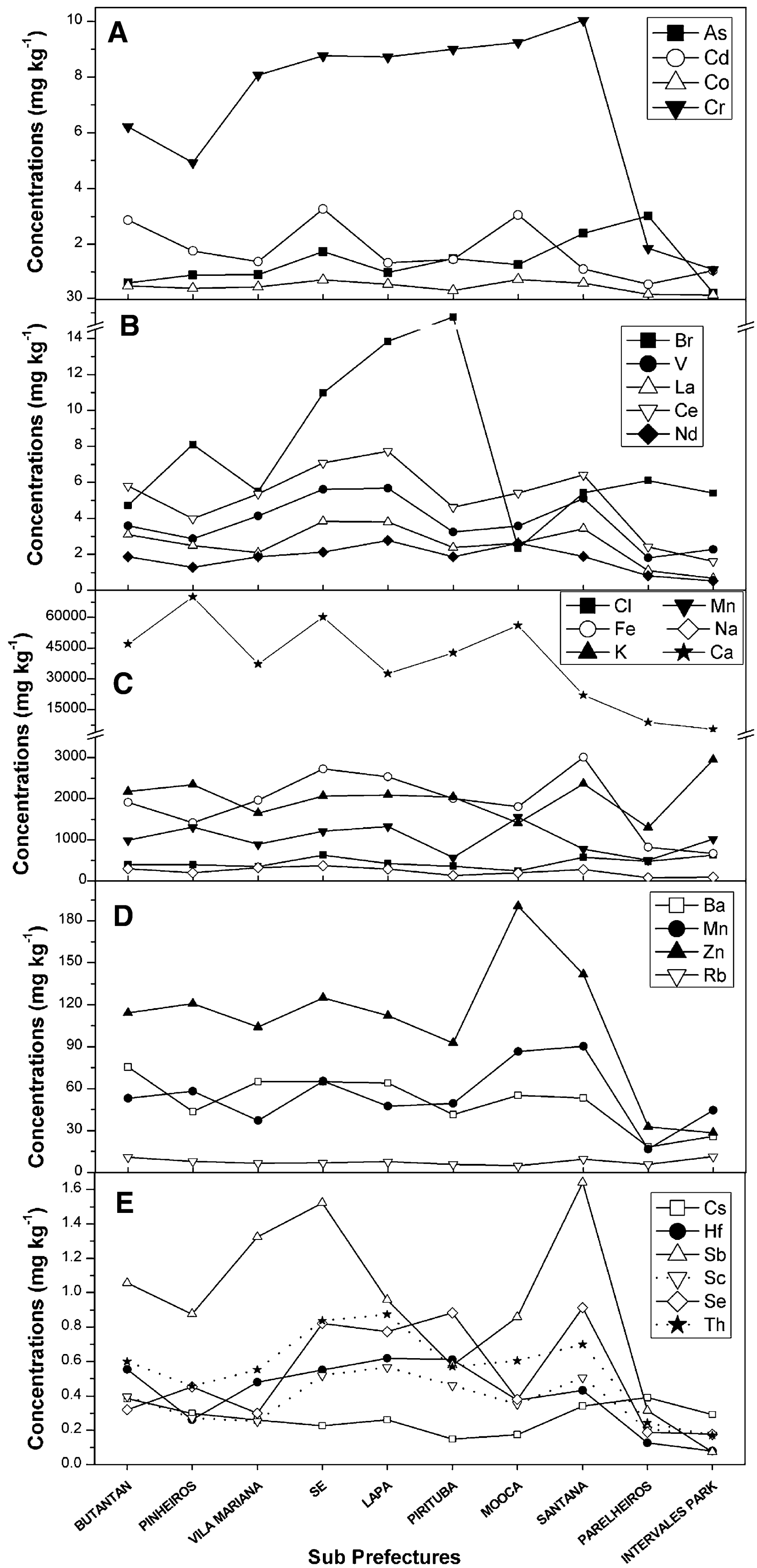
In this study we decided to verify if there is an association between air pollution and mortality rates due to cardio-respiratory diseases in an adult group over 45 years of both genders. In the municipality of São Paulo, it is possible to obtain mortality data separated by sanitary districts in a database maintained by the municipal government, in which causes of death are coded according to the International Code of Diseases (ICD10). These data are available in the website [8]. The population data for each sub prefecture were also obtained from a specific website [9].

Thus, we determined the mortality rates due to the diseases of circulatory and respiratory systems (ICD I00 to I99 and J0 to J99) for a group of adults over 45 years of age and living in the sub prefectures of São Paulo city where the lichens were collected. Based on slow growth of lichens and their exposure period to pollution, we use mortality data for a period of 5 years from 2005 to 2009. Mortality rate data obtained presented in Table 1 were used to correlate with element concentrations obtained in the analyses of lichens.

Table 1 Mortality rates due to circulatory and respiratory diseases during the period from 2005 to 2009 for adults over 45 years and residing in different sub-prefectures of São Paulo city

Sub prefectures	Group of adults (>45 years old)		
	Population	Number of deaths	Mortality rate due to respiratory and circulatory disease
Butantan	109,044	4,485	0.0411
Lapa	103,757	4,708	0.0453
Moóca	116,273	6,870	0.0591
Parelheiros	25,734	970	0.0376
Pinheiro	117,320	4,968	0.0423
Pirituba	114,720	4,890	0.0426
Santana	113,002	5,772	0.0511
Se	132,492	6,835	0.0516
Vila Mariana	128,389	5,462	0.0425

Fig. 2 Concentrations of elements in lichens from different sub prefectures of São Paulo city and from Intervales Park



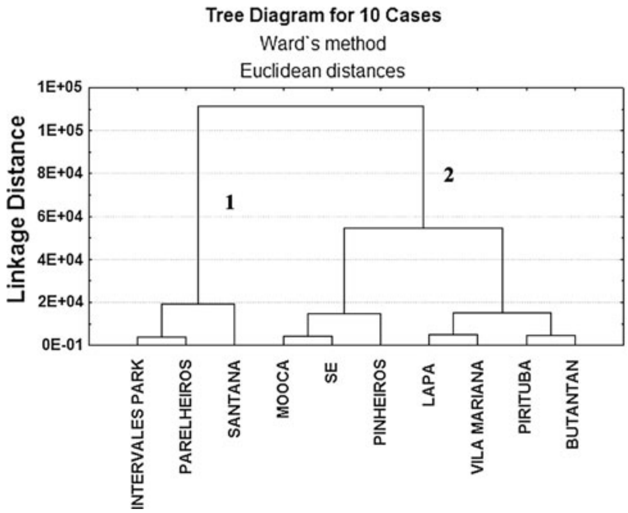


Fig. 3 Dendrogram obtained in cluster analysis for different sub prefectures and Intervales Park

Results and discussion

Element concentrations obtained in lichens from sub prefectures of São Paulo city

Results obtained in lichens are presented in Fig. 2. Elements Ca presented the highest concentrations at the percentage levels, As, Ba, Br, Cd, Ce, Cr, Cl, Fe, K, La, Mg, Mn, Na, Nd, Rb, V and Zn at the levels of mg kg^{-1} and the elements Co, Cs, Hf, Sb, Sc, Se and Th presented the lowest concentrations at the levels of μg kg^{-1}.

The most of elements found in lichens from São Paulo city presented higher concentrations than those from control site of Intervales Park (Fig. 2). The exceptions were Cl and K (Fig. 2C). There was a decrease of Cl concentration in samples from São Paulo city sites indicating that the lichens could have been affected by the pollution. K is an essential element for lichens but in polluted area this element is present in low level due to the stress caused by pollutants affecting its uptake. According Garty et al. [10], a decrease of K is expected in lichen exposed to heavy pollution.

High concentration of as (Fig. 2A) found in Parelheiros may be attributed to the use of pesticides containing organic compounds of this element in agriculture activities in this area. Parelheiros is located far from São Paulo downtown in rural area near a region of native vegetation (Fig. 1). The presence of as from pesticides in the atmosphere is reported [11, 12].

The higher concentration Sb in lichens from São Paulo city than that from reference site of Intervales Park (Fig. 2E) can be associated to the emission of plastic material or waste incinerations. Brazilian plastic materials contain Sb, since the compound of this element is used in plastic processing [13–15]. The origins of Cr and Fe in lichens can be associated to industrial emissions and soil origin, derived from the deposition on lichen of solid particles stirred up by the wind.

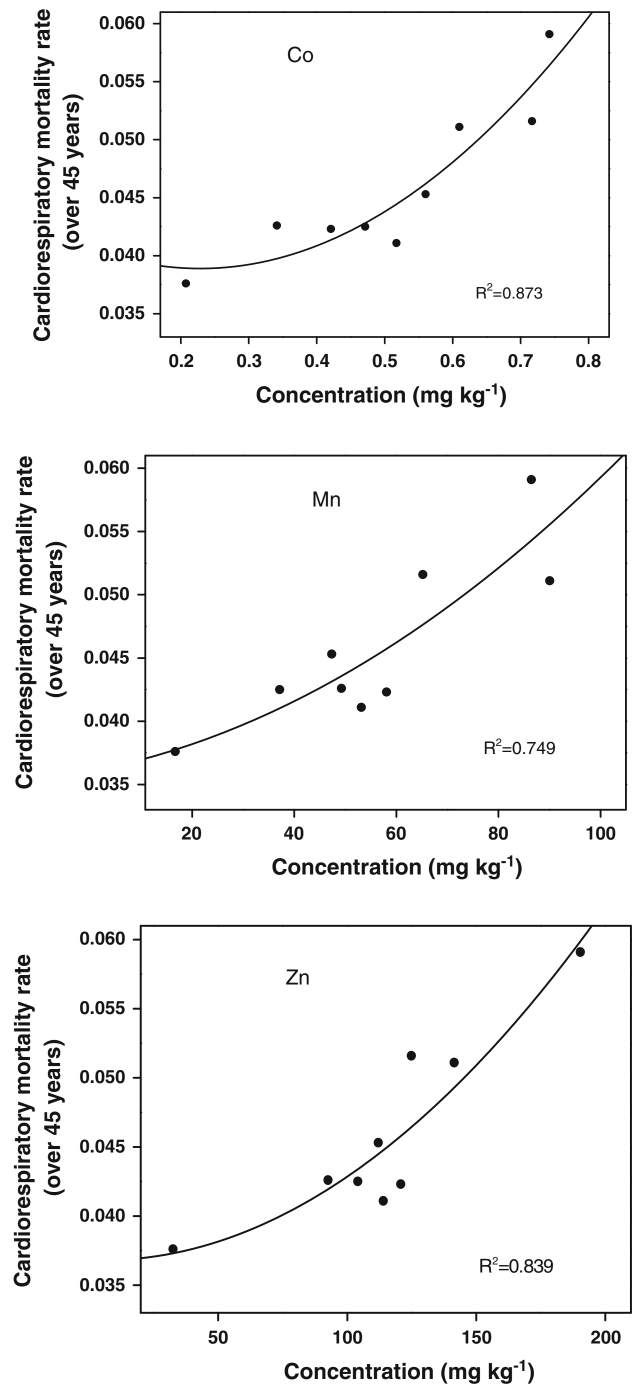


Fig. 4 Correlation between Co, Mn and Zn concentrations in lichen and cardio-respiratory mortality rates for adults over 45 years

The occurrence of high levels of Ba, Mn, V and Zn in lichens from the studied areas may be associated to vehicular and industrial sources. The origin of Ba can be attributed to the use of diesel as a fuel and V of gasoline. Mn and Zn may be associated to industrial origins and to a lesser extend by motor vehicle and tire rubber wear emissions [16, 17]. High concentrations of Ca (Fig. 2C) found in lichens from studied area can be associated to powder of cement used in construction of buildings in São Paulo city.

The lichen analysis data were submitted for sampling site classification by cluster analysis. The resulting dendrogram from this treatment revealed two main groups of sites, as can be seen in Fig. 3. The first group 1 is formed by sampling sites of Intervales Park (considered clean area), Parelheiros and Santana. Parelheiros is a sub prefecture of São Paulo Municipality located near a rural region, about 50 km from downtown and Santana is a sub prefecture located in a region not covered by rationing car use based on license number and this location is far from intense vehicular traffic. The second group 2 is formed by sub prefectures of Mooca, Sé, Pinheiros, Lapa, Vila Mariana, Pirituba and Butantan where are industrial and urban areas with heavy traffic. The cluster analysis substantially confirmed coherent groups of pollution levels.

Mortality rate data obtained presented in Table 1 were used to study correlation with element concentrations obtained in the analyses of lichens. The results through statistical treatment of Pearson′s correlation indicated for adults over 45 years significant correlation for the elements Co ($R^2 = 0.873$), Mn ($R^2 = 0.749$) and Zn ($R^2 = 0.839$) as can be seen in Fig. 4. R is correlation coefficient for data following a quadratic distribution.

However, the present study has an ecological design and thus has limited power to fully characterize the causality between the accumulation of trace elements and observed health effects. In the complex environmental scenario of a large urban center, it should be prudent to ascribe to a single element the responsibility of causing an adverse health effect, but rather representing in a proxy estimate of the complex mixture emitted by the sources present in urban environment. In our case, Zn, Mn and Co are elements present in both automotive and industrial emissions [18] and may be considered as estimates of the spatial gradient of air pollution across the city of São Paulo.

Conclusion

Results obtained demonstrated that biomonitoring is a simple, efficient and cost-effective method with the possibility of identifying emission sources and risks. It can be applied in areas without infrastructure of conventional air pollution monitoring.

The significant correlations found between the element concentrations in the lichen samples and mortality rates can provide indications of possible causes of the mortality due to cardio-respiratory diseases. These findings suggest the application of air pollution biomonitoring to assess risk and adverse health effect of chemical elements and mainly in areas where a conventional automatic network is not available.

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