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The influence of atmospheric particles on the elemental content of vegetables in urban gardens of Sao Paulo, Brazil☆

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ABSTRACT

Although urban horticulture provides multiple benefits to society, the extent to which these vegetables are contaminated by the absorption of chemical elements derived from atmospheric deposition is unclear. This study was designed to evaluate the influence of air pollution on leafy vegetables in community gardens of Sao Paulo, Brazil. Vegetable seedlings of *Brassica oleracea* var. *acephala* (collard greens) and *Spinacia oleracea* (spinach) obtained in a non-polluted rural area and growing in vessels containing standard uncontaminated soil were exposed for three consecutive periods of 30, 60 and 90 days in 10 community gardens in Sao Paulo and in one control site. The concentrations of 17 chemical elements (traffic-related elements and those essential to plant biology) were quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). *Tillandsia usneoides* L. specimens were used as air plant biomonitors. The concentrations of As, Cd, Cr and Pb found in vegetables were compared to the recommended values for consumption. Principal Component Analysis (PCA) was used to cluster the elemental concentrations, and Generalized Linear Models (GLMs) were employed to evaluate the association of the factor scores from each PCA component with variables such as local weather, traffic burden and vertical barriers adjacent to the gardens. We found significant differences in the elemental concentrations of the vegetables in the different community gardens. These differences were related to the overall traffic burden, vertical obstacles and local weather. The Pb and Cd concentrations in both vegetables exceeded the limit values for consumption after 60 days of exposure. A strong correlation was observed between the concentration of traffic-related elements in vegetables and in *Tillandsia usneoides* L. An exposure response was observed between traffic burden and traffic-derived particles absorbed in the vegetables. Traffic-derived air pollution directly influences the absorption of chemical elements in leafy vegetables, and the levels of these elements may exceed the recommended values for consumption.

1. Introduction

The popularization of community gardening for growing food in urban centres has fostered multiple health benefits, such as better social integration and eating habits and an increase in physical activity (George et al., 2015; Guitart et al., 2014; Harris et al., 2014; Nitta et al., 2015; Whatley et al., 2015). Furthermore, urban gardens can potentially improve the resilience of urban food systems, considering the United Nations projection that by 2050, more than 6 billion people or 65 percent of the world's population will live in cities, generating an unprecedented requirement for sustainable food production (United Nations, 2014). The Food and Agriculture Organization estimates that urban agriculture is currently practiced by 800 million people worldwide (FAO, 2016).

Sao Paulo has experienced a tremendous growth in the number of community gardens in the last 5 years. In this city, air pollution

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sources are primarily derived from the vehicular fleet (CETESB, 2015), frequently exceeding the levels established by WHO guidelines (WHO, 2006). Therefore, the practice of horticulture in urban areas must address the issue of potential contamination hazards of the produced food by the absorption of chemical elements derived from particulate matter present in the urban environment (De Temmerman et al., 2015; Säumel et al., 2012).

Chemical elements present in vegetable crops grown in urban environments are usually derived from previous soil contamination or airborne-derived pollution. Metal absorption may occur by root or foliar uptake and is influenced by several physico-chemical and physiological conditions. Earlier studies on this topic analysed the metal content of vegetables grown in the local soil (Peris et al., 2007; Säumel et al., 2012). Moreover, in these studies, the period of air exposure was not controlled, which did not enable a full comprehension of the isolated role of air pollution in the metal concentrations in vegetables.

Therefore, the contamination of crops as a result of traffic-related air pollution poses the following questions: To what extent are vegetables contaminated by the absorption of chemical elements from atmospheric deposition? What is the influence of the local urban environment on elemental absorption?

Brassica oleracea var. *acephala* (collard greens) and *Spinacia oleracea* (spinach) are frequently cultivated in community gardens and are extensively consumed in Brazil (Ministry of Health of Brazil, 2014; Tomita and Cardoso, 2002). These vegetables accumulate higher concentrations of metals compared with root vegetables (*Umbelliferae* and *Liliaceae*) and legumes (*Fabaceae*) (Alexander et al., 2006; Kachenko and Singh, 2006; Leake et al., 2009; Szolnoki and Farsang, 2013).

Therefore, in this study, we quantified the concentrations of 17 elements (traffic-related and those essential to plant biology) in the edible tissues of *Brassica oleracea* var. *acephala* (collard greens) and *Spinacia oleracea* (spinach) in 10 urban community gardens in Sao Paulo. We used multivariate analysis to correlate the elemental concentrations and the characteristics of the local urban environment, such as weather variables, traffic burden and vertical obstacles adjacent to the gardens.

In addition, to verify a correlation between the chemical elements found in collard greens and spinach and those from air pollution particles, we simultaneously exposed specimens of the air plant *Tillandsia usneoides* L. (TU) (*Bromeliaceae*), widely used as an air pollution biomonitor (Alves et al., 2008; Figueiredo et al., 2007; Martínez-Carrillo et al., 2010).

2. Methods

2.1. Study sites description

Ten community gardens within the inner city neighbourhoods of Sao Paulo, Brazil (Fig. 1a) and one control site – an organic farm in Piracaia, Brazil, a city with low atmospheric pollution in the rural area of the Sao Paulo state province - were selected for this study. These sites were chosen due to their different local settings (presence or absence of vertical obstacles, such as buildings or trees/hedges surrounding the gardens), geographical distribution and availability of the surrounding traffic data.

A geographical information system (GIS) census tract was used to establish a 500 m buffer from each garden to locate roads, major avenues, trees/hedges and buildings in the surrounding areas of the community gardens. These data were linked to a geocoded database created in ArcGIS software (version 10.3 ESRI, Redlands, CA, USA). An overall traffic burden (OTB) was calculated as proposed by von Hoffen and Säumel (2014) considering traffic-related variables within the buffer area, such as the daily average speed (km/h) during morning/afternoon periods, the number of vehicular fleets per day (buses, cars, trucks, motorcycle parcels) classified as 1 (low) $\leq$ 5000; 2 = 5001–10,000; 3 = 10,001–15.000; 4 = 15,001–20000; 5 = 20,001–30000; 6 = 30,001–40000; and 7 (high) $\geq$ 40,001, the presence or absence of vertical obstacles, such as buildings and trees/hedges, as well as their average height/width (m), and the Euclidean distance (m) relative to the gardens and the closest roads/avenues (Fig. 1b). Traffic data were obtained from the Traffic Engineering Company of Sao Paulo (CET, 2013).

The daily mean values of temperature (°C), relative humidity (%), rainfall (mm) and wind velocity (m s^{-1}) in the region surrounding each garden were obtained from the Emergency Management Centre of Sao Paulo Municipality during the study period.

2.2. Exposure experiment and sampling design

Organic seedlings of *Brassica oleracea* var. *acephala* (collard greens) and *Spinacia oleracea* (spinach) were cultivated for 15 days in a non-polluted rural area without the previous use of fertilizers or chemical pesticides. After this period, six parallel replicates of each species were transplanted simultaneously to washed and decontaminated (20% – HNO_3) high density polyethylene vessels (width: 50 cm, length: 90 cm and volume: 0.1 m^3) using an uncontaminated standard soil substrate (density of 500 kg cm^{-3}; 9% organic matter, pH of 5.8 ± 0.5 and electric conductivity of 2.5 ± 0.3 mS cm^{-1}). The seedlings and soil were characterized by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to detect the background concentration (BG) of the chemical elements prior to exposure in the gardens. The vessels were located in a central area of each community garden, 1 m above soil level (Fig. 1c). To avoid soil suspension by water droplets, we covered the soil in the vessels with coir (a natural fibre extracted from the husk of coconuts, which is used in horticulture – Fig. 1d). This material was also analysed prior to exposure and did not show signs of metal contamination.

The vegetables were exposed from September to November 2014. After every 30 consecutive days of exposure (for three periods of 30, 60 and 90 days), the “oldest” leaves were harvested from the bottom of the vegetables, washed for 3 min with distilled and ionic load-free water, gently brushed with a soft nylon dental brush, frozen (−20 °C) and submitted to elemental characterization using ICP-MS. The soil was also sampled every 30 days at eight defined points in each vessel at a depth extraction range of 0–30 cm, and a total amount of 500 g was collected, as recommended by Boulding (1994). The pH measurements of the sampled soils were obtained using a glass electrode (2:1 soil to water ratio by weight) according to the methodology proposed by Van Raij et al. (1987) to verify the correlation of the soil pH and the elemental concentrations in the vegetables over time. No manure, fertilizers or pesticides were used throughout the study period. In addition, samples of these two species offered in four local supermarkets were analysed as a comparative study. The results were compared to the limit values of As, Pb, Cr and Cd for washed green pods established by the Joint FAO/WHO (Codex Alimentarius), ANVISA (Brazilian Health Surveillance Agency), EU (Commission regulation (EC)) and AU/NZ (Australia and New Zealand Food Standards Code).

All vegetable sample analyses in this study are reported as fresh weight (f.w.).

2.3. Bromeliaceae biomonitoring

The specimens were collected at an unpolluted control site (Atlantic Rainforest), and the elemental content was characterized prior to exposure to determine the background values. TU plants were transplanted adjacent to the raised-bed vegetables at a height

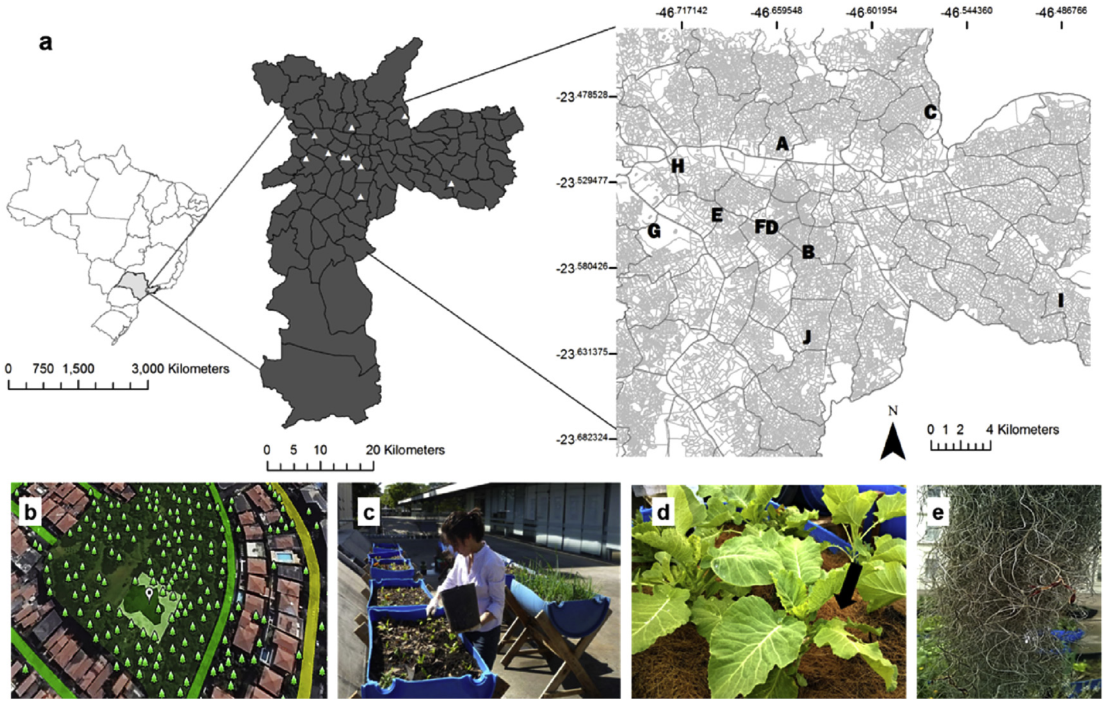

Fig. 1. (a) Overview of the Sao Paulo community garden locations represented by alphabetical codes; (b) traffic burden per day (green line <5000 and yellow line 5001–10,000 vehicles per day) and vertical obstacles (trees/hedges represented as green dots and buildings represented in white) from community garden E; (c) experimental setting in garden F; (d) soil of the vessel covered with coir; and (e) *Tillandsia usneoides* L. used as a biomonitor.

of 1.60 m above the ground in each garden (Fig. 1e). Leaf fragments of TU (50 g) were collect after 60 and 90 days of exposure and were frozen and subjected to elemental characterization by ICP-MS. The data from the 30-day exposure were not used because this period was regarded as the time required to acclimate to the 11 exposure sites. The employment of TU is appropriate for in situ monitoring studies in urban environments, such as the city of Sao Paulo (Figueiredo et al., 2007).

2.4. Analytical methods

The samples were ground, homogenized and placed in propylene metal-free Falcon® tubes (Becton Dickinson, Franklin Lakes, NJ, USA) prior to evaluating the chemical element composition.

Briefly, samples (75 mg) were dissolved in 1 mL of a 50% (v/v) tetramethylammonium hydroxide (TMAH) solution, incubated at room temperature for 12 h and brought to a 10 mL volume with a solution containing 0.5% (v/v) HNO_3 and 0.01% (v/v) Triton X-100.

High purity deionized water (resistivity of 18.2 MΩ cm) obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout the measurements.

The plant and soil samples were analysed in triplicate by ICP-MS (ICP-MS - ELAN DRC II, Perkin Elmer SCIEX, Norwalk, CT, USA) operating with high-purity argon (99.999%) to determine the concentrations of 17 elements (sodium (Na), magnesium (Mg), aluminium (Al), phosphorus (P), potassium (K), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), rubidium (Rb), cadmium (Cd), barium (Ba), and lead (Pb)), which were selected because of their relevance to plant biology (Kabata-Pendias, 2011) and because of their presence in traffic-related particulates (Moreira et al., 2016). Selected duplicates were also included to check for reproducibility. The ICP-MS method used in this study was based on the assay described by Batista et al. (2009).

All reagents used were of high-purity analytical grade, except for HNO_3, which was previously double distilled in a quartz sub-boiling still (Kürner Analysentechnik - Rosenheim, Germany). Multi-element (10 mg L^{-1}) and rhodium (1000 mg L^{-1}) solutions were obtained from Perkin Elmer (Shelton, CT, USA).

The analytical quality control of the data was guaranteed by analysing NIST Standard Reference materials (NIST SRM 1515 Apple Leaves, NIST 1547 Peach Leaves, NIST Tomato Leaves 1573a and NIST 2710 Montana soil l) from the National Institute of Standards and Technology (NIST, USA). The reference materials were analysed before and after sample determinations.

The detection limits of the elements (LoD) and the limits of quantification (LoQ) are shown in the supplementary material (Table S9).

2.5. Statistical analysis

The Shapiro-Wilk test and Normal Q-Q Plots were used to determine the data distribution, and Levene's test was used to test for homogeneity.

One-way analysis of variance (ANOVA) for repeated measurements followed by a Bonferroni adjustment for multiple comparisons was used to test for differences in elemental content in the vegetable, soil and TU samples among the different community garden locations over time. Log transformations were applied when necessary to comply with the assumptions of variance of homogeneity and residual normality.

Regression models were generated to test the association between the elemental concentrations obtained in the vegetables and TU after 60 and 90 days of exposure.

A Principal Component Analysis (PCA) using varimax orthogonal rotation with Kaiser normalization was applied to 'cluster' the 17 elemental concentrations from each vegetable to estimate their potential common sources (Wei et al., 2011) and to reduce the number of highlighted correlated variables (multicollinearity) to be applied in the GLMs. PCA was conducted using the mean concentration values for the total exposure period. The Kaiser-Meyer-Olkin (KMO) measurement of sampling adequacy and Bartlett's test of sphericity were applied prior to the PCA analysis to test the data suitability.

GLMs (Mccullagh, 1984) were employed to evaluate the obtained PCA factor scores from each component on the local weather, traffic-related, and vertical obstacle variables. The GLM was fitted using the identity-link function and normal scale response. 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

The descriptive results of the chemical elements throughout the exposure periods are summarized in Table 1. The background concentrations in the vegetable, soil and TU samples are presented in Tables S1, S2 and S3 as supplementary material.

The concentrations of Na, Mg, Al, P, K, Cr, Mn, Fe, Ni, Cu, Zn, Rb and P in spinach for the 30-day group were statistically lower ($p < 0.05$) than those for the 60- and 90-day groups. The concentrations of Na, Mg, Al, Cd, and Pb in collard greens in the 30-day group were statistically lower ($p < 0.05$) than those in the 60- and 90-day groups. Arsenic concentrations in collard greens were lower in the 30-day group than in the 60-day group ($p < 0.05$). Similarly, Mn, Ni and Ba concentrations in collard greens in the 30-day group were statistically lower ($p < 0.05$) than those in the 60- and 90-day groups.

In all of the sampled gardens, after 30 days of exposure, Al ($p = 0.02$) and Pb ($p = 0.05$) concentrations in collard greens were lower than those obtained in local supermarkets. In contrast, Cu ($p = 0.02$), Mn ($p = 0.01$) and Rb ($p = 0.02$) concentrations were higher in the community gardens. There were no significant differences for the elements Na, Mg, P, K, Ca, Cr, Fe Ni, Zn, As, Cd, and Ba. The elemental concentrations in spinach from the gardens were lower than those obtained from spinach in local supermarkets for the elements Al ($p = 0.04$), Pb ($p = 0.006$) and Zn ($p = 0.03$), while Cu ($p = 0.02$), K ($p = 0.04$), Mg ($p = 0.01$), Mn ($p = 0.03$), P ($p = 0.04$) and Rb ($p = 0.04$) were lower in the garden samples. There were no significant differences for Na, Ca, Cr, Fe, Ni, As, Cd, and Ba ($p > 0.05$) between gardens and supermarkets.

The elemental content of both vegetables was significantly lower in the control site than in the urban gardens ($p < 0.05$), except for Ca, K, Na and P (non-traffic-related elements); therefore, the elemental content is suitable for use as a basis for comparisons.

Cadmium concentrations in spinach exceeded the regulatory values established by Anvisa, FAO/WHO and EU (0.20 μg g^{-1}) in one garden during the 60-day exposure period. Considering the more restrictive levels adopted by AU/NZ (0.10 μg g^{-1}), three gardens during the 60-day exposure and one garden during the 90-day exposure surpassed the maximum permissible values (Fig. 2). The results indicate that none of these limits was exceeded for collard greens.

The Pb concentrations obtained in the spinach exceeded the Anvisa and EU limits in one garden during the 60-day exposure and in three gardens during the 90-day exposure. In contrast, the AU/NZ levels for spinach were surpassed in three gardens in the 60-day

Table 1
Elemental concentrations in spinach (SPI) and collard greens (CG) (μg g^{-1} biomass - wet weight (ww)). (†total number of samples per period; ‡Control site (CS): N = 6 per period; ‡‡Supermarket (SM): N = 4 for SPI and CG. *Minimum and maximum concentrations in the total period. SD = Standard Deviation.

Element	Species	N†	Mean (SD) 30 days	Mean (SD) 60 days	Mean (SD) 90 days	Minimum*	Maximum*	CS Mean (SD) 30 days‡	CS Mean (SD) 60 days‡	CS Mean (SD) 90 days‡	SM mean‡‡
Na	CG	66	128.96 (118.3)	260.64 (118.9)	183.22 (85.1)	55.7	412.03	472.40 (118.31)	427.97 (31.16)	156.23 (71.90)	64.08
	Spi	66	275.66 (159)	909.45 (597)	807.01 (383.4)	100.44	2404.24	443.02 (12,299)	847.70 (102.09)	1239.54 (15.40)	176.36
Mg	CG	66	501.8 (204.21)	669.77 (122.9)	791.1 (556.5)	323	2235.90	1064.99 (171.89)	763.42 (38.31)	597.36 (13.59)	402.74
	Spi	66	1044.68 (876.7)	2591.8 (1158.3)	2443.06 (878.4)	192.19	4738.09	2812.59 (657.14)	1174.28 (85.40)	822.65 (8.74)	384.99
Al	CG	66	4.06 (1.9)	9.02 (4.7)	9.14 (7.2)	1.87	23.04	2.83 (0.6)	4.21 (0.88)	3.28 (0.21)	8.12
	Spi	66	9.24 (9.4)	23.05 (17.6)	29.65 (19.3)	2.22	65.74	2.52 (1.2)	5.55 (1.22)	7.53 (2.54)	16.07
P	CG	66	482.09 (298.4)	596.07 (555)	458.74 (243)	222.91	2210.93	1248.96 (54.09)	505.36 (25.41)	309.40 (40.92)	339.95
	Spi	66	354.92 (355.2)	919.1 (594.9)	803.1 (328.3)	72.17	2410.34	1126.13 (239.72)	491.59 (49.51)	507.54 (13.86)	169.75
K	CG	66	1564.01 (572.3)	2295.9 (1537.1)	1298.87 (389.3)	628.76	5896.08	2709.76 (505.17)	3307.98 (178.26)	1870.47 (99.92)	1709.22
	Spi	66	1523.94 (909.6)	3860.6 (1530)	3008.38(1578.8)	421.91	7654.81	3533.67 (870.77)	2453.16 (281.20)	3593.85 (74.87)	2689.25
Ca	CG	66	2300.52 (660)	2265.66 (963.5)	3077.6 (1513.4)	955.87	5906.46	4170.78 (546.06)	3996.04 (209.83)	3045.65 (138.03)	2812.65
	Spi	66	1782.6 (2049.9)	2415.2 (1009.6)	2288.9 (1578.8)	405.3	7543.81	2415.37 (420.12)	2244.00 (86.15)	2305.09 (27.05)	1172.56
Cr	CG	66	0.16 (0.1)	0.28 (0.1)	0.23 (0.1)	0.04	0.48	0.10 (0.002)	0.17 (0.54)	0.10 (0.002)	0.17
	Spi	66	0.17 (0.2)	0.32 (0.1)	0.4 (0.1)	0.07	0.63	0.03 (0.009)	0.02 (0.09)	0.02 (0.01)	0.15
Mn	CG	66	12.16 (5.7)	14.66 (7.3)	27.04 (18.9)	3.33	63.4	8.8 (0.2)	7.68 (1.89)	7.57 (0.27)	4.71
	Spi	66	43.33 (28.7)	151.86 (67.9)	127.1 (52.99)	11.54	317.15	10.75 (2.55)	50.57 (4.88)	50.30 (0.54)	13.41
Fe	CG	66	41.72 (16.9)	56.01 (26.1)	46.19 (26.6)	10.9	111.08	57.51 (9.44)	83.93 (3.36)	55.98 (4.39)	37.35
	Spi	66	31.79 (30.27)	64.67 (32.7)	76.94(40.2)	1.21	151.16	47.32 (9.90)	42.32 (4.73)	36.02 (0.46)	27.49
Ni	CG	66	0.17 (0.05)	0.29 (0.2)	0.44 (0.71)	0.08	2.54	0.12 (0.05)	0.14 (0.01)	0.10 (0.29)	0.25
	Spi	66	0.1 (0.1)	0.28 (0.2)	0.29 (0.1)	0.01	0.74	0.008 (0.002)	0.098 (0.023)	0.13 (0.03)	0.12
Cu	CG	66	0.52 (0.2)	0.64 (0.3)	0.52 (0.3)	0.33	1.26	0.10 (0.1)	0.25 (0.12)	0.11 (0.59)	0.35
	Spi	66	0.78 (0.9)	2.31 (1.1)	2.35 (1.0)	0.27	5.57	0.12 (0.07)	1.05 (0.01)	1.03 (0.01)	0.45
Zn	CG	66	9.82 (3.3)	10.77 (5.5)	12.04 (6.9)	4.93	25.36	5.89 (0.25)	4.53 (0.26)	6.55 (0.94)	7.85
	Spi	66	11.72 (7.8)	31.63 (18.8)	26.86 (13.5)	4.68	58.23	3.57 (0.58)	16.52 (1.59)	12.83 (0.01)	23.52
As	CG	66	0.01 (0)	0.03 (0.04)	0.02 (0.01)	0	0.14	0.007 (0.002)	0.01 (0.02)	0.01 (0.002)	0.01
	Spi	66	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0	0.05	0.02 (0.006)	0.01 (0.004)	0.01 (0.004)	0.01
Rb	CG	66	3.84 (1.2)	5.58 (3.8)	5.33 (1.9)	2.06	14.04	1.59 (0.58)	1.09 (0.17)	1.55 (0.15)	1.44
	Spi	66	5.12 (3.9)	16.02 (6.2)	12.11 (5.0)	1.82	30.87	1.22 (0.15)	6.89 (1.20)	2.30 (0.8)	1.29
Cd	CG	66	0.01 (0)	0.01 (0)	0.01 (0)	0	0.01	0.006 (0.001)	0.002 (0.001)	0.001 (0.002)	0.02
	Spi	66	0.03(0.02)	0.09 (0.05)	0.07 (0.03)	0.01	0.21	0.009 (0.005)	0.02(0.004)	0.01 (0.002)	0.05
Ba	CG	66	2.37 (0.9)	3.14 (1.2)	4.38 (2.2)	0.76	7.96	1.89 (0.1)	2.10 (0.23)	1.15 (0.99)	3.07
	Spi	66	1.47 (1.9)	1.94 (2.11)	2.08 (1.3)	0.28	7.59	0.80 (0.59)	1.02 (0.08)	1.00 (0.02)	1.58
Pb	CG	66	0.03 (0.003)	0.1 (0.3)	0.09 (0.08)	0	1.03	0.01 (0.01)	0.01 (0.001)	0.02 (0.005)	0.11
	Spi	66	0.06 (0.08)	0.12 (0.1)	0.18 (0.16)	0.01	0.42	0.002 (0.003)	0.052 (0.006)	0.03 (0.002)	0.11

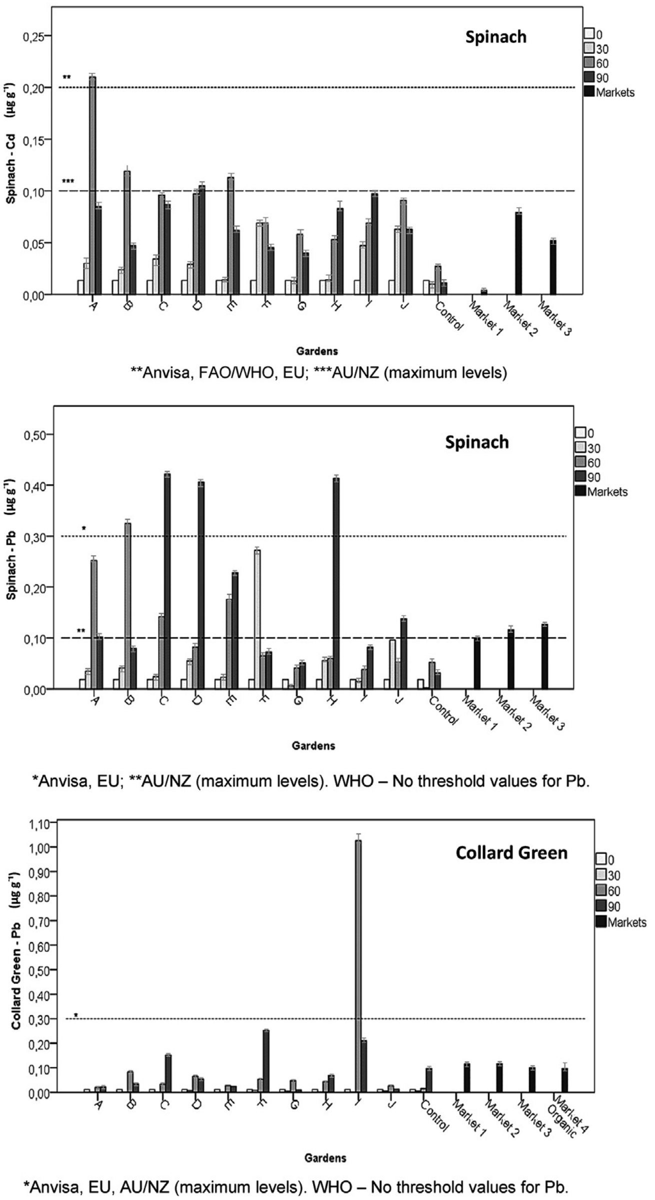

Fig. 2. Elemental content above the maximum levels considering the exposure periods of 0, 30, 60 and 90 days and values obtained for local market food stores (μg g^{-1}).

exposure group and in four gardens in the 90-day group, which included two supermarkets. The AU/NZ levels for collard greens (0.30 μg g^{-1} for *Brassica oleracea* var. *acephala*) were surpassed in only one garden in the 60-day group.

Arsenic concentrations ranged from 0 to 0.14 μg g^{-1} in the collard greens and from 0 to 0.05 μg g^{-1} in the spinach during the total period of exposure. There are no thresholds established by WHO/FAO for this element.

Chromium concentrations did not surpass the limits in any of the periods for both vegetables.

In addition, no limit value was exceeded for these four elements (cadmium, lead, chromium, arsenic) during the 30-day exposure period.

All element concentrations in the gardens' soil were below the ranges of the Trigger Action Value (TAV) for agricultural cultivation (Kabata-Pendias, 2011), which are used to indicate the presence of potentially harmful elements in uncontaminated sites. After the experiments were conducted for 3 months, non-significant soil enrichment was observed, as shown in Table S2 ($p = 0.42$). Further, there were no significant differences in the soil metal content between the urban community gardens and the control site in the rural area at the end of the exposure periods.

There were no significant differences for all elements in the collard green soil, except for Cr between 30 and 90 days ($p = 0.007$) and 60–90 days ($p < 0.05$). Spinach soil presented no differences for all elements in the three periods of exposure ($p > 0.05$). No correlation was found between the soil and vegetable concentrations.

The soil pH ranged from 4.9 to 5.8 (collard greens) and from 4.4 to 5.7 (spinach). The pH measurement results showed a decreasing trend over the exposure period for collard greens ($p < 0.001$) and for spinach ($p < 0.001$), except for the period between 60 and 90 days ($p = 0.60$) (Fig. S5 - supplementary material). There was no significant relationship between the elemental concentrations in the vegetables and the soil pH.

The regression models used to test the correlation between TU and collard green concentrations demonstrated that seven elements had a positive significant ($p < 0.05$) correlation and an adjusted $R^2 > 0.35$ (Fig. 3a), while 15 elements in spinach had a positive significant ($p < 0.05$) correlation with an adjusted $R^2 > 0.39$ (Fig. 3b).

The preliminary analysis for the PCA tests considering the multicorrelation, the significant Bartlett test ($p < 0.05$) and the criterion of Kaiser Meyer-Olkin (KMO = 0.69 for collard greens and KMO = 0.83 for spinach) demonstrated the suitability of the data for this statistical procedure. The application of the Rotated Component Matrix resulted in five components for collard greens (total cumulative variance of 78.9%) and three components for spinach (total cumulative variance of 81.5%) with eigenvalues greater than 1. Factor loadings <0.3 were considered insignificant and are omitted from Table S8 – supplemental material. The first component of collard greens accounted for 42.2% of the variance and showed the highest loadings for Mn, Ca, Fe, Mg, Zn and P, which are typically traffic-related elements as well as plant nutrients. The second component had higher loadings for Pb, Al, Ni and to a lesser extent, Ba and Cr, explaining 13.98% of the total variance. These elements typically indicate anthropogenic contamination. The third component explained 8.9% of the variance and had the highest loadings of the elements Rb and Cu. The fourth and fifth components explained 7.5% and 6.3% of the variance, respectively, and were composed of K and Cd, respectively.

The Rotated Component Matrix for spinach resulted in three components with a total cumulative variance of 86.9%. The first component accounted for 62.0% of the variance and had the highest loadings of Cu, Mn, Cd, Pb, Ni, Zi, Al, Cu and Rb. The majority of these elements are highly correlated with vehicular sources in urban centres. The second component accounted for 10.2% of the variance and had higher loadings of K, Na, P and to a lesser extent, Mg and Ca. This component clustered elements related to plant micro- and macronutrients. The third component (explaining 9.3% of the variance) had loadings of Ba, Ca, As, and to a lesser degree, Fe and Ca.

The GLM analysis showed that components composed of high loadings of elements related to traffic emissions were positively associated with OTB and were negatively associated with climate variables, VOs and distance from the nearest roads/avenues (traffic distance). However, the elements commonly related to plant constituents were positively associated with climate variables such as wind velocity, temperature, rainfall and humidity (Table 2).

These results suggest an exposure-response relationship between OTB and traffic-related elements accumulated in collard greens and spinach (Figs. 4 and 5). However, no significant differences were verified between the concentrations of some

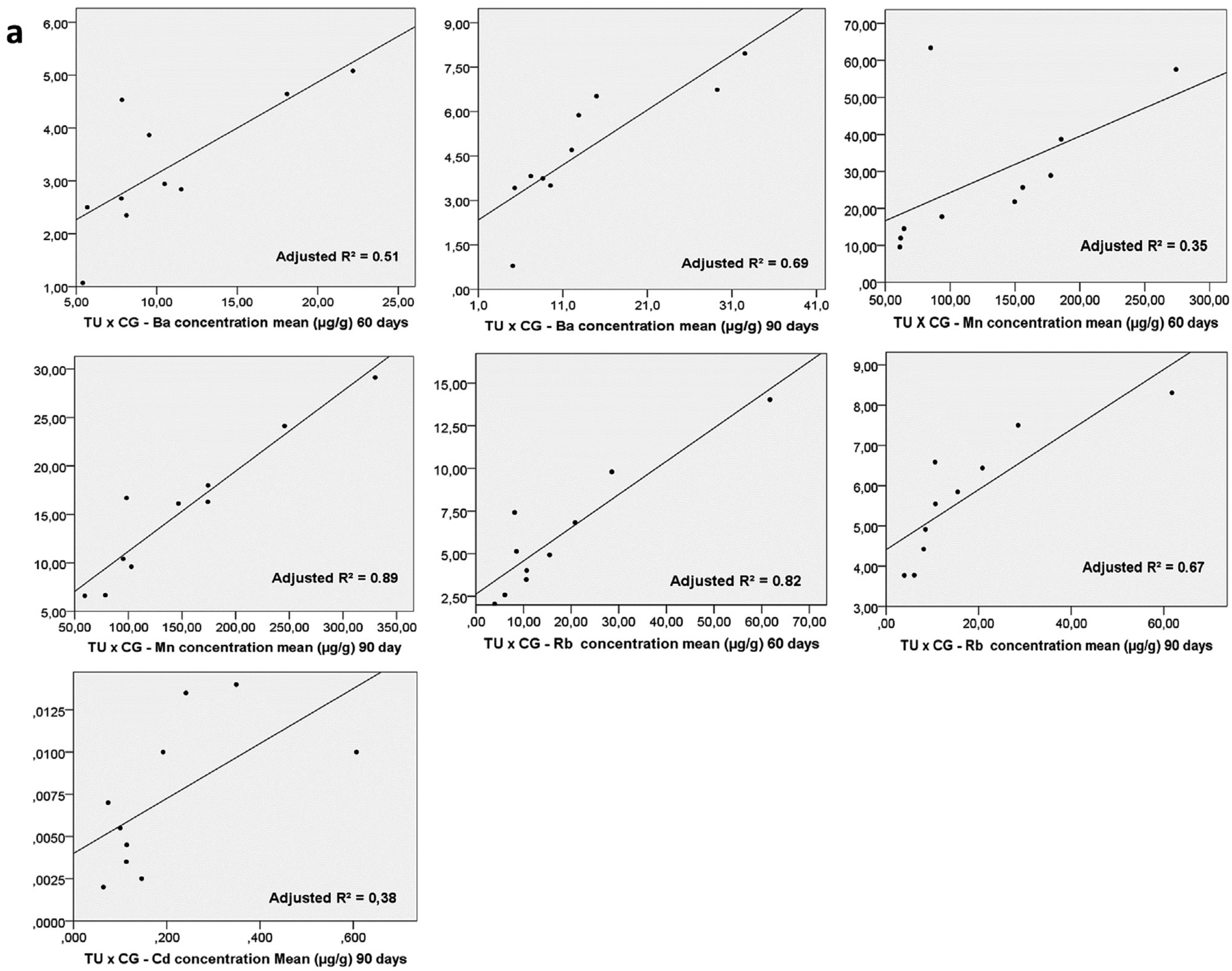

Fig. 3. a and b. Correlation between elemental concentrations in the vegetables and in *Tillandsia usneoides* L. (TU = *Tillandsia usneoides* L., CG = collard greens, SPI = spinach).

constituent elements of vegetable tissues, such as P, K, Na, Mg, and Ca, relative to the category of OTB ($p > 0.05$) (Figs. S11 and S12 - supplementary material).

4. Discussion

In this study, we showed a significant association between traffic-derived elemental concentrations, traffic burden and weather variables in *Brassica oleracea* var. *acephala* (collard greens) and *Spinacia oleracea* (spinach) in ten community gardens of Sao Paulo. The accumulation increased over time for most of the traffic-related elements. After 30 days of exposure, the metal accumulation was below the recommended maximum intake levels for Cd, Cr and Pb. However, after 60 or 90 days of exposure, the content of some of these elements surpassed different legal standards.

To our knowledge, this study is the first to consider the specific role of air pollution in the elemental content of leafy vegetables in urban community gardens. Säumel et al. (2012) found similar results between metal accumulation in vegetables and the influence of traffic variables and vertical obstacles in the urban gardens of Berlin. Their study, however, was not designed to determine the isolated role of air pollution deposition because the vegetables were collected from different soils/periods in Berlin.

The strong correlation between elemental concentrations in leafy vegetables and *Tillandsia usneoides* L. reinforces the hypothesis of the absorption of atmospheric particles derived from traffic emissions as a major source of vegetation contamination. Our data encourage the use of this air plant as an air pollution biomonitor.

Metal accumulation was significantly higher in spinach compared to collard greens. For collard greens, only Pb levels at 60 days exceeded the recommended intake values, whereas for spinach, Cd and Pb levels were higher than the recommended values after 60 and 90 days of exposure. The reasons for these differences are unclear but could reflect different absorption mechanisms in the plants, as well as indicating different chemical forms of the contaminants (Lim and McBride, 2015).

There was a variation in the content of traffic-related metals in the different studied gardens, which was significantly related to the characteristics of the local urban environment, such as vertical obstacles, distance from major avenues, fleet characteristics, number of vehicles and local weather conditions. The three gardens with higher concentration values for almost all elements were gardens A, C and D, which are located in areas classified as high OTB. Garden A is located close to an area with high traffic density and constant traffic jams and is near an area where small airplanes take off and land. Garden C is characterized by the lowest number of VOs. In the area surrounding this garden, there is a high circulation of heavy load trucks and an interstate roadway. Garden D is a small public garden located in a median strip of a main traffic

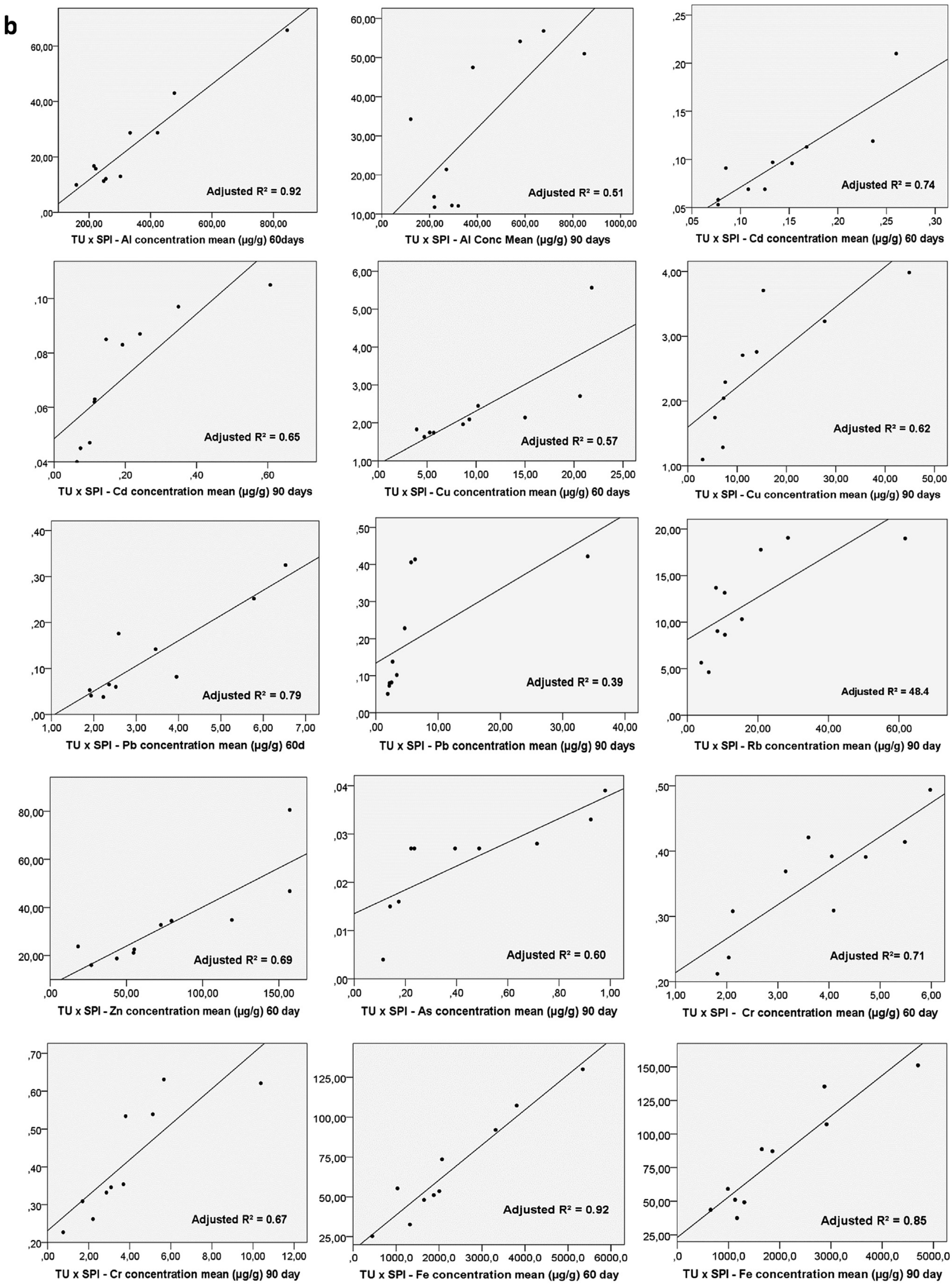

Fig. 3. (*continued*).

Table 2
Coefficients estimated through the generalized linear model (GLM) (OTB = overall traffic burden; VO = vertical obstacle; S.E. = standard error, β = beta coefficient). Significant values are presented in bold.

Vegetable	PCA component	Predictors	β	S.E.	*p*-value
Collard Green	Component 1	**Wind Velocity**	**−0.84**	**0.04**	**0.03**
		OTB	**0.12**	**0.05**	**0.02**
	Component 2	**Temperature**	**−0.63**	**0.17**	**<0.001**
		Humidity	0.06	0.04	0.13
		Rainfall	**−0.32**	**0.14**	**0.02**
		OTB	**0.45**	**0.19**	**0.02**
		VO	**−0.35**	**0.15**	**0.01**
	Component 3	**Traffic Distance**	**−0.30**	**0.04**	**<0.001**
		VO	−0.09	0.06	0.09
		OTB × VO	**0.001**	**0.00**	**0.04**
	Component 4	**Rainfall**	**0.58**	**0.05**	**0.02**
	Component 5	**Temperature**	**−0.91**	**0.16**	**<0.001**
		Humidity	**−0.19**	**0.05**	**0.001**
		Rainfall	−0.74	0.52	0.15
Spinach	Component 1	**Temperature**	**−0.33**	**0.06**	**0.002**
		Rainfall	**−0.20**	**0.13**	**0.01**
		OTB	**0.23**	**0.09**	**0.02**
	Component 2	**Temperature**	**0.79**	**0.14**	**<0.001**
		Humidity	**0.10**	**0.03**	**0.003**
		Rainfall	**0.97**	**0.33**	**0.003**
		OTB	0.05	0.21	0.80
	Component 3	Rainfall	0.02	0.08	0.75
		Traffic Distance	0.002	0.00	0.06
		VO	**−0.24**	**0.12**	**0.05**

avenue in the city.

Garden G presented the lowest concentration values for both vegetables. This garden is located in an area surrounded by many trees as VOs and is a greater distance from high traffic areas compared with all of the other gardens. Our results also showed the importance of vertical obstacles considering the negative relationship between their presence and the elemental concentration of anthropic contaminants. In a previous study, von Hoffen and Säumel (2014) showed the efficiency of hedges or walls to act as barriers against the contamination effects within high traffic areas. In our study, garden F, characterized as being located a short distance from a high traffic avenue and containing vertical obstacles, was not among the most contaminated gardens.

Weather variables exerted a substantial influence on the elemental absorption for both vegetables. Negative coefficients obtained in the GLMs demonstrate that periods of low relative humidity, rainfall, wind velocity and temperature, when there is a large decrease in pollutant dispersion in Sao Paulo, are directly linked to an increase in the uptake of traffic-related elements in both vegetables.

The lack of a correlation between the vegetable and soil content can be attributed to the complex relationship among soil properties, organic matter content, and pH, which affect the phytoavailability of metals to root uptake (Sauvé et al., 2000). McBride et al. (2014) found no correlations between the concentrations of Ba, Pb and Cd in soil and vegetables grown in urban gardens of New York City. The results reported by De Temmerman et al. (2015) revealed no differences in the element concentrations in the soils from different exposure areas, indicating the leaves of bush beans as the primary absorption mechanism. Atmospheric deposition is the dominant contamination pathway of certain metals, such as Pb and As (Alexander et al., 2006; Nabulo et al., 2006), whereas for Cd, root uptake is important (De Temmerman et al., 2015; Smolders, 2001). It is possible that the exposure time was not long enough to cause soil contamination by atmospheric deposition, although Warming et al. (2015) demonstrated that high elemental concentrations in soil do not necessarily reflect an increase in plant absorption.

The oldest leaves were sampled every 30 days, but our results showed an increase in concentration over time for many elements. The findings of De Temmerman et al. (2015) may help explain our results. These authors demonstrated that despite atmospheric deposition accumulation primarily on the leaves, stems play an important role in the accumulation and translocation of leaves-stems.

This study has some limitations because it considers only two species; thus, our results cannot be generalized to other

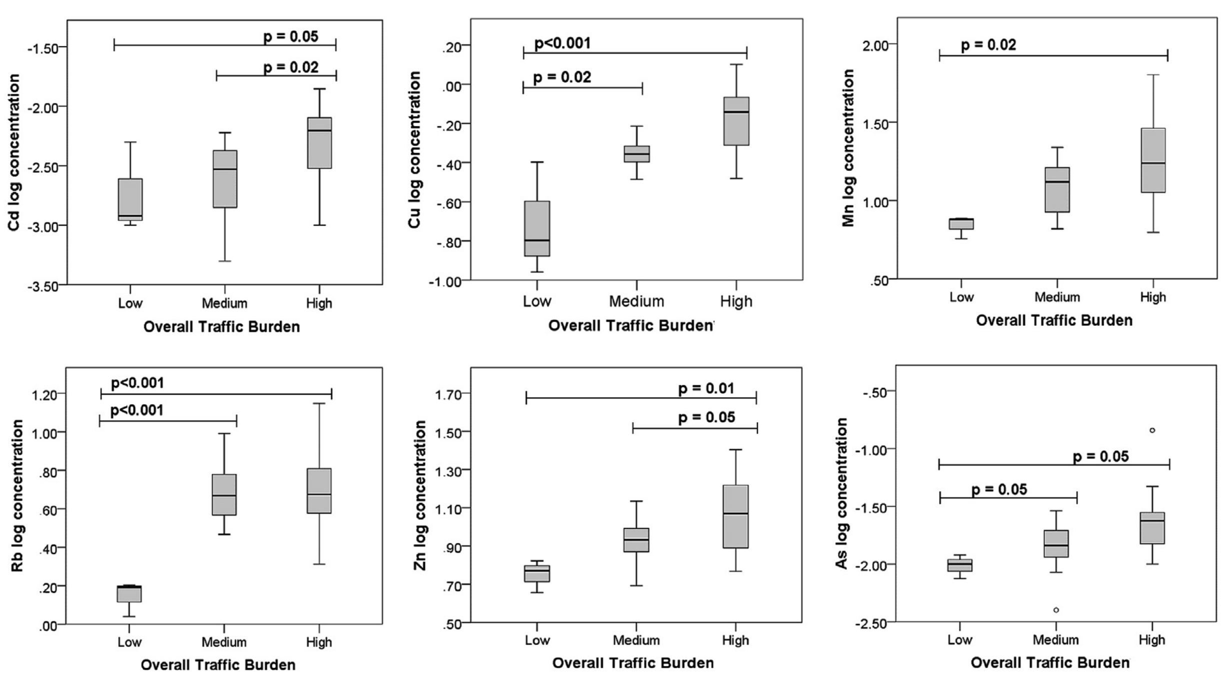

Fig. 4. Elemental content in collard greens (μg g^{-1} biomass wet weight (ww)) and categorized overall traffic burden.

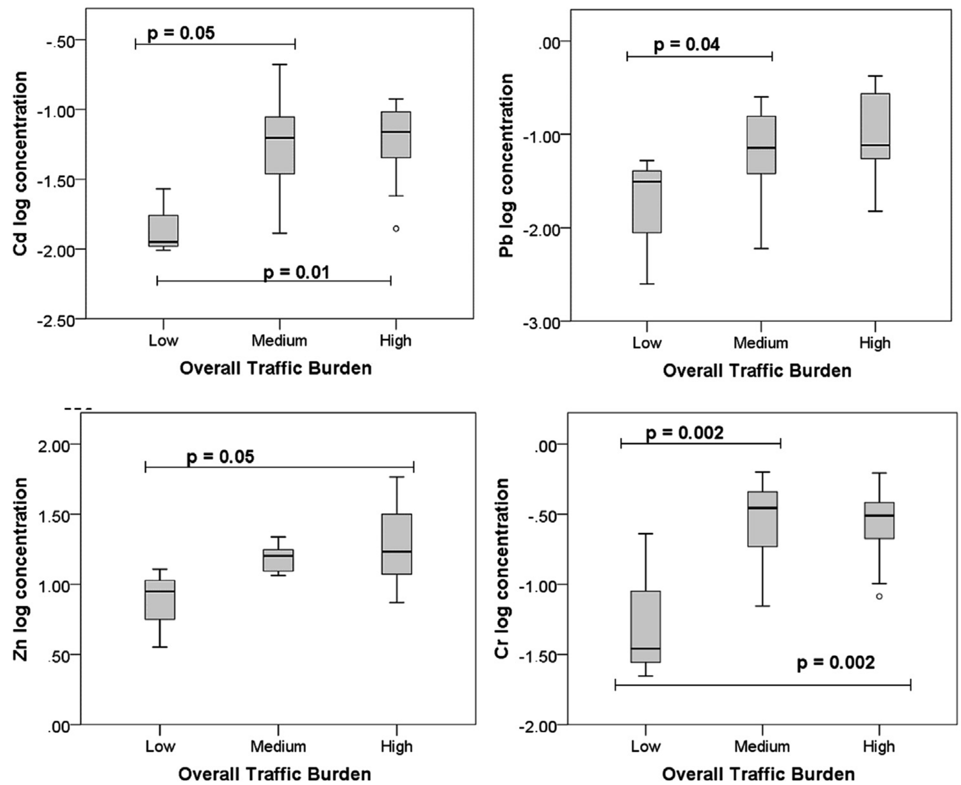

Fig. 5. Elemental content in spinach (μg g^{-1} biomass wet weight (ww)) and categorized overall traffic burden.

vegetables. We decided to study leafy vegetables because atmospheric particles accumulate primarily on the leaves. Our data suggest that foliar uptake plays an important role in metal absorption in these vegetables because we could not detect differences in soil enrichment and soil pH after 90 days of exposure. We also found no correlations between soil and plant vegetable elemental content. However, we cannot exclude a role for soil absorption in our data because the transfer mechanisms of metals and their fate in different plant leaves remain unclear (Schreck et al., 2012).

5. Conclusion

In summary, our study demonstrated that traffic-derived air pollution exerts a direct influence on the elemental content of spinach and collard greens grown in uncontaminated soil. The traffic burden is associated with increased elemental absorption, whereas vertical obstacles appear to negatively affect elemental absorption. Metal accumulation increases over time, exceeding the regulatory standards for some elements (such as Pb and Cd) after 60 days of exposure.

Vegetable gardening has greatly increased in recent years in several cities worldwide, providing indisputable social, environmental and health benefits. We believe that our study contributes to a better understanding of the complex relationship between the urban environment and vegetable growth to provide a basis for safer urban horticulture.

Financial interests

The authors declare that they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.05.036.

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