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Essential and non-essential elements in lettuce produced on a rooftop urban garden in São Paulo metropolitan region (Brazil) and assessment of human health risks

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Abstract

This study evaluated the essential and non-essential elements in lettuce cultivated on a rooftop urban garden in the metropolitan region of São Paulo. In addition, the human health risks associated with the potentially toxic metals based on the estimated daily intake (EDI), the target hazard quotient (THQ), and the possible sources of heavy metal contamination by multivariate statistical were analyzed. The lettuces contain essential macronutrients such as K, Ca, and Mg. The Cd, Cu, and Pb concentrations did not exceed the Brazilian legislation limit. Ba, Ni, Cr, Co, and Pb presented low levels compared to oral reference dose and they may be associated to vehicles emissions. Both EDI and THQ values suggested minimal risk upon consumption of lettuce.

Keywords Rooftop · Horticulture · Potentially toxic metals · Urban agriculture · Food safety

Introduction

The rapid growth of cities in the developing world is placing enormous demands on urban food supply systems. Urban agriculture provides fresh food, generates employment, recycles urban wastes, creates greenbelts, and strengthens cities' resilience to climate change [1]. Urban agriculture is considered an important solution to food security in the increasingly urbanized world. Rooftops are a new addition to possible places to grow vegetables and fruit, house honeybees, and even have small animals like chickens, rabbits, and fish [2, 3]. Roof gardens help increase availability of and facilitate access to fresh fruits and vegetables, helping to provide a balanced diets for all and to reduce malnutrition, especially for those living in poverty [4].

Food contamination can occur either by contact with contaminated soils or by air pollution, wheeled transport emissions, atmospheric deposits from industrial activities and incinerators, and pest treatments [5–8]. Obviously, this contamination is also observed in urban agricultural products and can exceed precautionary levels, and a dietary exposure to trace metals can result in significant human health risks [9]. Food contamination by heavy metals depends on their mobility in the soil and their bioavailability [10]. Heavy metal absorption from soil and translocation to edible plant parts is a potential risk for the food chain and must be evaluated based on soil metal availability and plant efficiency for metal uptake and translocation [11]. Heavy metals such As, Ba, Cd and Pb are non-essential, while Fe, Cu, Cr, Mn, and Zn are referred to as essential micronutrients for humans, animals, and plants to regulate and maintain their health.

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These are required in small quantities, but in excess they can cause toxic effects [12–15].

Lettuce (*Lactuca sativa* L.) is a widely grown and a popularly consumed vegetable worldwide and the main leafy vegetable marketed in Brazil [16–18]. It is a leafy vegetable that is a good low calorie source of fiber and contains nutrients such as vitamin C, folic acid, potassium, calcium, sodium, magnesium, manganese, zinc, and iron [18–20].

Some studies have raised the concern of urban environmental pollution, as for food safety. A recent study in Brazil was conducted in an urban garden on the roof of a large shopping mall in the city of São Paulo, surrounded by heavy vehicular traffic. The mean concentrations of As, Cd, Cr, Pb and Zn in the samples were quantified and it was concluded that lettuces grown in an urban garden presented no risk to human health [19]. In China, researchers evaluated hydroponically grown vegetables in a rooftop screen house and the results showed that none of the rooftop hydroponic vegetables exceeded the maximum residue limit for lead, arsenic, cadmium, chromium, mercury, or nitrate [21]. Tested rooftop gardening in Paris used local urban organic waste as crop substrates and concluded low heavy metals accumulation [22]. Ercilla-Montserrat et al. [23] have proven the feasibility of growing leaf crops on the rooftops in Barcelona and its surroundings using soilless systems in high-traffic areas, and Cd, Ni, and As concentrations were under the detectable levels, however, Pb was the only heavy metal detected in the lettuce leaves. On the other hand, a study on an urban garden in Italy demonstrated that lettuce cultivated near the road increased the risks of heavy metal accumulation compared to those at further distances [24]. Similar results were reported by Mancarella et al. [25], which found that in an urban garden in the city of Recife, Brazil, the distance from the street decreased the accumulation of many potentially toxic elements. However, information on the health risk assessment studies of toxic metals through consumption of lettuce cultivated in urban areas is quite limited. Once vegetables are one of the most applicable sources of essential and toxic elements it is required to analyze the estimated daily intake (EDI), as well as the human health risks based on the target hazard quotients (THQ) for potentially toxic elements [13, 26, 27].

In this context, this study aimed to evaluate the essential and non-essential elements in lettuce (*Lactuca sativa var. crispa*) cultivated on a rooftop urban garden developed by Agronomy College within the metropolitan region of São Paulo and the human health risks posed by potentially toxic metals intake associated with lettuce consumption was estimated through the EDI of metals, as well as the THQ. Additionally, the hierarchical cluster analysis (HCA) was applied to identify possible sources of heavy metals associated with road traffic.

Experimental

Collection and preparation of lettuce samples

The lettuce samples were harvested in 2019 from the experimental urban garden on the rooftop of Agronomy College, as shown in Fig. 1, localized in São Paulo, Brazil (Latitude 23°31′52″S and 46°36′15″W) near a commercial and urban center, with low, moderate, and high traffic intensity, and high-density road [28]. This point is 760 m from this important road, such as *Marginal Tietê* (see Fig. 2).

Lettuce samples ($n = 10$) were harvested and the edible parts were washed with sufficient purified water to remove impurities present in all plant structures, and oven-dried at 100 °C until achieving a constant weight. The samples were ground to 200 mesh size particles using an agate mortar and pestle. The powdered composite samples were placed in plastic containers and stored in a dry cupboard prior to analysis.

Fig. 1 **a** Lettuce cultivated on the rooftop and **b** experimental urban garden overview on the rooftop at Agronomy College

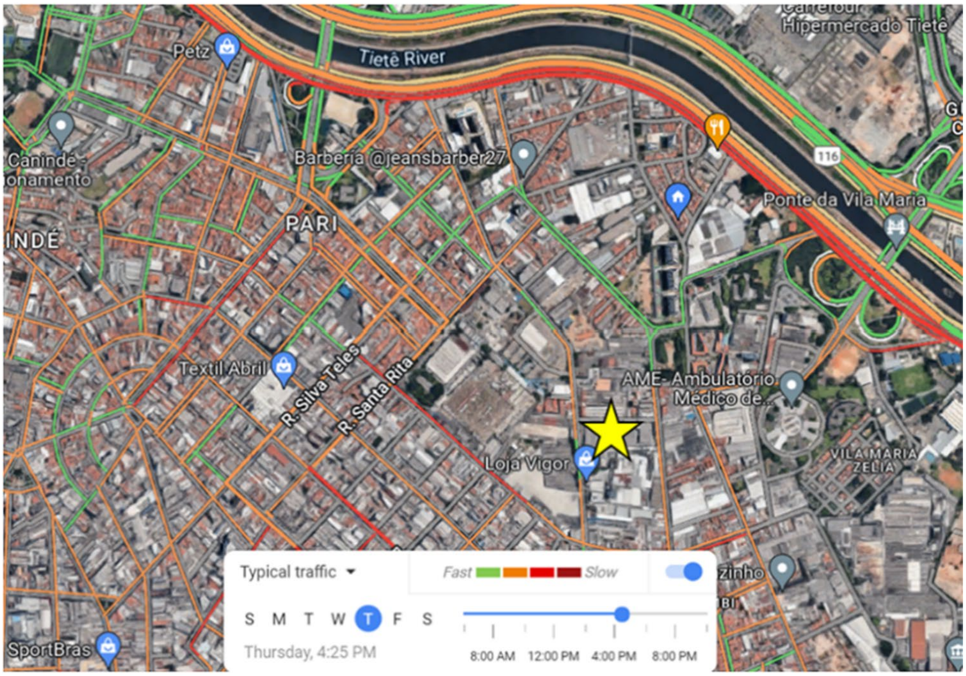


Fig. 2 Google Maps Traffic near the study site (yellow star) in São Paulo for a typical Thursday rush hour (16:25). Low traffic intensity (green color line); medium traffic intensity (orange color line); and high traffic intensity (red color line)

INAA measurement

The elemental concentrations in lettuce samples were determined by a relative method of Instrumental Neutron Activation Analysis (INAA). Instrumental Neutron Activation Analysis has been frequently used to evaluate inorganic contents in different types of matrices, finding applications in the areas of environment, mineralogy, agriculture, health, and archeology [19, 29].

The lettuce samples, certified reference materials (CRMs), and synthetic standards were irradiated at the nuclear research reactor IEA-R1 at *Instituto de Pesquisas Energéticas e Nucleares,* Brazil (IPEN) under short and long irradiations, 20 s, and 8 h, respectively.

For the long irradiation about 150 mg of the powdered lettuce samples, 100 mg of the powdered IAEA-336 Lichen and NIST 1547 Peach Leaves CRMs, and synthetic standards prepared by pipetting convenient aliquots of standard solutions (SPEX Certiprep Inc., USA) onto small filter paper sheets, were all sealed in polyethylene bags. These samples were irradiated for 8 h under a thermal neutron flux of 10^{12} cm^{-2} s^{-1} and were measured after 7 and 15 days of cooling, for 3600 s each. For the short irradiation approximately 60 mg of the powdered lettuce samples, 60 mg of the powdered standard reference material (SRM) NIST 1573a Tomato Leaves, and synthetic standards were irradiated for 20 s with a thermal neutron flux of approximately 10^{11} cm^{-2} s^{-1} at the pneumatic facility. These samples were measured for 180, 120, and 120 s respectively. The gamma spectrometry was performed using the EG&G Ortec HP-Ge detector, with a nominal efficiency of 20% and a resolution (FWHM) of 0.80 keV for the photopeak of 122 keV of ^{57}Co and 1.80 keV for the photopeak of 1332 keV of ^{60}Co and analyzed using the VISPECT2 software.

GF AAS and ICP OES measurements

About 300 mg of each of the powdered lettuce samples and the SRM NIST 1646a (Estuarine sediment) were digested in microwave digestion system (MARS 5-CEM Corporation), according to [30].

The content of the metals Cd, Cu, and Pb, in the digestion solutions, was analyzed by Graphite furnace atomic absorption spectrometer (GFAAS, PerkinElmer AAnalyst™ 800). The calibration of the instrument was done by external standard solutions ranging from 0.001 to 0.2 mg L^{-1}.

The quantification of Ni element, in the digestion solution, was performed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Varian MPX 710ES model). The calibration of the instrument was done by external standard solutions ranging from 0.01 to 10 mg L^{-1}

Quality assurance and control

The precision and accuracy were verified by a comparative method between the CRMs (NIST-SRM 1547, NIST-SRM 1573a, IAEA-336, and NIST-SRM 1646a) and synthetic standards (SPEX Certiprep Inc., USA).

The Z-score parameter was determined to evaluate the laboratory, according to the following Eq. (1):

$$Z_{\text{score}} = \frac{|X_{\text{lab}} - X_{\text{ref}}|}{\sigma_{\text{ref}}} \tag{1}$$

where X_{lab}: element concentration in the CRM analysis; X_{ref}: certified value of the element in the reference material; σ_{ref}: uncertainty of the certified value of the element in the reference material.

The laboratory performance is evaluated as: Satisfactory if $Z_{\text{score}} \leq 2$, questionable for $2 < Z_{\text{score}} < 3$ and unsatisfactory for $Z_{\text{score}} \geq 3$ [31].

Daily metals intake estimates (EDI) and target hazard quotients (THQ)

For the EDI evaluation, the average element content was multiplied by the average daily lettuce consumption of 2.6 g (wet mass) [32]. The conversion factor (0.056 ± 0.004) from fresh weight values to dry weight values was based on 94.4% ± 0.4 water content in lettuce [13, 18, 33].

To evaluate the health risks of potentially toxic metals associated with the consumption of lettuce, the THQ was calculated (Eq. 2). The methodology for estimating THQ is described in detail by the USEPA [26].

$$\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{DI}_l \times C_{\text{m}} \times 0.001}{\text{R}_{\text{f}}\text{D} \times bw \times \text{TA}_{\text{nc}}} \tag{2}$$

EF—exposure frequency (365 days per year); ED—exposure duration (76.3 years, equivalent to the average lifetime) [34]; DI_l—daily intake of lettuce (2.6 g per day, on the fresh weight × 0.056); C_{m}—the mass fraction of potentially toxic metal in the edible parts of lettuce (mg kg^{-1}, in this study); $R_{\text{f}}D$—oral reference dose (mg kg^{-1} day^{-1}); bw—average body weight (65.9 kg) [35]; Ta_{nc}—average exposure time for non-carcinogens (365 days per year × 76.3 years).

The health protection standard of lifetime risks for the THQ is 1. Therefore, at THQ values above 1, there is a possibility that adverse health effects may occur in the long term [13, 26, 36, 37].

The $R_{\text{f}}D$ for the potentially toxic elements are presented in Table 1.

Source identification

The Hierarchical cluster analysis was performed to identify the relationships and possible sources of heavy metals in samples, using Statistica 7 software. Ward's method was performed in combination with Euclidian distance to present a dendrogram of HCA [40].

Table 1 Oral reference doses ($R_{\text{f}}D$) for the potentially toxic elements

Element	$R_{\text{f}}D$ (mg kg^{-1} day^{-1})	$R_{\text{f}}D$ (mg day^{-1} for an adult 65.9 kg)	References
Ba	0.2	13.2	[38]
Co	0.0003	0.02	[27]
Cr	0.003	0.2	[36]
Ni	0.02	1.32	[39]
Pb	0.0035	0.23	[39]

Results and discussion

To assess the accuracy and precision of the methodologies, four CRMs (IAEA-336, NIST 1573a, NIST 1646a and NIST 1547) were analyzed. The Z-score values calculated for the elements determined in the reference materials are shown in Fig. 3.

The Z-scores obtained in the results of CRMs are between -2 and 2, indicating that the results are satisfactory for most of the elements and agree with the certified values. Ni is the only element that is not certified in the CRMs, thus the error relative (ER < 10%) was verified.

Eighteen chemical elements were determined in samples, representing fourteen essential elements Br, Ca, Cl, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Rb, and Zn, and four non-essential elements Ba, Cd, Cs, and Pb [14, 31]. The element concentrations determined in edible parts of the lettuce samples are presented in Table 2 and the literature reported values for the potentially toxic elements in lettuce from rural areas, markets, urban gardens, uncontaminated areas, and hydroponic productions are listed in Table 3.

As can be seen in Table 2, in order of abundance, the accumulation trend of macronutrients and sodium in lettuce leaves was K > Ca > Mg > Na.

The average contents of K, Mg, and Na are within reported values for the culture [13, 41]. Only Ca levels are below the reported range [33, 41]. Kraemer et al. [42] reported Ca, K, and Na contents in lettuce grown inorganic systems were 0.5%, 4.2%, and 0.1%, respectively. For lettuce acquired in markets in the city of Campinas, SP, the Ca, K, Mg, and Na contents were 0.84%, 5.7%, 0.32%, and 0.09% [16]. Kano et al. [43] studied the macronutrients in lettuce planted in rural areas and the results showed that the Ca, K, and Mg contents were 1.2%, 1.4% and 0.31%, respectively. Similarly, Abbey et al. [44] reported calcium (1.4%), potassium (3.8%), and magnesium (0.6%) contents in lettuce cultivated in rural areas.

León-Cañedo et al. [13] reported Ca (1.6%), K (5.4%), Mg (1.02%), and Na (1.6%) contents in lettuce grown in hydroponic solution. Mancarella et al. [25] studied heavy metals in urban gardens in the city of Recife and reported

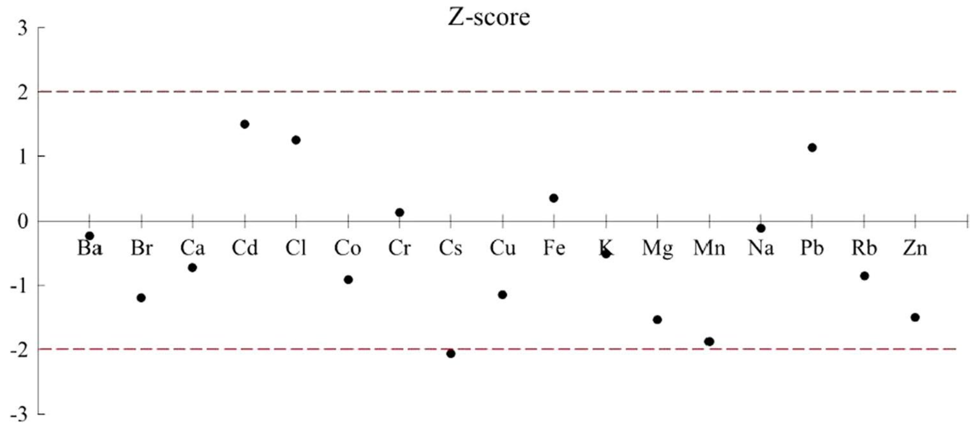


Fig. 3 Z-score values for elements determined in reference materials ($n = 4$)

Table 2 Descriptive statistics for element mass fractions in lettuce (mg kg^{-1}), exceptions in % (dry weight)

	Ba	Br	Ca %	Cd	Cl %	Co	Cr	Cs	Cu	Fe	K%	Mg%	Mn	Na%	Ni	Pb	Rb	Zn
Min	7	17	0.43	0.03	5.4	0.10	1	0.16	3.5	91	3.6	0.23	10	0.12	1.7	0.4	68	26
Max	27	32	0.76	0.27	12.5	0.28	21	0.45	5.7	393	9.3	0.42	23	0.37	10.7	9.8	142	52
Mean	17	23	0.60	0.11	7.4	0.17	7	0.27	4.6	169	6.2	0.28	18	0.26	3.6	3.0	104	38
SD	8	5	0.10	0.08	2.2	0.07	7	0.12	0.7	89	2.2	0.06	5	0.10	2.8	3.4	28	9
Reported values (mg kg^{-1}, dry weight)																		
Lit.^{a}	9–11*	20–22*	1.5–2.5	0.029–0.4*	–	0.008–0.18	30–60*	–	6–8*	–	5–8	0.4–0.6	400–1000	0.1–0.6	0.1–5	0.7–3.6*	(14)* 20–70	40–73*
DRIs (mg day^{-1})	1.98^{b}	–	700–1300^{c}	–	1500–2300^{c}	0.015^{b}	0.015–0.035^{d}	–	0.34–1.3^{c}	7–27^{c}	2000–3400^{c}	80–420^{c}	1.2–2.6^{d}	800–1500^{d}	0.2–1.0^{c}	–	1.4^{c}	3–13^{c}
EDI (μg day^{-1})	2.4	3.4	877	0.02	11,000	0.02	1.1	0.04	0.7	24.6	9030	414	2.6	375	0.5	0.4	15.2	5.5

[a] Literature [13, 33, 41, 45]. *For lettuce culture. DRIs—Dietary Reference Intakes, EDI—Estimate Daily Intake (wet weight)

[b] Tolerable upper intake level (UL)[46]

[c] RDA—the average daily dietary intake level[47]

[d] Adequate intake (AI). Life-stage group 1–70 years. SD—standard deviation

Ca (0.86 and 0.82%), K (0.5 and 0.48%), Mg (0.38 and 0.41%), and Na (0.56 and 0.76%) contents in lettuce grown at different distances from the road. Comparison with the essential elements in lettuce in the present study reveals that the concentration levels are comparable to those reported by other authors. Enrichment in some samples can also be observed for K. Another essential element for plants, Cl concentration varied from 5.4 to 12.5% in the samples.

The consumption of lettuce (2.6 g day^{-1}) cultivated on rooftops would provide approximately between 0.07 to 0.13% for Ca, 0.47 to 0.72% for Cl, 0.27 to 0.45% for K, 0.10 to 0.52% for Mg, and 0.02 to 0.05% for Na of the Dietary reference intakes (DRIs) (see Table 2).

Table 3 Reported values for the potentially toxic elements in lettuce (mg kg^{-1}, fresh and dry weight)

Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Country	Author
mg kg^{-1} (fresh weight)										
0.02			0.76	13.6	3.46	0.1	0.08	5.32	Romania	[39]
0.08	0.15	0.20				0.33	0.48		Brazil	[52]
0.02		0.01					0.09	3.4	Brazil	[19]
0.002–0.018			0.40–0.82				< 0.001–0.11		Brazil	[53]
< 0.01			3.7–4.8			< 0.02–0.30	< 0.06	28.5–32.7	Brazil	[54]
0.002–0.015 (fw)	0.01 (fw)	0.40 (fw)	0.20–0.32 (fw)	9.5 (fw)	1 (fw)	0.2 (fw)	0.02–0.55 (fw)	2.13 (fw)	Brazil	This study
0.05 (fw) (Lettuce)			10 (fw) (Leafy vegetable)				0.3 (fw) (Lettuce)		Brazil	ANVISA [57]
mg kg^{-1} (dry weight)										
			5.6–9.3		104–127			27–41	Mexico	[13]
0.02		1.25	24.3		19	3.1	0.6	52	Norway	[36]
2.5			5.9	219.6	24.1	0.8	1.85	48.5	Pakistan	[55]
		4.38	5.38	157	78.2	0.71	0.71	52.7	Japan	[56]
0.02–0.25									Brazil	[37]
		2.27–0.69	11.62–10.45	170–160	20		2.42–2.09	65–59	Brazil	[25]
0.03–0.27 (dw)	0.17 (dw)	7 (dw)	4–6 (dw)	169 (dw)	18 (dw)	3.6 (dw)	0.4–9.8 (dw)	38 (dw)	Brazil	This study

fw and dw—fresh and dry weight, respectively

Based on an approximate total food consumption of 1620 g day^{-1} for the Brazilian population's dietary habits from the South-eastern region [48], the lettuce consumption of 2.6 g corresponds to 0.16%. Cl, K, and Mg are the elements which present major contributions to DRIs.

Information about the Ba, Br, Cs, and Rb presence in lettuce is scarce. The mean mass fraction of Ba is above the values determined by Mancarella et al. [25] (10 mg kg^{-1}). The average concentration of Br is in accordance with previous values in lettuce culture. The data obtained for Rb is higher than those obtained by Kabata–Pendias [41]. Particularly, lettuce accumulates rubidium contents up to 68 mg kg^{-1} [49]. Recent data suggests the essentiality of rubidium to humans. Experimental studies also suggest pharmacological implications, especially in the prevention and treatment of certain types of pancreas and liver tumors. Lettuce consumption would contribute 1.1% of the Rb amount for recommended daily intake (1.4 mg day^{-1}) [50]. The average estimated daily intake calculated for Cs was 0.04 µg day^{-1}. This value contributes 0.42% of the global intake value (9.4 µg day^{-1}) [51]. The mean concentrations of Cd, Cr, Cu, Mn, and Zn were less than the reported values for the culture and those of Co, Ni, and Pb are within reported values for the culture.

Comparing the results obtained in this study with the literature's reported values for potentially toxic elements presented in Table 3, the average concentrations of Cr, Ni, and Pb are above the literature's reported values. For Cd, Fe, and Zn the mean concentrations are within the literature's reported values, whereas those of Co, Cu, and Mn are below the literature's reported values. When comparing mean concentrations obtained with the acceptable limits established by ANVISA—National Agency of Sanitary Vigilance [57], the Cd, Cu, and Pb concentrations did not exceed the limit. Although the mean mass fraction of Pb is below the acceptable limit, two samples of lettuce (0.42 and 0.55 mg kg^{-1} fresh weight) presented values higher than the established limit.

For the Ba, Co, Cr, Ni, and Pb elements that present concentrations above permissible limits and literature data, the average estimated daily intake was calculated and compared with R_fD. The results obtained are given in Fig. 4.

The estimated daily intake of Ba, Co, Cr, Ni, and Pb presented approximately 0.02%, 0.12%, 0.55%, 0.04%, and 0.2% of the R_fD value, respectively. The Cr content in the lettuce would contribute to high values for the total food consumption of the Brazilian population's dietary habits from the South-eastern region.

The target hazard quotients values of the studied potentially toxic elements were all much lower than 1, suggesting that the health risks associated with potentially toxic elements exposure is not significant. In addition, the sum of the relative contributions of potentially toxic elements (TTHQ) were also calculated, as shown in Fig. 5.

The values varied from 0.001 to 0.02. The TTHQ values were generally less than 1, which suggested an acceptable level of risk where non-carcinogenic health effects are not important. The mean relative contributions of Ba, Co, Cr, Ni, and Pb to the TTHQ were also calculated. Cr is a major risk contributor in these samples, accounting for 50.8% of the total THQ, while the risk contribution from Ba and Ni is relatively low, accounting for 1.7% and 3.6%, respectively. Harmanescu et al. [39] showed that the THQ for Pb was higher than Ni in lettuce. Mancarella et al. [25] presented results of THQ below the safety limit of 1 in the following order Pb > Ba > Cr. The major risk contributor elements due to lettuce consumption were Cr > Pb > Ni in the study conducted by [36]. Similar contributions were found in the present study.

Fig. 4 Estimated daily intake and R_fD for lettuce edible parts

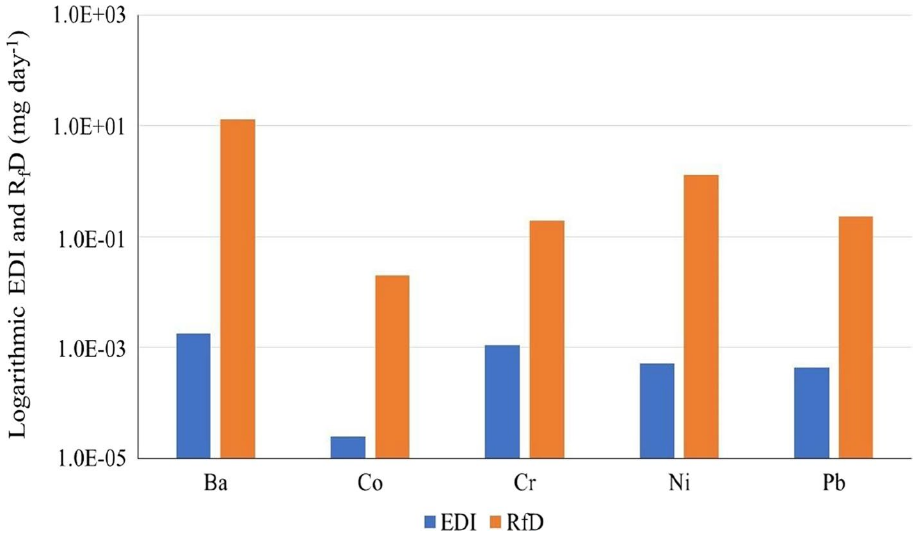
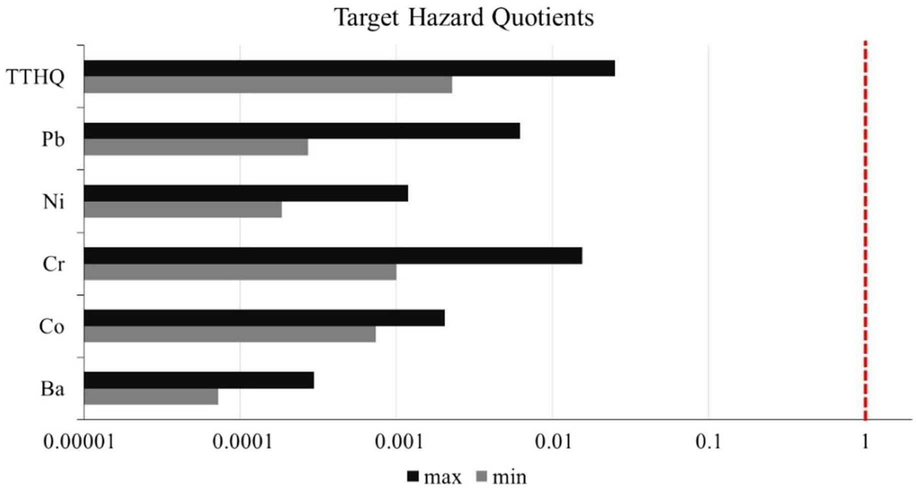


Fig. 5 Estimated target hazard quotients (THQ) and total target hazard quotients (TTHQ) of potentially toxic elements due to lettuce consumption



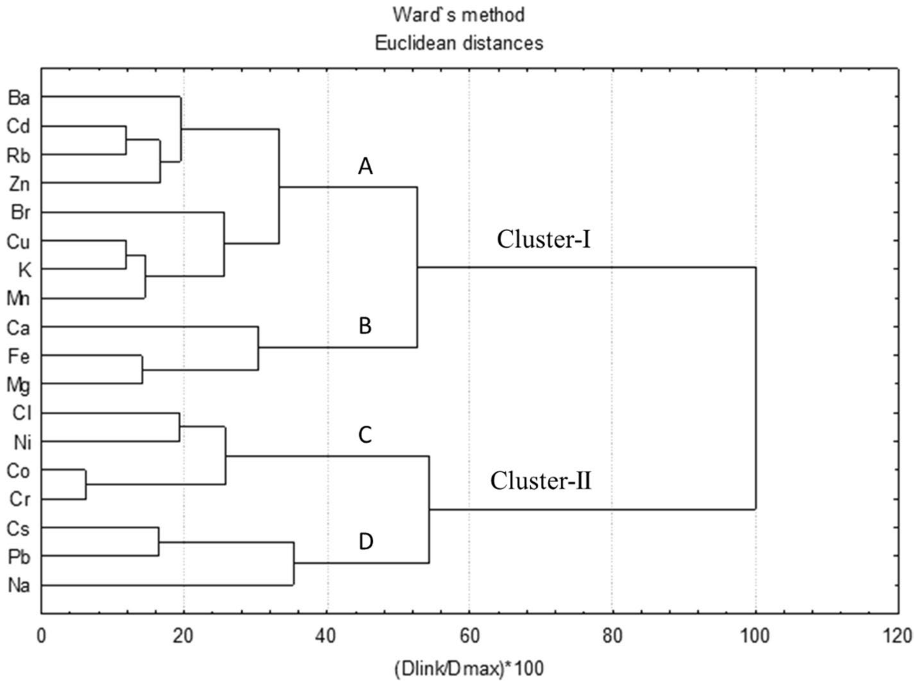
Cluster analysis with dendrogram, using Ward's Method, was employed to analyze the distribution and the possible sources of heavy metals as shown in Fig. 6.

Based on cluster analysis, the results show that there are two main distinctive clusters among the considered variables. The cluster I splits into two subclusters: A (Ba, Cd, Rb, Zn, Br, Cu, K, and Mn) and B (Ca, Mg, and Fe), which were found in highest values average concentrations, also being essential for plants [14, 58]. The confirmed association between macro and micronutrients may suggest these elements have anthropogenic influence in to a certain extent, as agrochemicals application, possible road traffic emissions [58–60], besides soil-forming parent materials. The cluster II includes Cl, Ni, Co, Cr in subcluster C, and Cs, Pb, and Na in subcluster D. The elements Ni, Cr, Co, and Pb are frequently associated with pollutants emitted by vehicles, as brake and tire wear emissions, resuspended road dust, in the vicinity of the sampling sites [58, 61].

Conclusions

The lettuces grown on rooftops, in the Metropolitan region of São Paulo, contain essential macronutrients such as potassium, calcium, and magnesium and have lower concentrations of trace elements. The Cd, Cu, and Pb concentrations did not exceed the Brazilian legislation limit. Ba, Co, Cr, Ni, and Pb are present at low levels compared to R_fD. The Cl, K, Mg, and Cr element contents are major contributors to the total food consumption of the Brazilian population's

Fig. 6 Dendrogram showing cluster analysis for metal concentration found in lettuce samples cultivated on rooftop urban garden



dietary habits from the South-eastern region. The results of the cluster analysis showed that Ni, Cr, Co, and Pb may be associated with vehicles emissions. Both EDI and THQ values suggested minimal risk upon consumption of lettuce. Despite the small number of samples, the results were promising. Increasing the sample size, adding other vegetables, and analyzing other pollutants are suggested for further studies.

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Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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