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Biomonitoring of genotoxic effects and elemental accumulation derived from air pollution in community urban gardens

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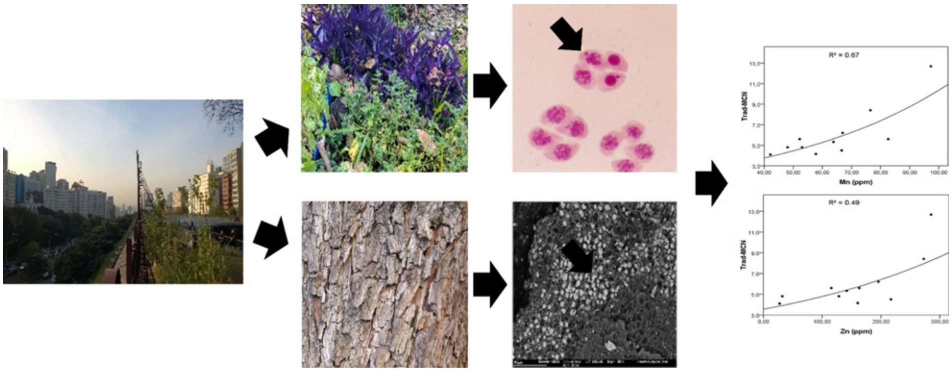
HIGHLIGHTS

- A positive exponential relationship between traffic-related elements deposited on tree barks and Trad-MCN was observed.
- Mn/Zn concentrations on tree barks were associated with an increase in Trad-MCN.
- Negative associations between Trad-MCN and traffic distance/absence of vertical obstacles were observed in the gardens.

GRAPHICAL ABSTRACT



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ABSTRACT

Urban gardening is a growing global phenomenon with a positive impact on society. Despite several associated benefits, growing vegetables in urban gardens that are localized in highly polluted areas poses questions about the safety of the produced food. Therefore, the identification of risk factors that result in possible deleterious effects to human health is important for realizing all of the benefits to society. We evaluated the use of two biomonitoring methods in ten urban gardens of Sao Paulo city and one control site: the micronuclei frequencies for early tetrads of *Tradescantia pallida* (Rose) Hunt. cv. "Purpurea" Boom (hereafter, Trad-MCN) as a short-term indicator of genotoxic response and tree barks to quantify the accumulation of traffic-related chemical elements as a long-term biomarker of air pollution in urban gardens. Mature plants of *Tradescantia pallida* were exposed in each garden, and their inflorescences were sampled over three months. A random set of 300 early tetrads in 13 to 21 slides per garden were evaluated for micronuclei frequencies. Elemental concentrations in 428 tree barks samples from 107 different trees in the areas surrounding urban gardens were quantified using an energy dispersive X-ray fluorescence spectrometer. The frequency of Trad-MCN has a significant correlation with traffic variables and chemical elements related to road dust and tailpipe emissions deposited in tree barks. Negative associations between Trad-MCN and both the distance through traffic and the presence of vertical obstacles were observed in the community gardens. The Mn/Zn concentrations in tree barks were associated with increased Trad-MCN.

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1. Introduction

Urban gardening for growing food is a global phenomenon with a positive impact on society from different economic, social and environmental perspectives (Cameron et al., 2012; CoDyre et al., 2015; Ernwein, 2014). Despite several associated benefits that have been attributed to urban gardens (Bendt et al., 2013; Guitart et al., 2014; Tsilini et al., 2015), some studies have demonstrated a link between high concentrations of metals absorbed in different vegetables and negative externalities of urban environments, such as traffic-related sources (De Temmerman et al., 2015; Säumel et al., 2012). A previous study by our group in the community gardens of Sao Paulo (Amato-Lourenco et al., 2016) has demonstrated an exposure-response between urban characteristics and traffic-related elements that are absorbed in two leafy vegetables. In this city, air pollution concentrations frequently exceed the levels that are established by World Health Organization (WHO) guidelines (WHO, 2006), and the pollutants are primarily derived from a vehicular fleet of 8 million vehicles (CETESB, 2015).

This problem poses questions about the safety of food produced in urban gardens in air polluted areas. The identification of risk factors that are associated with deleterious effects to human health is important for providing a safer basis for urban agriculture. Therefore, biomonitoring of urban garden sites can be an auxiliary tool for choosing adequate urban spaces.

Biomonitoring methodologies such as the micronuclei frequencies on early tetrads of *Tradescantia pallida* (Rose) Hunt. cv. "Purpurea" Boom (Trad-MCN) have been successfully employed in studies of air pollution as an indicator of genotoxic response (Carvalho-Oliveira et al., 2005; Pereira et al., 2013; Spósito et al., 2015). Recently, the quantification of metals that have accumulated in urban tree barks have been employed as a long-term bioaccumulator of traffic derived air pollution (Catinon et al., 2012; Ejidike and Onianwa, 2015; Moreira et al., 2016). Plants may function as precocious sentinels and since they are next to people, they might provide a realistic exposure panorama.

In this study, we evaluated the use of these two biomonitoring methods in urban gardens as preliminary indicators of contamination by air pollution and its relation to the surrounding environment in ten urban gardens of Sao Paulo and one control site.

2. Material and methods

2.1. In situ biomonitoring

Ten community gardens in the Sao Paulo municipality were selected for this study (Fig. 1a); they were part of a previous study that assessed the role of air pollution on leafy vegetables (Amato-Lourenco et al., 2016). All gardens are located in urban areas in which the vehicular fleet is the main source of air pollution (CETESB, 2015). These locations are characterized by different rates of vehicular traffic (buses, trucks, cars and motorcycles) and by different proximities to major avenues and roads (CET, 2013).

For the in situ biomonitoring, seven flowerbeds (width: 13 cm, length: 45 cm and depth: 15 cm) that contain four to six mature *Tradescantia pallida* (Rose) Hunt. cv. "Purpurea" Boom plants grown in an uncontaminated and standardized (3:1) mixture of sieved soil substrate (density of 500 kg cm^{-3}; 9% organic matter, pH of 5.8 ± 0.5 and electrical conductivity of 2.5 ± 0.3 mS cm^{-1}) and vermiculite were allocated in each urban garden (Fig. 1c). The flowerbeds were located in a central area of each community garden and placed on the ground, in the same level of vegetables. The soil substrate was elementally characterized using ICP-MS and energy dispersive X-ray fluorescence spectrometer (for sulphur concentrations) prior to exposure and did not exhibit metal contamination (table S1 - supplementary material) according to the Kabata-Pendias (2011) parameters. The plants were maintained in open air and watered twice a week from September to December 2014. An organic farm in Piracaia, which is a city with low atmospheric pollution in the rural area of the Sao Paulo state province in Brazil, was employed as a control site for the micronuclei assay.

2.2. Trad-MCN assay

The Trad-MCN assay was conducted using an adapted protocol as proposed by (Ma, 1981). Young inflorescences were collected every 30 days throughout the period of three months (from September to December 2014) and immediately fixated in a 1:3 acetic acid to 92% ethanol solution for 24 h. The inflorescences were dissected, and the early tetrads of the meiotic microspore of pollen mother cells were squashed in aceto-carmine stain on a microslide. The counting was performed

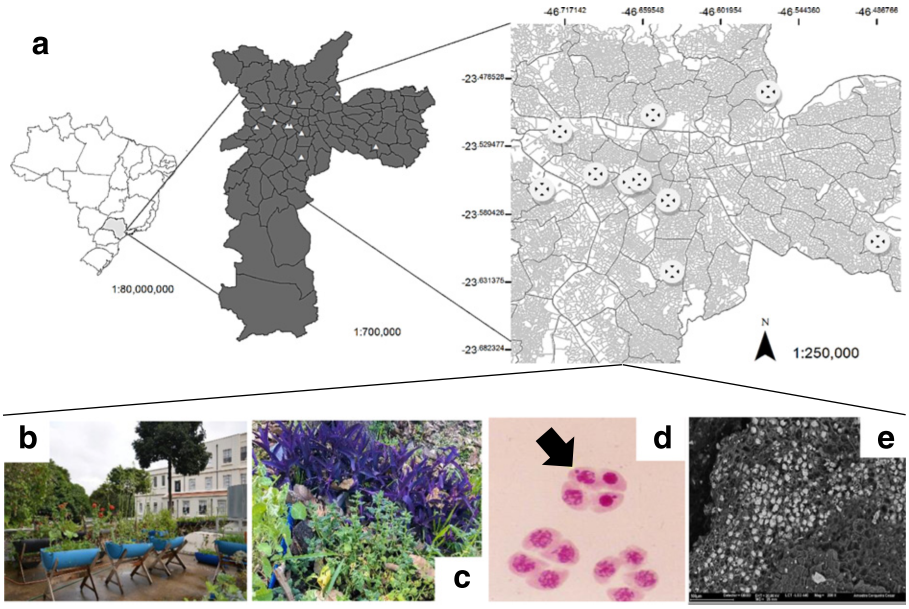

Fig. 1. (a) Locations of exposure and sampling in Sao Paulo municipality. (b) Perspective of urban garden "F". (c) Specimens of *T. pallida* during the exposure in garden "H". (d) Micronuclei highlighted in tetrads of *T. pallida*. (e) Micrograph of a sample of tree bark in garden "F" obtained by scanning electron microscopy (SEM).

with 400× magnification (Olympus H-2, Japan) in a random set of 300 early tetrads in each of the 13 to 21 slides per garden. Fig. 1d shows a micrograph of a micronuclei in a tetrad of *T. pallida*. All slides were coded to ensure that the analysis was performed blind.

2.3. Measurement of chemical elements in tree barks

A geographical information system (GIS) census tract was used to establish a buffer of 600 m from each community urban garden to localize and select the trees within these areas. Samples of tree barks were also collected in a control site at the forest reserve "*Mata da Câmara*"—a rural area 50.2 km from São Paulo municipality.

The samples of tree barks from the species *Tipuana tipu, Caesalpinia pluviosa* and *Ligustrum* sp. were collected within a 600 m radius from each garden. To standardize sample collection of bark trees, we have selected trees with diameter at breast height (DBH) (Lukaszkiewicz and Kosmala, 2008) between 30 and 45 cm, reflecting mature trees. All tree barks were removed by hand at a height of 1.5–2.0 m from the soil. From each selected tree, one piece of bark was collected from each quadrant of the trunk. In the laboratory, all foreign material was removed, and the barks were manually grated with a titanium grater until a thickness of approximately 3 mm was achieved. Each sample was ground to powder using a vibratory micromill with an agate mortar (*Fritsch Pulverisette* 0, Fritsch GmbH, Idar-Oberstein, DE). The bark samples were sieved using a 400 mesh sieve, and the average particle size distribution was determined using low-angle laser light scattering (Malvern Instruments Ltd., Worcestershire, UK) to ensure that the particles were smaller than 100 μm. Then, 0.5 g of tree bark powder and 1 g of boric acid (H_3BO_3, puriss p.a. ACS reagent) were transformed into a 20 mm diameter pellet by applying 15 t of pressure for 40 s (Moreira et al., 2016).

The content of 15 non-metals and metals — traffic-related elements and elements that are essential to plants biology, such as Sodium (Na), Magnesium (Mg), Aluminium (Al), Phosphorus (P), Sulphur (S), Chlorine (Cl), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Cooper (Cu), Zinc (Zn), Rubidium (Rb), Strontium (Sr), and Barium (Ba)—were quantified using an energy dispersive X-ray fluorescence spectrometer (EDX 700-HS, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) as proposed by Carneiro et al. (2011). The instrument consisted of a low-power Rh-target tube at a voltage of 5 to 50 kV and a current of 1 to 1000 μA. The characteristic emitted X-ray radiation was detected by a Si(Li) detector. X-ray fluorescence emission spectra were collected for 400 s for elements Na-Sc and 240 s for the range of elements Ti-U on a 10 mm surface area of samples in a vacuum atmosphere. All analyses were performed in triplicates. The sample intensities were converted to element concentrations ($\mu g\ g^{-1}$) according to fundamental parameter calibrations using the NIST Standard SRM 1547 Peach Leaves (National Institute of Standards, Gaithersburg, MD, USA). A carbon in cellulose form was used as the mass balance. The uncertainties of concentrations were calculated considering the errors in the measurements of the sample and the standard reference material (Table S2 – Supplementary material). Fig. 1e shows a micrograph of a sample of tree bark from garden "F" and the presence of foreign material deposited on its surface.

2.4. Characterization of the urban environments around the gardens

Traffic-related variables from the nearest roads or avenues, such as the daily average speed ($km\ h^{-1}$), the Euclidian distance in relation to the gardens and the nearest road or arterial avenue (m) and the number of vehicles per day (considering buses, cars, trucks, and motorcycles) were obtained from the Traffic Engineering Company of Sao Paulo (CET, 2013). Vertical obstacles between the gardens and avenues, such as buildings or tall vegetation (trees/hedges), and their average height/width (m) were quantified in terms of presence/absence to assess their influence on the tree bark depositions and Trad-MCN. An overall traffic burden (OTB) was calculated as proposed by von Hoffen and Säumel (2014) considering a buffer of 600 m and categorized as low, medium or high (Amato-Lourenco et al., 2016).

Daily means of temperature (°C), relative humidity (%), rainfall (mm) and wind velocity ($m\ s^{-1}$) were obtained for each garden from the Emergency Management Centre of Sao Paulo Municipality during the biomonitoring period. The data of particulate matter (PM_{10}) concentrations during the months of study for each biomonitoring location were obtained from the monitoring stations of the Sao Paulo State Environmental Agency (CETESB).

All data were linked to a geocoded database that was created with ArcGIS software (version 10.3 ESRI, Redlands, CA, USA).

2.5. Statistical analysis

Prior to the data analysis the test Kolmogorov-Smirnov with Lilliefors Significance Correction and residual analysis were performed to verify the distribution of the data and homogeneity of variance. The non-parametric data were log transformed and reanalysed to verify if the normality conditions were attended. Analysis of variance (ANOVA) was conducted to compare the Trad-MCN frequencies between the gardens and the control site. A Pearson correlation matrix was constructed to test the correlations among the chemical elements, Trad-MCN, PM_{10}, and the OTB. Then, exponential regressions were performed considering the quantified chemical elements and the Trad-MCN frequencies. Generalized linear models (GLM) including explanatory variables such as traffic-distance, vertical obstacles and chemical elements, were constructed. The GLM was fitted using a log-link function, and Akaike's Information Criterion (AIC) was applied to indicate the better-fitting model.

Statistical analyses were performed using IBM SPSS software (version 22 IBM Corp., Chicago, IL, USA).

3. Results

Descriptive analysis results for the micronuclei frequency (Trad-MCN) are summarized in Table 1. The Trad-MCN frequencies at the control site were significantly lower than those at gardens "A", "C", "E", "I" and "J" ($p < 0.05$).

A total of 428 tree barks samples from 107 different trees were collected, and the elemental contents were quantified. The descriptive results of the elemental concentration, weather and traffic variables are summarized in Table 2. Elements such as Al, Fe, Mn, Rb, S, Sr and Zn presented higher concentrations than the elements from the control site.

The daily means of air temperature oscillated from 17.2 °C to 35.2 °C in the period, whereas the relative humidity ranged from 16% to 78%. The rainfall varied from 0.0 mm to 20.4 mm, and the wind velocity varied from 1.0 $m\ s^{-1}$ to 4.0 $m\ s^{-1}$. No significant differences in the meteorological variables were observed among the gardens ($p > 0.05$). Fig. 2 shows the mean values of the meteorological variables and Trad-MCN

Table 1
Micronuclei frequency in *Tradescantia pallida* var. *purpurea* inflorescences (Trad-MCN) exposed in Sao Paulo city ($N = 142$ slides) and control site ($N = 29$ slides) (SD; standard deviation, Min; minimum, Max; maximum).

Sites	Mean	SD	Min	Max
A	6.2	1.12	3.00	14.46
B	3.8	0.67	2.00	9.67
C	10.6	5.46	2.00	55.67
D	3.8	1.21	1.00	12.33
E	5.6	1.16	2.00	15.69
F	4.8	0.56	1.33	7.69
G	4.8	3.02	0.69	35.00
H	4.4	0.47	2.67	7.67
I	5.6	0.61	2.33	9.67
J	8.4	1.56	3.00	22.70
Control	2.4	0.29	1.00	4.00

during the months when the inflorescences were collected in the in situ biomonitoring.

Pearson's matrix results (Table 3) showed a significant positive correlation between the Trad-MCN and the OTB. Similarly, the micronuclei frequencies were positively correlated with the concentrations of the elements: Mn, Fe, Zn, Sr and Al in tree barks. We evaluated the association between PM_{10} and the biomonitoring variables and noted a strong correlation between the elements obtained from tree barks, such as Mn, Fe, Cu, Zn, Rb, S and Al. A correlation between PM_{10} and Trad-MCN (Table 2) was observed. There was a trend ($p = 0.058$) between OTB and PM_{10}.

The exponential regression results demonstrated that traffic-related elements can statistically significantly predict the Trad-MCN frequencies ($p < 0.05$). The models exhibited a reasonable fit for the majority of these elements (Fig. 3). The residuals of the regression models did not show a systematic pattern, and therefore, no heteroscedasticity was present in the errors. The Durbin-Watson values ranged from 0 to 3.

Table 4 presents the results of the GLM, which considered nonlinear relationships, and included traffic-related variables. The best explanatory model showed a negative and significant association between the distance from high traffic avenues or roads ($p < 0.05$), and the presence of vertical obstacles ($p < 0.001$) with the Trad-MCN frequencies. The interaction between the elements Mn and Zn showed a positive association with the micronuclei rates. The analyses of the residuals against the fitted values suggest that the assumed variance function was appropriate.

4. Discussion

In this study, we evaluated the use of Trad-MCN frequencies as short-term biomonitor and the elemental content retained on tree bark samples as long-term biomonitor of air pollution in urban gardens. The frequency of Trad-MCN had a significant correlation with the traffic variables and the chemical elements related to tailpipe emissions and road dust adhered in tree barks. These methodologies may be useful biomonitors in urban gardens. The Trad-MCN bioassay estimates the genetic instability caused by vehicular pollutants, whereas tree barks indicate long-term accumulation of metals. This study is the first study to address the use of biomonitors in urban gardens.

The Trad-MCN quantifies the rate of chromosomic damage, which can be caused by stress factors, such as pollutants and climatic variables (Spósito et al., 2015). During the period of exposure, the temperature, relative humidity, wind velocity and rainfall did not statistically differ among the gardens. This condition minimized a potential bias caused by different climatic conditions. The mean values obtained in the control site were consistent with the results of previous studies that were conducted in areas of low air pollution concentrations in the state of Sao Paulo (Guimarães et al., 2000).

Garden "C" presented the highest frequencies of micronuclei and the highest concentrations of Al, Fe, Mn, Sr and Zn on the tree barks. This area is characterized by proximity to an interstate highway, a lack of vertical obstacles (such as trees or buildings) and a high circulation of

Table 2
Elemental concentration (ppm), weather and traffic variables. Standard deviation in parenthesis.

	A	B	C	D	E	F	G	H	I	J	Control
Al	1234.4 (652.74)	2075.11 (982.94)	3074.02 (955.12)	949.43 (522.13)	1125.25	887.97 (223.52)	318.78 (110.23)	1936.45 (362.51)	444.78 (89.55)	1879.63 (400.03)	275.13 (85.88)
Ba	980.49 (124.00)	1080.24 (586.71)	1005.10 (402.22)	911.76 (456.32)	628.2 (108.66)	667.96 (124.99)	183.09 (95.41)	786.71 (101.12)	89.78 (9.26)	662.84 (96.52)	164.24 (20.36)
Ca	26,330.00 (5854.23)	24,228.00 (12,342.11)	22,888.00 (10,220.18)	27,490.00 (11,565.89)	29,053.00 (15,502.22)	28,341.00 (13,889.44)	32,982.00 (6208.24)	26,700.00 (7523.20)	27,220.00 (6541.99)	29,314.00 (6412.50)	19,826.70 (1241.52)
Cl	84.44 (12.25)	127.22 (88.44)	165.10 (15.14)	416.63 (102.71)	101.79 (44.12)	138.02 (88.23)	88.87 (19.24)	188.04 (24.66)	234.04 (44.09)	374.69 (102.33)	240.44 (20.22)
Cu	7.86 (1.17)	7.21 (1.85)	8.36 (3.44)	7.44 (1.08)	7.64 (1.05)	7.3 (0.89)	4.46 (1.20)	10.14 (2.01)	7.00 (0.64)	7.61 (1.30)	4.00 (0.66)
Fe	2359.74 (575.20)	1949.45 (386.23)	2611.03 (785.00)	1818.49 (454.45)	1320.04 (412.23)	1628.25 (551.08)	1338.15 (452.22)	1947.17 (109.55)	2151.94 (452.82)	1787.12 (223.14)	405.48 (74.11)
K	1963.00 (256.84)	2688.00 (1082.16)	2195.00 (452.22)	2013.00 (751.61)	2007.00 (965.22)	1507.00 (894.55)	825.00 (192.37)	1942.00 (125.39)	420.00 (82.55)	1547.00 (352.96)	1915.33 (321.08)
Mg	970.00 (577.05)	1696.07 (641.20)	1406.67 (333.43)	1367.00 (548.22)	799.00 (164.24)	998.00 (444.63)	2235.59 (772.34)	1325.00 (298.11)	3060.00 (523.93)	1741.82 (452.69)	258.37 (55.80)
Mn	66.98 (12.13)	82.63 (10.10)	97.33 (12.09)	63.84 (14.52)	57.77 (19.08)	53.01 (37.55)	48.03 (12.14)	66.63 (8.99)	52.20 (8.09)	76.5 (7.23)	42.09 (11.10)
Na	15.88 (8.12)	17.83 (2.56)	16.05 (4.55)	13.43 (7.15)	18.55 (1.33)	16.79 (2.54)	19.44 (6.12)	18.94 (4.66)	14.05 (4.51)	19.08 (3.55)	21.36 (14.36)
P	1307.00 (323.88)	1060.00 (316.98)	1295.00 (325.44)	1200.00 (481.32)	1135.00 (222.30)	1016.00 (697.32)	763.00 (136.02)	1184.00 (663.55)	990.00 (45.36)	1106.00 (32.96)	859.33 (105.71)
Rb	18.73 (5.22)	16.45 (4.12)	15.58 (6.22)	15.16 (6.14)	11.65 (3.21)	13.98 (9.88)	10.98 (3.22)	15.27 (3.34)	11.37 (4.44)	14.85 (3.98)	9.37 (2.04)
Sr	100.22 (36.42)	127.6 (97.97)	162.36 (88.87)	91.62 (11.32)	110.03 (12.11)	146.89 (14.52)	97.79 (8.36)	130.34 (18.21)	84.48 (11.30)	152.85 (14.15)	51.66 (10.63)
S	5057.00 (930.44)	3893.00 (878.55)	4619.00 (1002.05)	5677.00 (2034.88)	4005.00 (1899.00)	3944.00 (1112.21)	2332.00 (563.36)	4387.00 (288.25)	4400.00 (602.08)	2627.00 (115.33)	2098.00 (302.44)
Zn	195.67 (32.41)	162.95 (20.22)	285.37 (23.19)	141.74 (14.07)	160.48 (45.65)	128.59 (66.60)	32.26 (2.95)	216.98 (115.63)	115.67 (41.02)	272.84 (11.11)	27.61 (8.51)
Temperature (°C)	26.7 (0.93)	28.3 (1.04)	27.0 (0.67)	28.3 (0.90)	28.4 (0.52)	28.4 (0.79)	27.9 (1.04)	27.1 (0.91)	27.1 (1.10)	25.9 (1.06)	27.9 (1.05)
Humidity (%)	48.4 (4.14)	42.1 (3.96)	46.5 (4.10)	41.91 (3.03)	44.7 (3.23)	41.45 (3.37)	52.96 (9.16)	47.38 (4.30)	48.68 (2.96)	54.64 (6.33)	55.21 (6.89)
Pluviosity (mm)	2.25 (2.15)	2.94 (3.48)	2.19 (1.73)	3.16 (2.11)	3.49 (2.26)	2.67 (1.00)	2.95 (2.84)	3.14 (3.01)	3.08 (2.57)	2.74 (1.60)	3.45 (1.58)
Wind (m s^{-1})	2.60 (0.01)	2.05 (0.03)	1.68 (0.04)	2.03 (0.02)	2.03 (0.02)	2.02 (0.02)	2.02 (0.02)	2.41 (0.06)	2.01 (0.02)	2.90 (0.03)	–
Vehicular average speed (km h^{-1})	33.65 (15.20)	30.00 (10.85)	25.23 (7.22)	40.56 (2.10)	27.13 (14.44)	17.8 (9.75)	60.00 (12.50)	45.00 (10.50)	17.30 (10.12)	25.00 (15.10)	–
Traffic volume	94,969 (11,852)	45,500 (8632)	33,916 (7520)	30,100 (6842)	28,848 (6005)	36,110 (7950)	1200 (252)	24,600 (3488)	21,843 (3994)	22,500 (5442)	–
Distance from traffic (m)	65.0	5.0	6.0	600.0	475.0	110.0	30.0	200.0	100.0	105.0	–

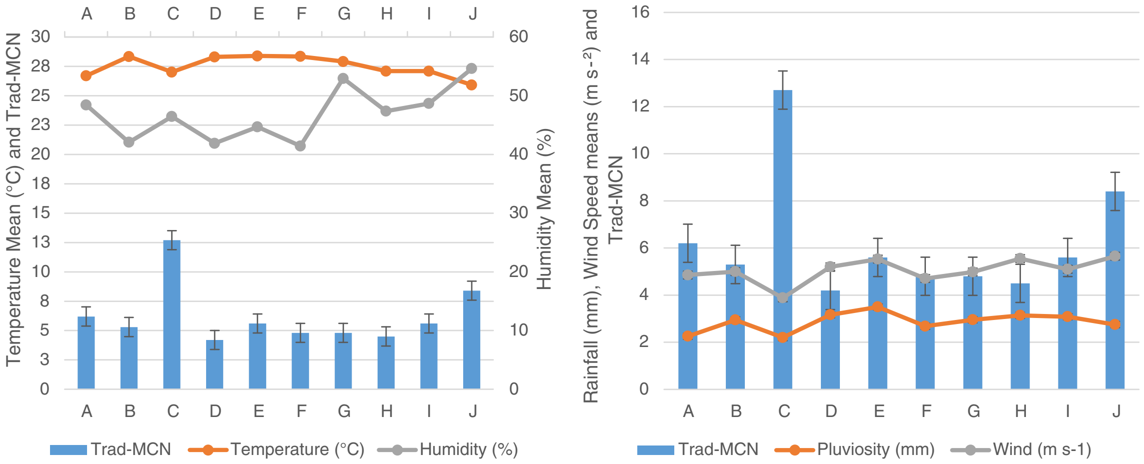

Fig. 2. Meteorological variables: temperature, humidity, rainfall, wind velocity and micronuclei (Trad-MCN) average for the ten urban gardens during the period of exposure.

heavy load trucks. The control site presented the lowest concentrations of all traffic-related elements in tree barks, which demonstrates the enrichment of these elements in the urban gardens. The micronuclei bioassay presented the lowest frequencies at the control location. Our Trad-MCN findings are similar to the findings by Klumpp et al. (2006), who have shown that the genotoxic effects were higher in sites with heavier traffic. The micronuclei bioassay can be a suitable tool for detecting 'hot spots' of mutagenic air pollution in urban areas. It has to be stressed that the mutagenic effects observed in the *T. pallida* cannot be directly associated with carcinogenicity due to different metabolic patterns in higher plants and animals (Ma, 1981).

The multivariate model indicates a negative association between micronuclei frequencies and the traffic distance and vertical obstacles. These results demonstrate the positive impact of the presence of barriers on minimizing the contamination of air pollutants and the decrease of Trad-MCN frequencies with increasing distances from high traffic avenues/roads (von Hoffen and Säumel, 2014). Säumel et al. (2012) demonstrated the reduction of elements, such as Cd and Pb, in areas where vertical barriers were adjacent to the vegetable gardens.

Our results indicate a positive correlation between the OTB and the deposition of these elements on tree barks. One of the important ambient services provided by trees is PM capture. Consequently, the accumulation of metals in tree barks has been employed as an indicator of source apportionment (Guéguen et al., 2012; Sawidis et al., 2011; Faggi et al., 2011; Carneiro et al., 2011; Suzuki, 2006). In our samples, the concentrations of Al, Fe, Mn, S, Rb, Sr and Zn can be attributed mainly to road dust and traffic emissions (Moreira et al., 2016). de Miranda et al. (2002) have shown that the high contents of S, Fe and Zn in São Paulo can be explained by heavy-duty diesel fleet emissions. In addition, Mn could be associated with light-duty vehicle emissions (Andrade et al., 2012). Sr can be attributed to asphalt materials and break-wear in urban areas (Schauer et al., 2006). It is also important to stress that sulphur is added to gasoline and diesel to improve fuel combustion in Brazil (S10 to S50 in Sao Paulo); this element is considered to be a marker of fossil fuel emissions (Andrade et al., 2012). Some of the presented elements are indeed part of the crustal surface such as Rb, that can be also derived from the degradation of catalytic converters (Lough et al., 2005). Other authors (Garg et al., 2000; Sternbeck et al., 2002) have also classified these elements as traffic related (tailpipe and non-tailpipe) when analyzing urban PM.

The positive relationship between Trad-MCN and the concentrations of Mn and Zn in tree bark indicate that these elements are potential aneugenic and clastogenic agents for *Tradescantia pallida*. One

Table 3
Pearson correlation matrix. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.001 level (2-tailed). §Marginally significant ($p = 0.058$). Significant values are presented in bold.

	PM_{10}	Trad-MCN	OTB	Mn	Fe	Cu	Zn	Rb	Sr	Ba	Na	Mg	Al	P	Sr	Cl	K	Ca
PM_{10}	1																	
Trad-MCN	**0.59***	1																
OTB	0.60§	**0.67***	1															
Mn	**0.69***	**0.81***	**0.66***	1														
Fe	**0.80***	**0.63***	**0.80***	**0.73***	1													
Cu	**0.82***	0.30	0.25	**0.60***	**0.71***	1												
Zn	**0.86***	**0.71***	**0.61***	**0.88****	**0.72***	**0.82****	1											
Rb	**0.69***	0.40	**0.65***	**0.71***	**0.76***	**0.67***	**0.70***	1										
Sr	0.35	**0.62***	0.20	**0.73***	**0.58***	**0.63***	**0.79****	0.53	1									
Ba	0.55	0.41	0.45	**0.79****	**0.60***	**0.69***	**0.70***	**0.89****	**0.59***	1								
Na	−0.29	−0.25	−0.49	−0.24	**−0.69***	−0.38	−0.24	−0.42	−0.08	−0.31	1							
Mg	−0.10	0.15	0.13	0.07	0.44	0.03	0.01	−0.06	0.08	−0.29	−0.41	1						
Al	**0.72***	**0.75***	**0.48***	**0.96****	**0.64***	**0.68***	**0.88****	**0.65***	**0.79***	**0.76***	−0.08	−0.06	1					
P	0.72	0.50	0.57	**0.70***	**0.72***	**0.82***	**0.83***	**0.79***	0.46	**0.81****	−0.50	−0.22	**0.69***	1				
S	**0.64***	0.17	0.35	0.38	**0.70***	**0.67***	0.42	**0.62***	0.16	**0.61***	**−0.85****	0.05	0.31	**0.78***	1			
Cl	0.29	0.13	0.43	0.06	−0.06	0.05	0.17	−0.03	−0.07	−0.03	−0.24	0.10	−0.01	0.05	0.08	1		
K	0.46	0.18	0.23	0.57	0.08	0.33	0.40	0.53	0.26	**0.80***	0.13	**−0.65***	**0.60***	0.54	0.26	−0.05	1	
Ca	−0.95	−0.22	−0.62	−0.23	0.09	0.04	−0.06	−0.05	0.19	−0.18	−0.10	0.46	−0.26	−0.20	−0.05	−0.08	−0.53	1

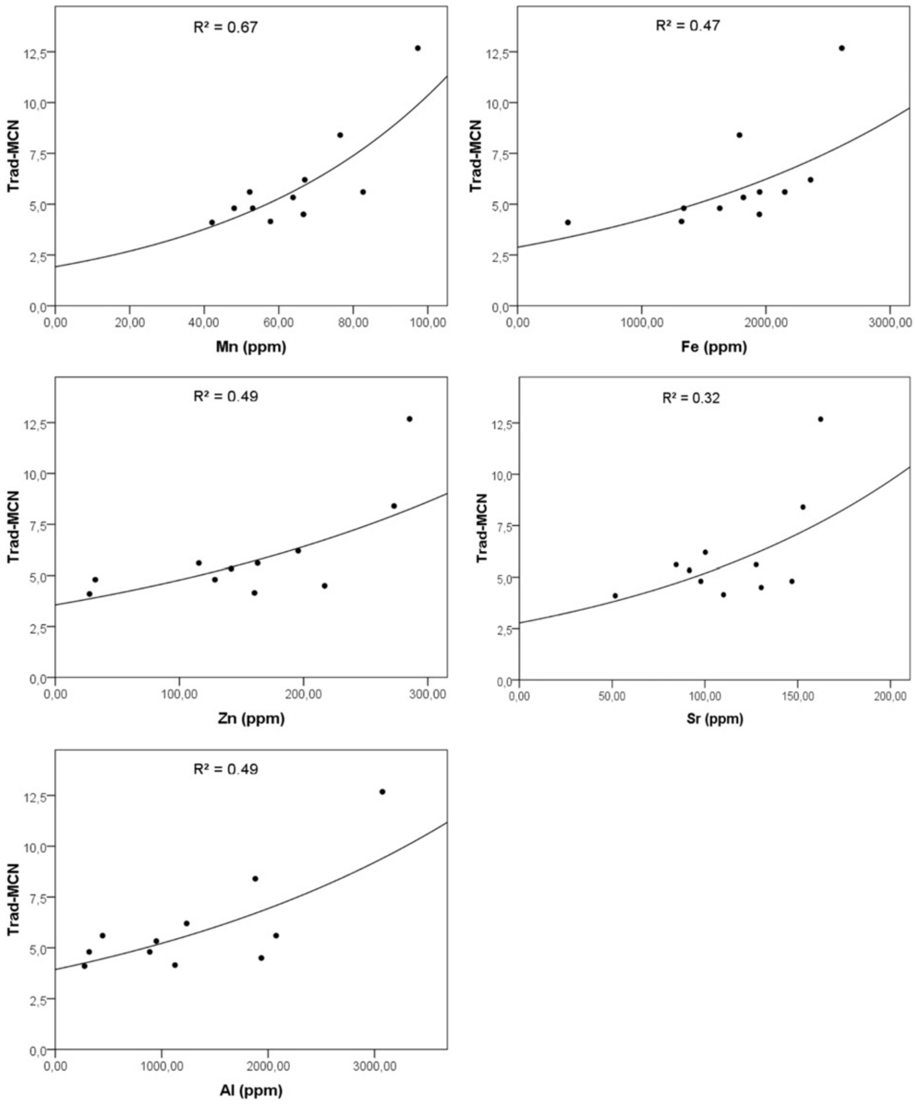

Fig. 3. Exponential regression curves between chemical elements in tree barks and Trad-MCN frequencies ($p < 0.005$) and respective R^2.

hypothesis based on our results is that particles derived from air pollution were deposited and absorbed by Tradescantia leaves or roots and deposited on tree barks. These two elements are micronutrients to plants but in high concentrations are considered phytotoxic (Beyer et al., 2013). However, no macroscopic symptoms of Mn and Zn toxicity were observed in the plants during the biomonitoring period.

We cannot exclude the role of soil uptake contribution to elemental composition in tree barks, but the methodology used probably minimized this effect. Throughout bark, elemental concentration gradients occur and elemental interchange exist within wood tissues. However, in cork the transport is limited if not totally inhibited, due to complete adcrusting suberization of the cell walls (Wolterbeek and Bode, 1995). For this reason, in this study, outer bark (cork) has been sampled as the outermost 3-mm layer of thick-barked tree (De Bruin and Wolterbeek, 1984; Sloof and Wolterbeek, 1993).

Table 4
Coefficients estimated using the GLM model (standard error in parenthesis).

Criteria	Traffic distance	Vertical obstacles	Mn ∗ Zn
ßGLM	−0.006[a] (0.002)	−0.532[a] (0.260)	0.033[ǂ] (0.052)

Dependent variable: Trad-MCN.
β_{GLM}: beta value from GLM.
[a] $p < 0.05$.
[ǂ] $p < 0.001$.

5. Conclusions

Our results indicate an association between traffic-related elements and the frequency of Trad-MCN, which might be a biomonitor of genotoxicity. In addition to the ambient services that are provided by trees, a barks analysis can be a surrogate for long-term air pollution and elemental contamination.

Biomonitoring can be a useful tool helping to incorporate safer agriculture sites in urban planning.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.09.221.

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