



Effectiveness of traffic-related elements in tree bark and pollen abortion rates for assessing air pollution exposure on respiratory mortality rates



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ABSTRACT

The majority of epidemiological studies correlate the cardiorespiratory effects of air pollution exposure by considering the concentrations of pollutants measured from conventional monitoring networks. The conventional air quality monitoring methods are expensive, and their data are insufficient for providing good spatial resolution. We hypothesized that bioassays using plants could effectively determine pollutant gradients, thus helping to assess the risks associated with air pollution exposure. The study regions were determined from different prevalent respiratory death distributions in the Sao Paulo municipality. Samples of tree flower buds were collected from twelve sites in four regional districts. The genotoxic effects caused by air pollution were tested through a pollen abortion bioassay. Elements derived from vehicular traffic that accumulated in tree barks were determined using energy-dispersive X-ray fluorescence spectrometry (EDXRF). Mortality data were collected from the mortality information program of Sao Paulo City. Principal component analysis (PCA) was applied to the concentrations of elements accumulated in tree barks. Pearson correlation and exponential regression were performed considering the elements, pollen abortion rates and mortality data. PCA identified five factors, of which four represented elements related to vehicular traffic. The elements Al, S, Fe, Mn, Cu, and Zn showed a strong correlation with mortality rates ($R^2 > 0.87$) and pollen abortion rates ($R^2 > 0.82$). These results demonstrate that tree barks and pollen abortion rates allow for correlations between vehicular traffic emissions and associated outcomes such as genotoxic effects and mortality data.

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1. Introduction

Air pollution remains an important public health problem in most of the world (WHO, 2013). In a review of the evidence on the health aspects of air pollution (WHO, 2013), experts concluded that among the main pollutants, particulate matter (PM), particularly particles with an aerodynamic diameter equal to or $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$), is responsible for adverse effects on human health and suggested that there is no safe exposure threshold. This finding confirmed the known relation between exposure to PM and the adverse effects on the respiratory and cardiovascular systems that occur in adults and children. Additionally, in 2013, the International Agency for Research on Cancer (IARC) at the World Health Organization considered outdoor air pollution to be carcinogenic to humans and identified PM as the component of air pollution most closely associated with the increased cancer prevalence over the

past decades, particularly lung cancer (Loomis et al., 2013). Assessing the risk of human exposure to air pollution requires adequate spatial monitoring of pollutants. In general, assessments of conventional air pollution conducted by network systems in cities are generally not capable of determining the spatial variation of exposure. In particular, Sao Paulo City, where air quality monitoring is performed by 16 monitoring stations in the urban area of 1523 km², clearly has low spatial representativeness. According to the Sao Paulo Official Monitoring Agency, in 2014, 296.34 thousand tons of pollutants (carbon monoxide, hydrocarbons, nitrogen oxides, particulate matter and sulfur oxides) were discharged into the atmosphere of the Sao Paulo Metropolitan Region, of which 259.98 tons were from vehicular emissions (CETESB, 2014).

Plant bioassays have been successfully used for in situ exposure studies of air quality monitoring (Carneiro et al., 2011; Mišik et al., 2007) because plants are generally highly sensitive to genotoxic effects of atmospheric pollutants (EPA, 1980). The widespread location of plants within the urban environment allows the most realistic assessment of the effects of exposure to air pollutants. Furthermore, tests of pollen abortion rates are less expensive and faster than traditional monitoring

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techniques and do not require sophisticated analytical laboratories. The use of tree barks for monitoring exposure has also been demonstrated to be a valuable method for determining the type and source of atmospheric chemical elements (Catinon et al., 2009; Guéguen et al., 2012; Schelle et al., 2008) for obtaining high spatial resolution (Moreira et al., 2016). Currently, there is strong evidence that the effects of atmospheric pollution exposure are modulated by the type of pollutants (Heo et al., 2014; Lippmann et al., 2013). Certain PM compounds, such as metals and organic compounds adsorbed in particles, play an important role in terms of air pollution toxicity (Gottipolu et al., 2008; Chen and Lippmann, 2009). Monitoring of atmospheric chemical elements using tree bark is more economically practical than traditional techniques and allows extensive and frequent monitoring of surveyed areas. In this manner, tree bark studies can assist decision makers on the subject of health and environmental protection against potentially hazardous air pollution exposure.

A previous study demonstrated a significant association between Trad-MCN and adjusted mortality rates for cardiovascular diseases and cancer (Mariani et al., 2009). This finding indicated that plant bioassays could be effective for assessing the risks to human health resulting from exposure to air pollution.

Moreover, pollen abortion assays and traffic-related elements accumulated in tree barks are appropriate for characterizing air quality gradients over a small distance (60 m) from high traffic roads (Carneiro et al., 2011).

In the present study, pollen abortion assays and related-traffic element accumulation in tree barks were used to evaluate the feasibility of these techniques in determining the relationship between air pollution exposure and human health effects. The hypothesis of this study is that the use of these plant bioassays can effectively determine pollution gradients, allowing the associated risks of air pollution exposure to respiratory diseases to be assessed. For this purpose, the correlation between pollen abortion assays and traffic-related elements accumulated in tree barks with the mortality data of lung cancer and chronic obstructive pulmonary disease (COPD) in Sao Paulo, Brazil, were evaluated.

2. Materials and methods

This study was approved by the Institutional Review Board, Number 1036/09.

2.1. Experimental design

Fig. 2 presents a schematic representation of the steps of the applied protocol. Initially, to select the biomonitoring sites, the mortality rates of lung cancer (ICD C34) and COPD (ICD J44) of the individuals were calculated. Individuals above 45 years of age were considered in all 31 regional districts of Sao Paulo, Brazil, from the period of January 2004 to December 2008. Then, four regional districts considering the low, mean and highest mortality rates from lung cancer and COPD were considered. In these four selected regional districts, the pollen abortion rates and the chemical elements accumulated in tree barks were determined. A pollen abortion assay was conducted at three sites in each regional district, considering the vehicular traffic density, as follows: one site with high vehicular traffic (arterial or express), one site with intermediate vehicular traffic (collector) and one with low vehicular traffic (local). The bark was collected on 73 streets and avenues representing all of the traffic densities (local, collector, arterial and express). Finally, the correlations between chemical elements accumulated in tree barks, pollen abortion rates, and mortality rates for lung cancer and COPD during the period from January 2008 to December 2012 were evaluated.

2.2. Mortality data

The database of lung cancer and COPD mortality was obtained from the Program of Improvement of Mortality Information System

(PROAIM) of the Health Department of Sao Paulo City. To select the sites for biomonitoring, the standardized mortality rate per 100,000 inhabitants was calculated separately for each cause of death. For this purpose, deaths during the period from January 2004 to December 2008 were considered.

To test the correlation between mortality and biomonitoring variables, the mortality rate was calculated using lung cancer and COPD together. For this purpose, deaths during the period from January 2008 to December 2012 were used. This variable is expressed as the mortality rate.

2.3. Study area

The following regional districts were selected (Fig. 1): Mooca (area of 35.2 km² and 343,980 inhabitants), Pinheiros (area of 31.7 km² and 289,743 inhabitants), Sé (area of 26.2 km² and 431,106 inhabitants), and Vila Marina (area of 26.5 km² and 344,632 inhabitants). In the study area, five monitoring stations of the Sao Paulo Sanitation Agency (CETESB) exist and are distributed as follows: one monitoring station each in the Mooca, Pinheiros and Vila Mariana regional districts and two in the Sé regional district. To minimize confounding effects on mortality, socioeconomic factors and air pollution sources were also considered during selection of the four regional districts. These regional districts have a similar human development index (HDI), ring from 0.90 to 0.95 (Prefeitura de Sao Paulo), and vehicular traffic is a primary source of air pollution in these districts (CETESB, 2004, 2005, 2007, 2012).

2.4. Collection and preparation of tree barks

The barks of 155 different mature trees located in the selected study domain areas, with a diameter at breast height of >30 cm, were collected during the period of 2012–2013 in the following streets of different vehicular traffic densities: express, arterial, collector and local streets. The minimum distance between trees was 10 m. The species *Tipuana tipu*, *Caesalpinia pluviosa*, *Ligustrum* sp., and *Tibouchina granulosa* were selected for this study because of their frequent use in Sao Paulo City for arborization and due to their similar bark characteristics. The samples were manually removed at a height of 1.5 to 2.5 m from topsoil. From each selected tree, one piece of bark was collected from each quadrant of the trunk, and these bark pieces were analyzed together. The samples were carefully cleaned using a white dental brush with soft nylon bristles to remove foreign material. The 3 mm outer layers of each bark sample were manually ground using a titanium grater and then ground to a fine powder using a vibratory micro mill with an agate mortar (Vibratory Micro Mill Pulverisette 0, FRITTSCH GmbH – Milling and Sizing Industriestrasse 8 55743 Idar-Oberstein). Approximately 0.5 g of each powdered sample was transformed into 20 mm diameter tablets by applying 15 tons of pressure for 60 s. For each sampling site, two tablets were prepared (Moreira et al., 2016).

2.5. Measurement of chemical elements in tree barks

The contents of metallic and non-metallic elements were measured using an energy-dispersive X-ray fluorescence spectrometer (EDX 700-HS, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). This instrument employed a low-power Rh-target tube at a voltage of 5 to 50 kV and a current of 1 to 1000 μ A. The characteristic X-ray radiation emitted was detected by a Si(Li) detector. X-ray fluorescence emission spectra were collected for 400 s for elements Na-Sc and for 240 s for elements ranging from T to U on a 10 mm surface area of samples in a vacuum atmosphere. Each tree bark tablet sample was measured twice. The sample intensity measurements were converted to element concentrations (μ g·g⁻¹) according to calibrations of fundamental parameters using the NIST Reference Material SRM 1547 Peach Leaves (National Institute of Standards, Gaithersburg, MD, USA). Finally,



Fig. 1. Study area showing the locations of regional districts: (1), Sé regional district; (2), Mooca regional district; (3), Vila Mariana regional district; and (4), Pinheiros regional district. Δ, Triangles mark the locations of the official air quality stations.

carbon, in cellulose form, was used for mass balance (Carneiro et al., 2011).

2.6. Pollen grain abortions in *Bauhinia* sp.

Bauhinia sp. was chosen for the pollen abortion assay because it is cytologically characterized by a uniform chromosome number of $2n = 28$ (Sharma and Raju, 1968) and because it is extensively used for ornamental purposes in the streets of Sao Paulo. The sampling was conducted in the months of May to September of 2012 at three different

sites in each study domain area. At each site, 15 flower buds were sampled once per month and immediately fixed in a (3:1) mixture of ethanol and acetic acid. After 24 h, slides were prepared according to Mičičeta and Murín (1996). Anthers were dissected and pressed onto slides, and pollen grains on the slides were stained with Carmine solution and scored using a light microscope. Exactly 3000 pollen grains were counted at each site. Pollen grains were evaluated in terms of size, form and staining ability, with deviations considered to be evidence of abortion. The criteria used to establish pollen abortions were a significantly larger than normal grain size and the presence of altered forms of pollen (e.g.,

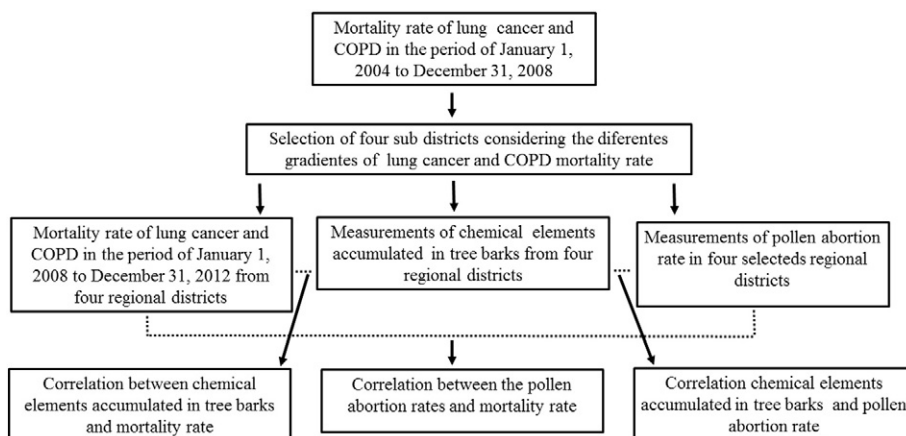


Fig. 2. Schematic representation of the study.

Table 1

Descriptive analysis of the chemical elements accumulated in tree barks. The results are reported in $\mu\text{g g}^{-1}$.

Elements	Median	Percentile		Minimum	Maximum
		25	75		
Na	19.40	16.39	22.63	4.22	126.80
Mg	1,598	880	2813	225	8,010
Al	470.87	282.16	873.66	57.11	6,818.75
P	1005	853	1120	333	2078
S	2,815	2,139	3633	1243	8954
Cl	88.30	47.82	158.11	6.89	801.00
K	1273	970	1840	278	25,070
Ca	34,043	26,560	39,941	7,558	59,628
Mn	35.28	25.36	49.85	4.39	139.60
Fe	775	405	1803	61	7356
Cu	6.14	5.22	7.40	3.53	16.30
Zn	81.97	34.21	153.33	13.72	595.84
Rb	12.20	10.11	16.37	4.49	55.84
Sr	93.96	71.25	134.13	25.91	316.09
Ba	267.40	120.95	593.16	0.00	21,089.04

many air bags) along with coloration imperfections. Young pollen grains were excluded from the analysis.

2.7. Statistical analyses

Differences between pollen abortion rates and concentrations of elements accumulated in tree barks in the regional districts were evaluated using the Kruskal-Wallis test with pairwise multiple comparisons. Principal component analysis (PCA) using the varimax orthogonal rotation method with Kaiser normalization was applied to the concentrations of elements accumulated in tree bark data to find the correlations of all elements and to determine their possible source by variable reduction. Exponential regression was applied to the studied elements, the pollen abortion rates and the respiratory mortality data. All statistical analyses were conducted using Statistical Package for Social Sciences for Windows (SPSS/version 15.0) software.

3. Results

Table 1 presents the descriptive analysis of chemical elements accumulated in tree barks. The elements sodium (Na), magnesium (Mg), aluminum (Al), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), and barium (Ba) were determined.

Table 2

Principal component analysis. Rotated factor matrix (varimax method with Kaiser normalization).

Elements	Factors				
	1	2	3	4	5
Na	-0.359	-0.112	-0.368	0.028	0.686
Mg	-0.052	-0.182	0.836	0.085	-0.037
Al	0.886	-0.088	0.000	0.031	-0.043
P	-0.062	0.335	0.054	0.792	0.115
S	0.465	0.433	0.559	-0.295	0.195
Cl	0.011	-0.087	0.234	0.152	0.840
K	0.113	-0.448	0.360	0.613	0.143
Ca	-0.251	0.868	-0.233	0.149	-0.139
Mn	0.769	-0.219	0.073	0.198	0.021
Fe	0.842	-0.049	0.309	-0.086	-0.191
Cu	0.705	0.219	0.430	-0.255	-0.130
Zn	0.796	0.212	0.194	-0.189	0.007
Rb	0.253	-0.074	0.633	0.359	0.091
Sr	0.145	0.870	0.025	0.030	-0.059
Ba	0.610	0.013	-0.270	0.169	-0.070
Eigenvalues	4.10	2.22	2.19	1.45	1.34
% of variance	27.35	14.81	14.58	9.67	8.93
Cumulative %	27.35	42.16	56.73	66.40	75.33

Among these elements, only P and Rb did not show differences with regard to regional districts. The elements Al, Mn, Fe, Cu, Zn and Ba have been reported to be elements related to vehicular traffic (Schauer et al., 2006). According to Suzuki (2006), the elemental content of Al, Mn, Fe, Cu and Zn in tree bark is an indicator of anthropogenic emissions such as fossil fuel combustion and motor vehicle and industrial emissions. Because sulfur is added to gasoline and diesel to improve fuel combustion in Brazil, this element is considered to be a marker of fossil fuel emissions (Andrade et al., 2012).

PCA identified five factors, which explain 75.33% of the data sets (Table 2). Factor 1 is characterized by the elements Al, Mn, Fe, Cu, Zn

Table 3

Descriptive analysis and of pollen abortion rates and of elements Al, S, Mn, Fe, Cu, Zn and Ba accumulated in tree barks in the four regional districts of Sao Paulo Municipality. Values of pollen abortion are given in %, and element concentrations are reported in $\mu\text{g g}^{-1}$.

Elements	Regional districts	N	Median	Percentile		Minimum	Maximum
				25	75		
Pollen abortion*	Sé	201	81.00	59.83	93.33	1.60	100.00
	Vila Mariana	182	7.00	4.33	10.08	0.67	45.00
	Mooca	139	18.00	12.00	32.00	4.33	94.67
	Pinheiros	142	9.67	7.33	14.08	2.33	47.67
Al ^{§*}	Sé	84	738.02	325.23	985.96	57.11	2,713.27
	Vila Mariana	45	400.13	285.73	796.66	83.68	2,478.16
S [#]	Mooca	24	724.56	393.05	986.41	200.32	2,974.75
	Pinheiros	54	307.76	174.73	618.72	97.77	6,818.75
	Sé	84	3,044	2,472	4,235	1,390	8,460
	Vila Mariana	45	2,836	1,985	3,884	1,243	5,065
Mn [£]	Mooca	23	2,631	2,090	2,850	1,413	8,954
	Pinheiros	54	2,594	1,955	3,413	1,520	6,058
	Sé	84	41.08	29.60	51.05	13.33	100.69
	Vila Mariana	45	31.75	22.18	46.06	9.52	96.47
Fe [¢]	Mooca	24	48.945	36.03	76.68	20.06	139.6
	Pinheiros	54	27.435	19.49	38.76	4.39	132.91
	Sé	84	941	438	2,144	61	5,241
	Vila Mariana	45	606	364	1,571	95	3,629
Cu ^{**}	Mooca	24	1,771	519	2,160	322	6,092
	Pinheiros	54	472	290	1,178	130	7,356
	Sé	84	6,485	5.50	8.52	3.65	14.28
	Vila Mariana	45	6.24	5.08	7.01	3.53	10.44
Zn ^{§§}	Mooca	24	6.63	5.49	8.94	4.09	16.3
	Pinheiros	54	5.645	5.02	6.51	3.74	10.42
	Sé	84	93.94	39.03	179.41	15.64	595.84
	Vila Mariana	45	97.94	28.92	130.38	20.77	362.55
Ba ^{###}	Mooca	24	69.41	44.26	181.23	18.63	383.02
	Pinheiros	54	60.23	23.24	115.15	13.72	414.56
	Sé	84	373.35	178.66	809.28	42.52	21,089.04
	Vila Mariana	45	268.74	117.99	563.88	25.77	3,326.6
	Mooca	24	288.54	136.00	646.06	22.76	2,351.41
	Pinheiros	54	129.17	55.87	367.50	0	3,157.76

* Pollen abortion exhibits a statistically significant difference between all regional districts ($p < 0.0001$).

§* Al exhibits a statistically significant difference between Sé and Vila Mariana ($p = 0.027$) and Pinheiros ($p < 0.0001$), and between Mooca and Pinheiros ($p = 0.001$).

Exhibits a statistically significant difference between Sé and Vila Mariana ($p = 0.027$), Mooca (0.009) and Pinheiros ($p = 0.002$).

£ Mn exhibits a statistically significant difference between Sé and Vila Mariana ($p = 0.016$), Mooca (0.067) and Pinheiros ($p < 0.0001$) and between Mooca and Vila Mariana ($p = 0.001$) and Pinheiros ($p < 0.0001$).

¢ Fe exhibits a statistically significant difference between Pinheiros and Sé ($p < 0.0001$) and Mooca ($p < 0.0001$) and between Vila Mariana and Mooca ($p = 0.015$).

** Cu exhibits a statistically significant difference between Pinheiros and Mooca ($p = 0.029$) and Sé ($p = 0.001$).

§§ Zn exhibits a statistically significant difference between Sé and Pinheiros ($p = 0.003$).

Ba exhibits a statistically significant difference between Pinheiros and Sé ($p < 0.0001$), Vila Mariana ($p = 0.022$) and Mooca ($p = 0.014$).

Table 4

Particulate matter aerodynamic diameter equal to or less than 10 μm (PM_{10}) and nitrogen dioxide (NO_2) obtained from the monitoring of five Sao Paulo Sanitation Agency (CETESB) stations in the study period. Pollutants values are expressed in $\mu\text{g m}^{-3}$.

Month	Average	PM_{10}				NO_2			
		Cerqueira Cesar ^a	Pq. Dom Pedro ^a	Vila Mariana	Mooca	Cerqueira Cesar ^a	Pq. Dom Pedro ^a	Vila Mariana	Pinheiros
May	Monthly	26.01	30.04	36.23	27.79	45.19	25.35	51.51	44.68
	Minimum daily	10.39	13.13	18.58	13.00	20.09	12.07	36.65	28.43
	Maximum daily	49.07	68.79	57.79	54.96	76.92	41.30	74.52	61.74
	N	31	31	29	31	31	30	30	31
June	Monthly	31.14	30.43	36.51	34.42	48.50	45.96	52.08	46.59
	Minimum daily	6.17	9.33	15.96	12.26	24.09	10.20	31.65	28.91
	Maximum daily	59.39	65.79	66.08	62.29	72.52	76.57	78.91	67.91
	N	30	30	28	30	30	30	30	30
July	Monthly	39.12	44.58	45.83	43.17	58.63	64.27	63.69	52.06
	Minimum daily	7.00	8.70	14.17	9.96	17.48	21.22	34.30	21.96
	Maximum daily	75.05	92.96	81.92	75.42	106.67	117.09	107.30	96.70
	N	31	31	31	31	30	31	31	31
August	Monthly	37.13	46.32	46.03	47.06	54.79	53.81	57.47	51.76
	Minimum daily	10.22	19.96	23.79	24.50	23.74	21.95	40.87	28.48
	Maximum daily	72.79	79.25	78.70	69.04	98.13	89.91	88.61	88.48
	N	31	28	29	28	31	31	31	31
September	Monthly	45.71	46.30	50.40	49.17	59.60	54.40	63.46	51.86
	Minimum daily	13.91	13.65	19.76	16.08	31.52	19.70	36.90	21.78
	Maximum daily	104.47	86.50	93.75	92.83	97.43	96.65	96.48	88.81
	N	24	29	30	27	24	29	21	30

^a Cerqueira Cesar and Pq. Dom Pedro Station are located in the Sé regional district.

and Ba, which explain 27.33% of the variance. The elements Ca and Sr characterize factor 2, and the elements Mg, Rb and S characterize factor 3. Each of these factors explains approximately 14% of the variance of the data set. Factor 4 is characterized by P and K, which explain 9.67% of the variance of the data set. Chlorine and sodium characterize factor 5, explaining 9.9% of the variance.

The results for the pollen abortion rates and the elements Al, Mn, S, Fe, Cu, Zn and Ba accumulated in tree barks from the four study areas are shown in Table 3. Pollen abortion was different among all regional districts ($p < 0.0001$). Al was different between Sé and Vila Mariana ($p = 0.027$) and Pinheiros ($p < 0.0001$) and between Mooca and Pinheiros ($p = 0.001$). Sulfur was different between Sé and Vila Mariana ($p = 0.027$), Mooca ($p = 0.009$) and Pinheiros ($p = 0.002$). Mn was different between Sé and Vila Mariana ($p = 0.016$), Mooca (0.067) and Pinheiros ($p < 0.0001$) and between Mooca and Vila Mariana ($p = 0.001$) and Pinheiros ($p < 0.0001$). Fe was different between Pinheiros and Sé ($p < 0.0001$) and Mooca ($p < 0.0001$) and between Vila Mariana and Mooca ($p = 0.015$). Cu was different between Pinheiros and Mooca ($p = 0.029$) and Sé ($p = 0.001$). Zn was different between Sé and Pinheiros ($p = 0.003$). Ba was different between Pinheiros and Sé ($p < 0.0001$), Vila Mariana ($p = 0.022$) and Mooca ($p = 0.014$). The highest pollen abortion rates were found in the Sé regional district ($p < 0.0001$). Similarly, S showed the highest concentrations in barks collected in the Sé regional district ($p < 0.05$). The lowest pollen abortion rates were found in the Vila Mariana regional district ($p < 0.0001$), but the lowest concentrations of Mn, Fe and Ba were found in barks collected in the Pinheiros regional district ($p > 0.05$).

Table 4 shows the values of PM_{10} and NO_2 in the period from May to September of 2012, obtained from five air quality monitoring stations within the CETESB. In this period, no monitoring was performed of PM_{10} in Pinheiros and of NO_2 in Mooca. The highest daily average of PM_{10} was measured in September, except for Pq. The highest average was measured in July at Dom Pedro Station (Sé regional district). The highest monthly average of PM_{10} was measured in September in the Vila Mariana regional district ($50.40 \mu\text{g m}^{-3}$). For NO_2 , the highest daily average was measured in July for all stations. Similarly, the highest monthly average of NO_2 was found in July, except for Cerqueira Cesar Station (located in the Sé regional district).

The highest mortality rate for lung cancer and COPD during the period 2008 to 2012 was found in the Sé regional district (Table 5).

Figs. 3–5 show the correlations between respiratory mortality rates, pollen abortion rates and concentrations of elements in tree barks obtained in the four regional districts. As shown in these figures, there were strong correlations between the parameters, pollen abortion rates, element concentrations determined in tree barks, and the respiratory mortality rate ($R^2 > 0.8$), with the mean values of the residuals being equal to zero.

4. Discussion

The most relevant findings of this study were the strong correlations found between traffic-related element concentrations in tree barks and pollen abortion and mortality rates related to lung cancer and COPD in the population of Sao Paulo. To the best of our knowledge, this is the first study regarding correlations between mortality data, element concentrations in tree barks and pollen abortion rates. Because the techniques used here are simpler and less expensive than conventional air quality monitoring, they allow for monitoring of extensive areas, resulting in improved spatial resolution of air quality monitoring. In addition, by considering both chemical and biological approaches simultaneously, the risk assessment accuracy can be significantly improved. This fact is of particular relevance for improving the understanding of air pollution effects on human health.

Note that in this study, the highest correlation was found between pollen abortion and mortality rates. The association between the increase of air pollution and risk of mortality has been well established (WHO, 2013). However, epidemiological studies in urban areas have demonstrated that chronic health effects related to air pollution exposure may be even three times greater than previously reported (Jerrett et al., 2005; Jerrett et al., 2013). Traditionally, epidemiological studies

Table 5

The mortality rates for the four districts in the study domain area.

Regional districts	Total deaths ^a	Population	Mortality rates (%)
Sé	225	88,582	0.254
Mooca	167	75,565	0.221
Vila Mariana	141	65,887	0.214
Pinheiros	133	65,517	0.203

^a Total deaths from lung cancer (C34) and COPD (J44) in the period from 2008 to 2012.

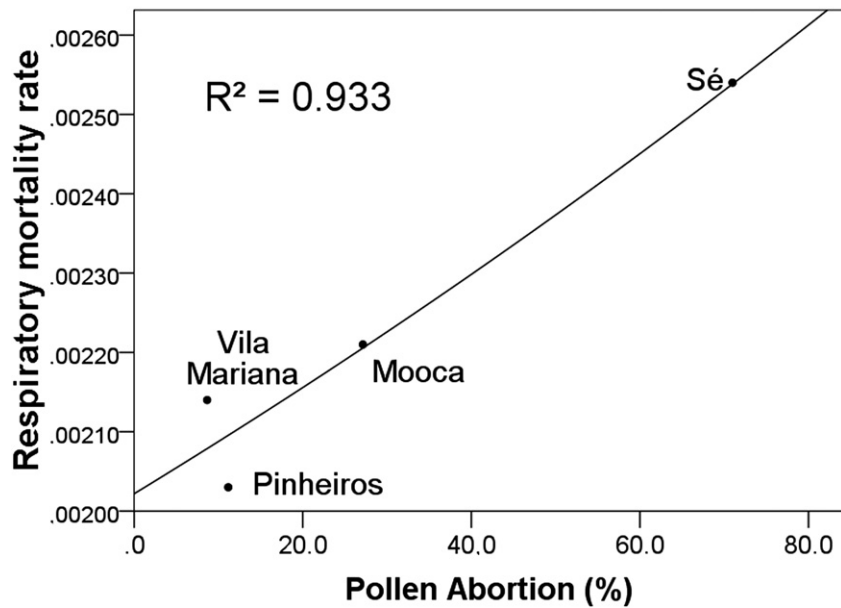


Fig. 3. Exponential correlation between pollen abortion rates and respiratory mortality rates considering the four study areas Mooca, Pinheiros, Sé, and Vila Mariana regional districts. The p value was <0.005 .

are based on conventional networks of air quality monitoring systems. However, such monitoring systems do not allow the intra-urban spatial variation of pollutants to be determined with the required accuracy to ensure minimal error in exposure risk assessment. One possibility to overcome this difficulty is to obtain direct measurements in a study area with better spatial resolution, but this approach can hinder the implementation of studies over large areas due to the high cost and complexity of logistic execution. Recently, some studies have employed spatiotemporal land-use regression models to improve the consistency of association between air pollution exposure and human health effects (Jerrett et al., 2005; Jerrett et al., 2013). Although this strategy improves the accuracy in terms of determining the air pollution exposure, these methodologies require accurate measurements of air pollutants over a long period of time. In this context, the use of plants as air pollution

biomonitors represents a good alternative for air quality management. These techniques also allow for monitoring of extensive areas, resulting in improved spatial resolution.

The use of tree barks in air pollution studies has been reported to be a good tool for indicating and characterizing depositions of air pollutants with improved spatial resolution (Carneiro et al., 2011; Moreira et al., 2016; Schulz et al., 1999). In this study, the factor analysis applied to the data of the accumulated chemical elements demonstrated that the main source of air pollutants in Sao Paulo is vehicular traffic. Among the five factors identified, four can be characterized by elements whose source is attributed to vehicular emissions (tailpipe emissions) or to the wear of vehicle parts, such as brake wear and tire wear. These four factors together are responsible for explaining 66.4% of the variance of the data sets, confirming that the main source of air

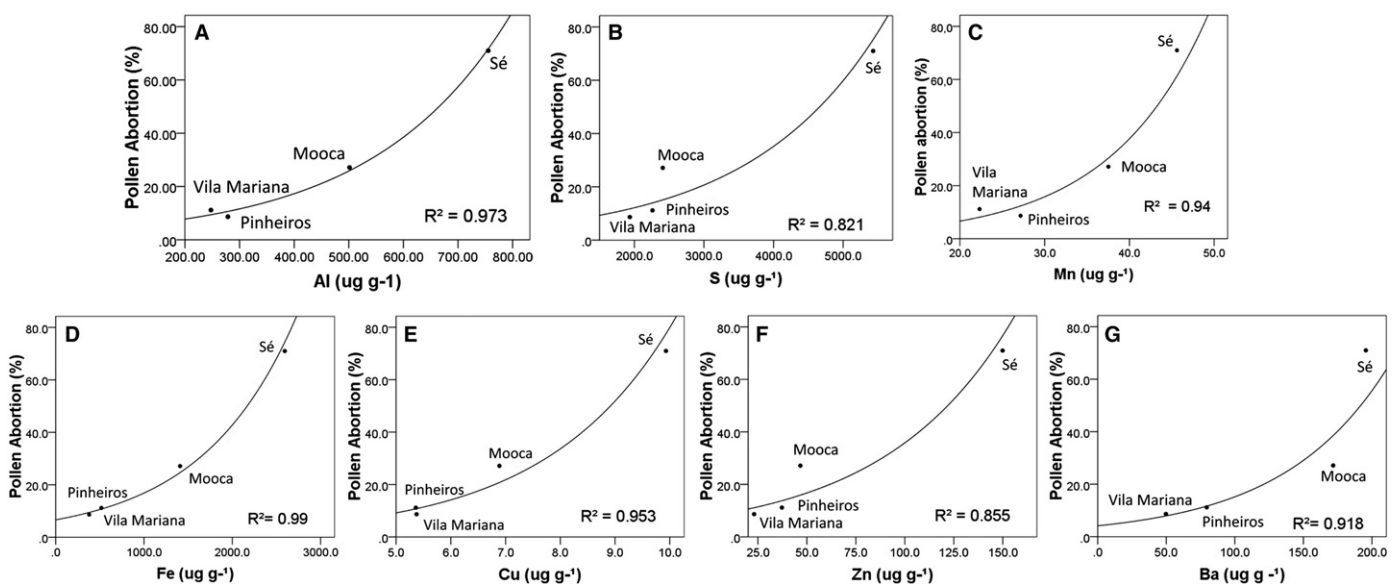


Fig. 4. Exponential correlation between pollen abortion rates and element concentrations in tree barks considering the four study domain areas Mooca, Pinheiros, Sé, and Vila Mariana regional districts. In panel A, correlation between pollen abortion rates and aluminum; panel B, correlation between pollen abortion rates and sulfur; panel C, correlation between pollen abortion rates and manganese; panel D, correlation between pollen abortion rates and iron; panel E, correlation between pollen abortion rates and copper; panel F, correlation between pollen abortion rates and zinc; and in panel G correlation between pollen abortion rates and barium. All p values were <0.005 .

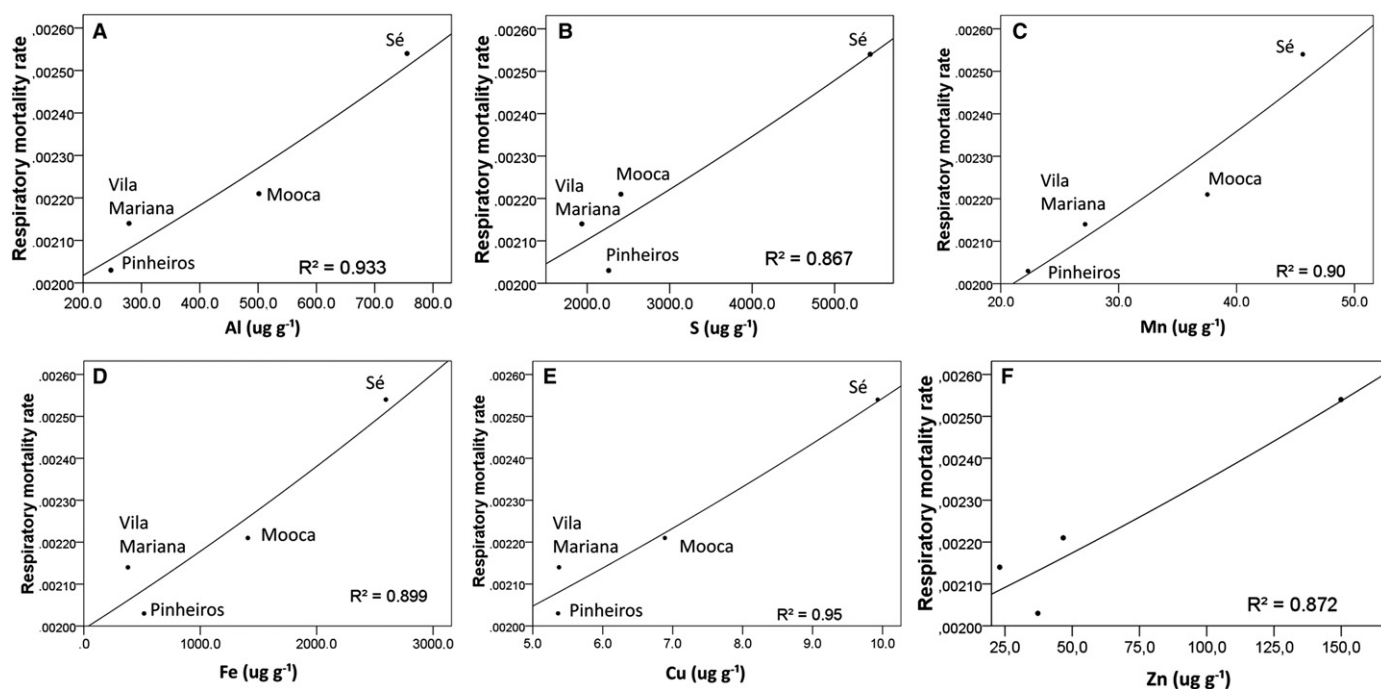


Fig. 5. Exponential correlation between respiratory mortality and element concentrations in tree barks obtained in the four study areas Mooca, Pinheiros, Sé, and Vila Mariana regional districts. In panel A, correlation between respiratory mortality rates and aluminum; panel B, correlation between respiratory mortality rates and sulfur; panel C, correlation between respiratory mortality rates and manganese; panel D, correlation between respiratory mortality rates and iron; panel E, correlation between respiratory mortality rates and copper; and in panel F, correlation between respiratory mortality rates and zinc. All p values were <0.005 .

pollutants in Sao Paulo is vehicular traffic. These results reinforce the plausibility of this technique as a sensitive tool for environmental monitoring. The elements Mn and Zn (factor 1), Ca (factor 2), and Mg and Rb (factor 3) are used as components of additives for engine lubricating oil. These elements found in urban PM are linked to tailpipe emissions. The presence of Al, Ba, Cu, Fe, and Zn (factor 1) and Sr (factor 2) is attributed to brake and tire wear (Schauer et al., 2006). Sawidis et al. (2011) attributed the Cu and Fe content accumulated in tree bark to emissions from vehicles and industrial activities. In their study, Schauer et al. (2006) demonstrated that the major contribution of K (factor 4) in urban PM is from resuspended road dust. However, Andrade et al. (2012), in a study that characterized the emissions in tunnels in Sao Paulo, Brazil, attributed the content of K found in PM_{2.5} to ethanol combustion. Since the 1980s, ethanol fuel produced from sugarcane has been used in Brazil. Therefore, this fuel contains K from vegetation. Currently, ethanol represents approximately 50% of the fuel used in Brazil, both in vehicles with flex engines and as a mixture with gasoline.

A significant finding of this study is that all elements of factor 1 found in the PCA showed high correlations with pollen abortion rates. The pollen abortion assay has been shown to be a sensitive tool for investigating air pollution effects (Fleck et al., 2016; Mičičeta and Murín, 1996; Mišiček et al., 2006; Mišiček et al., 2007). This technique is able to identify the physiological response to short changes in the spatial gradient of air pollutants (Carneiro et al., 2011). This methodology can be used in areas without the support of sophisticated analytical laboratories.

Another relevant aspect of this study is that both airborne and genotoxicity markers were obtained from passive biomonitoring. This offers a great advantage over other methodologies where the data related to air quality are obtained at fixed points and have the effect of indicators on health generally used, such as mortality or morbidity not accurately reflecting the exposure to the measured pollutant concentrations.

Various factors, including smoking and socioeconomic status, play important roles in the relationship between air pollution exposure and mortality.

In this study, we used the mortality rates due to lung cancer and COPD as an indicator of population exposure to air pollution. Although mortality resulting from lung cancer and COPD has been strongly associated with long-term tobacco use (USDHHS, 2014), these causes of mortality have shown strong correlations with air pollution exposure. Presently, there is strong epidemiological evidence that chronic exposure to air pollution has harmful effects on the respiratory system. Several panel studies have reported worsening in the symptoms in individuals with preexisting chronic pulmonary diseases, primarily in patients with COPD, increased lung cancer risk and increased total mortality. Among the pulmonary diseases, COPD is believed to be the pulmonary disease with the strongest association to chronic air pollutant exposure (Sunyer and Basagaña, 2001). The studies have shown a decrease in pulmonary function (Pope and Kanner, 1993), heightened night-time chest symptoms (Harré et al., 1997) and increased rescue bronchodilator use (Silkoff et al., 2005) due to air pollution exposure, leading to an increase in hospital admissions (Dominici et al., 2006).

Growing evidence indicates that socioeconomic status plays an important role in the effects of air pollution (Bell and Ebisu, 2012; Hajat et al., 2013; Marshall et al., 2014; Martins et al., 2004). Factors such as chronic medical conditions, health care access, nutrition, fitness, other pollutant exposures, drug and alcohol use and educational level are generally determined by socioeconomic status (Martins et al., 2004; O'Neill et al., 2003). These factors increase individual susceptibility to air pollutants. In the present study, we considered the human development index (HDI) to minimize the effects of socioeconomic factors on mortality.

Importantly, this study has several limitations. First, this study does not allow a causal relationship to be established between the mortality rates and pollution exposure estimators because of the ecological approach adopted. Second, this is a trend analysis study; therefore, the correlation found between mortality and plant bioassays may not be representative of citywide conditions. Finally, in plant bioassays, confounding factors such as smoking and socioeconomic status are not considered. Nevertheless, the correlations found between mortality and pollen abortion are strong. The similarities in socioeconomic status

and air pollution sources in the regional district study may minimize these confounding effects on mortality.

However, despite these limitations, the strong correlations between the elements and abortion rates in pollen grains with mortality from respiratory causes indicate that the use of these techniques may be important for determining the risk of human exposure to atmospheric pollution.

In conclusion, the vehicular traffic-related elements determined in tree barks have a significant positive correlation with mortality from respiratory causes and the cytotoxic effect expressed by the pollen abortion rates, providing indications of possible causes of mortality rates related to respiratory diseases.

The results of this work demonstrate the feasibility of using vehicular traffic elements identified in tree barks and pollen abortion rates in assessing the risks of exposure to air pollution.

Conflict of interest statement

We declare that none of the authors involved in writing this paper has any conflicts of interest with respect to the content of this article.

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