

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

Urban agriculture is considered an important solution to food security. Rooftops are a new addition to possible places to grow vegetables due to urban land being limited and expensive. However, urban agriculture can be a risk in the case that vegetables are contaminated with heavy metals via atmospheric deposition because of traffic emissions. Among the vegetables, lettuce stands out as one of the most popular in Brazilian food and it is a very important vegetable for the human diet, but its consumption is the most common route for human exposure to toxic metals. The objective of this study was to evaluate the essential and non-essential elements in lettuce cultivated on a rooftop urban garden developed by Agronomy College within the metropolitan region of São Paulo, Brazil. In addition, to assess the human health risks associated with the potentially toxic metals based on the estimated daily intake (EDI) and the target hazard quotient (THQ), and to access the possible sources of heavy metal contamination by multivariate statistical. Instrumental neutron activation analysis, graphite furnace atomic absorption spectrometer, and inductively coupled plasma optical emission spectrometry were applied to determine the elemental concentration in lettuce leaves. This study demonstrated that lettuces grown on a rooftop are sources of 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. The Ba, Co, Cr, Ni, and Pb presented low levels compared to oral reference dose (R_fD). The results of the cluster analysis showed that Ni, Cr, Co, and Pb may be associated to vehicles emissions. Both EDI and THQ values suggested minimal risk upon consumption of lettuce.

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 (Graeme and Makiko 2014). 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 (Doron 2005; Luc 2006). Roof gardens help to increase the availability, facilitate the access to fresh fruits and vegetables, which is perceived as a contribution to balanced diets for all and to the decrease of malnutrition affecting especially those living in poverty (Baudoin et al. 2017).

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 (Manta et al. 2002; Chen et al. 2005; Massaquoi et al. 2015; Khan et al. 2016). Obviously, this contamination is also observed in urban agriculture products and its products can exceed the precautionary values, and a dietary exposure to trace metals can result in significant human health risks (Säumel et al. 2012). Food contamination by heavy metals depends on their mobility in the soil and their bioavailability (Pančevski et al. 2014). 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 (Alleoni et al. 2005). 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. These are required in small quantities, but in excess, they can cause toxic effects (Sharma et al. 2016; León-Cañedo et al, 2019; Ngigi and Muraguri 2019; Solgi et al. 2019).

Lettuce (*Lactuca sativa* L.) is a widely grown and a popularly consumed vegetable worldwide and the main leafy vegetable marketed in Brazil (Kawashima and Soares 2003; Sala and Costa 2012; Kim et al. 2016). It is a leafy vegetable that presents in its constitution fiber source, vitamin C, folic acid, nutrients such as potassium, calcium, sodium, magnesium, manganese, zinc, iron, and it has low calorie values (Kim et al. 2016; Bortoletto et al. 2019; Camejo et al. 2020).

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 and 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 (Bortoletto et al. 2019). In China, researchers evaluated hydroponically grown vegetables in a rooftop screen house and the results showed that none of the roof hydroponic vegetables exceeded the maximum residue limit for lead, arsenic, cadmium, chromium, mercury, or nitrate (Liu et al. 2016). Tested rooftop gardening in Paris used local urban organic waste as crop substrates and concluded low heavy metals accumulation (Grard et al. 2015). Ercilla-Montserrat et al. (2018) 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 (Antisari et al. 2015). Similar results were reported by Mancarella et al. (2016), 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 metal through consumption of lettuce cultivated in urban areas is quite limited. Since vegetables are one of the most relevant sources of essential nutrients and toxic elements, it is very important to estimate the daily intake (EDI) of potentially toxic elements, as well as the human health risks based on the target hazard quotients (THQ) (León-Cañedo et al, 2019; Liang et al. 2019; Gebeyehu and Bayissa 2020).

In this context, this study aimed to evaluate the essential and non-essential elements in lettuce (*Lactuca sativa var. crispata*) 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 daily intake (EDI) of metals, as well as the target hazard quotient (THQ). Additionally, the hierarchical cluster analysis (HCA) was applied to identify possible sources of heavy metals associated with road traffic.

Materials And Methods

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, low, moderate, and high traffic intensity, and high-density road (CET 2019). 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 ultrapure water to remove impurities and soil particles present in all plant structures, 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.

Inaa Measurement

The elemental concentrations in lettuce samples were determined by a relative method of Instrumental Neutron Activation Analysis (INAA). INAA, a sensitive, nondestructive, and multi-element technique, has been frequently used to evaluate inorganic contents in different types of matrices, find applications in the areas of environment, mineralogy, agriculture, health, and archeology (Bortoletto et al. 2019; Silva et al. 2022).

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 two irradiation schemes, short (20 seconds) and long irradiations (8 hours).

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) using milli-Q water of 18.2 MΩ cm at 25°C (Millipore Corporation, USA) onto small filter paper sheets, were all sealed in polyethylene bags. These samples were carried out under a thermal neutron flux of $10^{12} \text{ cm}^{-2} \text{ s}^{-1}$ for 8 hours. 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 carried out at the pneumatic facility with a thermal neutron flux of approximately $10^{11} \text{ cm}^{-2} \text{ s}^{-1}$ for 20 seconds.

The gamma-ray spectra were obtained using an EG&G ORTEC counting system (high-resolution solid-state Ge detector, type POP TOP, Model 20190) with a resolution of 1.9 keV for the 1332 keV peak of ^{60}Co . In the short irradiation scheme, samples, SRM, and synthetic standards were measured for 180, 120, and 120 seconds respectively. In the long irradiation scheme, samples, CRMs, and synthetic standards were measured after 7 and 15 days of cooling, for 3600 seconds each. All counts were performed at a distance between 0–6 cm previously determined to give a dead time lower than 10%. Once the distance was chosen, all samples were counted at the same geometry. The detector was coupled to an EG&G ORTEC ACE8K card and associated electronics. Spectrum analysis was performed using the VISPECT2 software in TURBOBASIC language.

Gf Aas And Icp Oes Measurements

For determination of Cd, Cu, Ni, and Pb elements, about 300 mg of each of the powdered lettuce samples and the SRM NIST 1646a (Estuarine sediment) were digested in 22.5 ml of acid solution (HNO_3 , HCl , HClO_4 , H_2O_2 in ratio of 5:15:0.5:2) at 100°C during 120 min in microwaves digester (MARS 5 – CEM Corporation). Then, the clear solution was diluted up to 50 ml with distilled water and filtered with Whatman filter paper no. 1. 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 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 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 equation:

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

where X_{Lab} , X_{Ref} , σ_{Ref} are the laboratory results, the recommended and standards uncertainties, respectively.

The laboratory performance is evaluated as: Satisfactory if $Z_{score} \leq 2$, questionable for $2 < Z_{score} < 3$ and unsatisfactory for $Z_{score} \geq 3$ (Begaa and Messaoudi 2018).

Daily Metals Intakes Estimate (Edi) And Target Hazard Quotients (Thq)

For the estimate daily intake evaluation (EDI), the average element content was multiplied by the average daily lettuce consumption of 2.6 g (wet mass) (IBGE 2020). The conversion factor (0.056 ± 0.004) from fresh weight values to dry weight values was based on $94.4\% \pm 0.4$ water content in lettuce (Kim et al. 2016; Latif et al. 2018; León-Cañedo et al. 2019).

To evaluate the health risks of potentially toxic metals associated with the consumption of lettuce, the target hazard quotients (THQ) were calculated. 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 (Léon-Cañedo et al. 2019; Liang et al. 2019; Muniz et al. 2022). The methodology for estimating THQ is described in detail by the USEPA (Liang et al. 2019).

THQ are complex parameters used for the estimation of potential health risks associated with long term exposure to chemical pollutants in vegetables (Eregno et al. 2017; Muniz et al. 2022).

THQ are the ratios between the potentially toxic metals concentration and the oral reference dose (RfD), weighted by the duration and frequency of exposure, intake rate, and body weight. The THQ are calculated by the equation:

$$THQ = \frac{EF \times ED \times DI_l \times C_m \times 0.001}{R_fD \times bw \times TA_{nc}} \quad (2)$$

where EF is the exposure frequency (365 days per year); ED is the exposure duration (76.3 years, equivalent to the average lifetime) (IBGE 2019); DI_l is the daily intake of lettuce (2.6 g per day, on the fresh weight x 0.056); C_m is the potentially toxic metals concentration in edible parts of lettuce (mg kg^{-1} , in this study); R_fD is the oral reference dose ($\text{mg kg}^{-1} \text{ day}^{-1}$); bw is the average body weight (65.9 kg) (IBGE 2008); and TA_{nc} is the average exposure time for non-carcinogens (365 days per year x 76.3 years).

The used oral reference doses (R_fD) for the potentially toxic elements are presented in Table 1.

Table 1
Oral reference doses (R_fD) for the potentially toxic elements

Element	R_fD ($\text{mg kg}^{-1} \text{ day}^{-1}$)	R_fD (mg day^{-1} for an adult 65.9 kg)	Reference
Ba	0.2	13.2	USEPA (2005)
Co	0.0003	0.02	Gebeyehu and Bayissa (2020)
Cr	0.003	0.2	Eregno et al. (2017)
Ni	0.02	1.32	Harmanescu et al. (2011)
Pb	0.0035	0.23	Harmanescu et al. (2011)

Source Identification

In this study, a hierarchical cluster analysis (HCA) was performed to identify the relationships and possible sources of heavy metals in the lettuce samples, using Statistica 7 software. Ward's method was performed in combination with Euclidian distance to present a dendrogram of HCA (Gupta et al. 2018).

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. For Ni only the error relative (ER < 10%) was verified because the CRM used only provided non certified values.

Eighteen chemical elements were determined in the lettuce 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 (Begaa and Messaouri 2018; Ngigi and Muraguri 2019). 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 $K > Ca > Mg > Na$.

Table 2

Descriptive statistics for element concentrations in lettuce (mg kg⁻¹), exceptions in % (dry weight)

	Ba	Br	Ca%	Cd	Cl%	Co	Cr	Cs	Cu
Min.	7	17	0.43	0.03	5.4	0.10	1	0.16	3.5
Max.	27	32	0.76	0.27	12.5	0.28	21	0.45	5.7
Mean	17	23	0.60	0.11	7.4	0.17	7	0.27	4.6
SD	8	5	0.10	0.08	2.2	0.07	7	0.12	0.7
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*
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
EDI (µg day ⁻¹)	2.4	3.4	877	0.02	11000	0.02	1.1	0.04	0.7
	Fe	K%	Mg%	Mn	Na%	Ni	Pb	Rb	Zn
Min.	91	3.6	0.23	10	0.12	1.7	0.4	68	26
Max.	393	9.3	0.42	23	0.37	10.7	9.8	142	52
Mean	169	6.2	0.28	18	0.26	3.6	3.0	104	38
SD	89	2.2	0.06	5	0.10	2.8	3.4	28	9
Reported values (mg kg ⁻¹ , dry weight)									
Lit. ^a	-	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)	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)	24.6	9030	414	2.6	375	0.5	0.4	15.2	5.5
^a Literature (Kabata-Pendias 2010; Latif et al. 2018; León-Cañedo et al. 2019; Guzmán-Morales et al. 2012). DRIs - Dietary Reference Intakes. EDI – Estimate Daily Intake (wet weight). ^b Tolerable Upper Intake Level (UL) - the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population (WHO 2016). ^c RDA - the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group (Institute of medicine 2001). ^d Adequate intake (AI). Life-stage group 1–70 years. *For lettuce culture. SD – Standard deviation.									

The average content of K, Mg, and Na are within reported values for the culture (Kabata-Pendias 2010; León-Cañedo et al. 2019). Only Ca levels are below the reported range (Kabata-Pendias 2010; Latif et al 2018). Kraemer et al. (2020) reported Ca, K, and Na contents in lettuce grown in organic systems were 0.5, 4.2, 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% (Kawashima and Soares 2003). Kano et al. (2010) 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. (2021) reported calcium (1.4%), potassium (3.8%), and magnesium (0.6%) contents in lettuce cultivated in rural areas.

León-Cañedo et al. (2019) reported Ca (1.6%), K (5.4%), Mg (1.02%), and Na (1.6%) contents in lettuce grown in hydroponic solution. Mancarella et al. (2016) studied heavy metals in urban gardens in the city of Recife and reported 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).

Based on an approximate total food consumption of $1,620 \text{ g day}^{-1}$ for the Brazilian population's dietary habits from the South-eastern region (IBGE 2011), the lettuce consumption of 2.6 g corresponds to 0.16%. The 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 concentration of Ba is above the values determined by Mancarella et al. (2016) (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 (2010). Particularly, lettuce accumulates rubidium contents up to 68 mg kg^{-1} (Anke et al. 2005). 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 intakes (1.4 mg day^{-1}) (Antal et al. 2009). The average estimated daily intake calculated for Cs was $0.04 \text{ } \mu\text{g day}^{-1}$. This value contributes 0.42% of the global intake value ($9.4 \text{ } \mu\text{g day}^{-1}$) (Parr et al. 1992). The mean concentration of Cd, Cr, Cu, Mn, and Zn were less than the reported values for the culture and the 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 the Co, Cu, and Mn are below the literature's reported values.

When comparing mean concentrations obtained with the acceptable limits established by National Agency of Sanitary Vigilance (ANVISA 2021), the Cd, Cu, and Pb concentrations did not exceed the limit. Although the mean concentration 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.

Table 3

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

Author	Country	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
mg kg^{-1} (fresh weight)										
Harmanescu et al. (2011)	Romania	0.02			0.76	13.6	3.46	0.1	0.08	5.32
Guerra et al. (2021)	Brazil	0.08	0.15	0.20				0.33	0.48	
Bortoletto et al. (2019)	Brazil	0.02		0.01					0.09	3.4
Dala-Paula et al. (2018)	Brazil	0.002–0.018			0.40–0.82				< 0.001–0.11	
França et al. (2017)	Brazil	< 0.01			3.7–4.8			< 0.02–0.30	< 0.06	28.5–32.7
mg kg^{-1} (dry weight)										
Léon-Cañedo et al. (2019)	Mexico				5.6–9.3		104–127			27–41
Eregno et al. (2017)	Norway	0.02		1.25	24.3		19	3.1	0.6	52
Achakzai et al. (2011)	Pakistan	2.5			5.9	219.6	24.1	0.8	1.85	48.5
Itho et al. (2006)	Japan			4.38	5.38	157	78.2	0.71	0.71	52.7
Muniz et al. (2021)	Brazil	0.02–0.25								
Mancarella et al. (2016)	Brazil			2.27–0.69	11.62–10.45	170–160	20		2.42–2.09	65–59
This study	Brazil	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)
		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)
ANVISA (2021)		0.05 (fw) (Lettuce)			10 (fw) (Leafy vegetable)				0.3 (fw) (Lettuce)	
dw and fw – dry and fresh weight, respectively. ANVISA - National Agency of Sanitary Vigilance.										

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 THQ 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 TTHQ (sum of the relative contributions of potentially toxic elements) 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. (2011) showed that the THQ for Pb was higher than Ni in lettuce. Mancarella et al. (2016) 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 Eregno et al. (2017). Similar contributions were found in the present study.

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 (Ngigi and Muraguri 2019; Alexandrino et al. 2020). The confirmed association between macro and micronutrients may suggests these elements have anthropogenic influence in a certain extent, as agrochemicals application, possible road traffic emissions (Wang et al. 2018; Alexandrino et al. 2020; Payandeh et al. 2021), 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 (Sevik et al. 2019; Alexandrino et al. 2020).

Conclusions

This study demonstrated that lettuces grown on rooftops, in the Metropolitan region of São Paulo, Brazil, are sources of 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. The Ba, Co, Cr, Ni, and Pb presented 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 dietary habits from the South-eastern region. The results of the cluster analysis showed that Ni, Cr, Co, and Pb may be associated to vehicles emissions. Both EDI and THQ values suggested minimal risk upon consumption of lettuce. This indicates that in our study, the ingestion of lettuce produced on the rooftop does not represent a risk to health. However, increasing the sample size, adding other vegetables, and analyzing other pollutants are suggest in further studies.

Declarations

Author Contributions: F.V.S., M.V., and P.S.C.S principally conceived the idea for the study; M.R.F. was responsible for revising this manuscript; R.C.L.F. was responsible for ICP-OES analysis; All authors participated in some form in the concept, writing, and editing of this manuscript.

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References

1. Abbey L, Ijenyo M, Spence B, Asunni AO, Ofoe R, Amo-Larbi V (2021) Bioaccumulation of chemical elements in vegetables as influenced by application frequency of municipal solid waste compost. *Can J Plant Sci* 101:967–983. <https://doi.org/10.1139/cjps-2020-0291>
2. Achakzai AKK, Bazai ZA, Kayani SA (2011) Accumulation of heavy metals by lettuce (*Lactuca sativa* L.) irrigated with different levels of wastewater of Quetta City. *Pak J Bot* 43(6):2947–2951
3. Alexandrino K, Viteri F, Rybarczyk Y, Andino JEG, Zalakeviciute R (2020) Biomonitoring of metal levels in urban areas with different vehicular traffic intensity by using *Araucaria heterophylla* needles. *Ecol Indic* 117:106701. <https://doi.org/10.1016/j.ecolind.2020.106701>
4. Alleoni LRF, Borba RP, Camargo OA de (2005) Metais pesados: da cosmogênese aos solos brasileiros, In: Tópicos em Ciência do Solo, Viçosa: Escola Superior de Agricultura Luiz de Queiroz Universidade de São Paulo, São Paulo, Brazil, pp 1–42
5. Anke M, Angelow L, Müller R, Anke S (2005) Recent progress in exploring the essentiality of the ultratrace element rubidium to the nutrition of animals and man. *Biomed Res Trace Elements* 16(3):203–207. <https://doi.org/10.11299/brte.16.203>
6. Antal DS, Dehelean CA, Peev CI, Anke M (2009) Rubidium in medicinal plants: Contribution to the research of a potentially essential element. *Rev Chim* 60:156–159.
7. Antisari LV, Orsini F, Marchetti L, Vianello G, Gianquinto G (2015) Heavy metal accumulation in vegetables grown in urban gardens. *Agron Sustain Dev* 35:1139–1147. <https://doi.org/10.1007/s13593-015-0308-z>
8. ANVISA (2021) Agência Nacional de Vigilância Sanitária. Instrução Normativa IN N° 88 de 26 de março de 2021. <https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-88-de-26-de-marco-de-2021-311655598>. Accessed 16 May 2022
9. Baudoin W, Desjardins Y, Dorais M, Charrondièrre UR, Herzigova L, El-Behairy U, Metwaly N, Marulanda C, Ba N (2017) Rooftop Gardening for Improved Food and Nutrition Security in the Urban Environment, In: Orsini F, Dubbeling M, de Zeeuw H, Gianquinto G, (eds) Rooftop Urban Agriculture, Urban Agric. Springer Cham. https://doi.org/10.1007/978-3-319-57720-3_13
10. Begaa S, Messaoudi M (2018) Thermal neutron activation analysis of some toxic and trace chemical element contents in *Mentha pulegium* L. *Radiochim Acta* 106:769–774. <https://doi.org/10.1515/ract-2018-2942>
11. Bortoletto LA, Lima ES, Fávoro DIT, Ulrich JC, Souza VAF, Cotrim MEB, Bezerra FC (2019) Avaliação de metais tóxicos de alfaces cultivadas em horta urbana na cidade de São Paulo, São Paulo. *Braz J Environ Sci* 52:99–118. <https://doi.org/10.5327/Z2176-947820190462>
12. Camejo D, Frutos A, Mestre TC, Piñero MC, Rivero RM, Martínez V (2020) Artificial light impacts the physical and nutritional quality of lettuce plants. *Hortic Environ Biotechnol* 61:69–82. <https://doi.org/10.1007/s13580-019-00191-z>
13. CET (2019) Traffic Engineering Company. Pesquisa de Monitoração da Fluidez: Desempenho do Sistema Viário principal, Volume e Velocidade 2019, 1-146
14. Chen TB, Zheng YM, Lei M, Huang ZC, Wu HT, Chen H, Fan KK, Yu K, Wu X, Tian QZ (2005) Assessment of heavy metal pollution in surface soils of urban parks in Beijing China. *Chemosphere* 60(4):542–551. <https://doi.org/10.1016/j.chemosphere.2004.12.072>.
15. Dala-Paula BM, Custódio FB, Knupp EAN, Palmieri HEL, Silva JBB, Glória MBA (2018) Cadmium copper and lead levels in different cultivars of lettuce and soil urban agriculture. *Environ Pollut* 242(part A):383–389. <https://doi.org/10.1016/j.envpol.2018.04.101>
16. Doron G (2005) Urban agriculture: Small medium large. *Archit Des* 75(3):52–59. <https://doi.org/10.1002/ad.76>

17. Ercilla-Montserrat M, Muñoz P, Montero JI, Gabarrell X, Rieradevall J (2018) A study on air quality and heavy metals content of urban food produced in a Mediterranean city (Barcelona). *J Clean Prod* 195:385–395. <https://doi.org/10.1016/j.jclepro.2018.05.183>
18. Eregno FE, Moges ME, Heistad A (2017) Treated Greywater Reuse for Hydroponic Lettuce Production in a Green Wall System: Quantitative Health Risk Assessment. *Water* 9(7):454. <https://doi.org/10.3390/w9070454>
19. França FCSS, Albuquerque AMA, Almeida AC, Silveira PB, Filho CA, Hazin CA, Honorato EV (2017) Heavy metals deposited in the culture of lettuce (*Lactuca sativa* L.) by the influence of vehicular traffic in Pernambuco, Brazil. *Food Chem* 215:171–176. <https://doi.org/10.1016/j.foodchem.2016.07.168>
20. Gebeyehu HR, Bayissa LD (2020) Level of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. *PLoS One* 15(1):0227883. <https://doi.org/10.1371/journal.pone.0227883>
21. Graeme T, Makiko T (2014) Growing Greener Cities in Latin America and the Caribbean - An FAO report on urban and peri-urban agriculture in the region. FAO, Roma
22. Gard BJ-P, Bel N, Marchal N, Madre N, Castell J-F, Cambier P, Houot S, Manouchehri N, Besancon S, Michel J-C, Chenu C, Frascaria-Lacoste N, Aubry C (2015) Recycling urban waste as possible use for rooftop vegetable garden. *Future of Food: J Food Agric Soc* 3(1):21–34. <https://www.researchgate.net/publication/281723738>
23. Guerra F, Trevisan AR, Muraoka T, Marcante NC, Canniatti-Brazaca SG (2012) Heavy metals in vegetables and potential risk for human health. *Sci Agri* 69(1):54–60. <https://doi.org/10.1590/S0103-90162012000100008>
24. Gupta SK, Ansari FA, Nasr M, Chabukdhara M, Bux F (2018) Multivariate analysis and health risk assessment of heavy metal contents in foodstuffs of Durban, South Africa. *Environ Monit Assess* 190:151. <https://doi.org/10.1007/s10661-018-6546-1>
25. Guzmán-Morales R, Cruz-La Paz O, Valdés-Carmenate R, Valdés-Hernández PA (2021) Evaluation of heavy metal contamination and accumulation in lettuce (*Lactuca sativa* L.) plants. *Cult Trop* 42(4):3. <https://www.redalyc.org/articulo.oa?id=193270002003>. Accessed 23 May 2022
26. Harmanescu M, Alda LM, Bordean DM, Gogoasa I, Gergen I (2011) Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chem Cent J* 5:64. <https://doi.org/10.1186/1752-153X-5-64>
27. IBGE (2008) Instituto Brasileiro de Geografia e Estatística – Pesquisa de orçamentos familiares 2008–2009, Estimativas populacionais das medianas de altura e peso de crianças adolescentes e adultos por sexo situação do domicílio e idade - Brasil e Grandes Regiões. <https://sidra.ibge.gov.br/tabela/2645>. Accessed 07 April 2022
28. IBGE (2011) Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008–2009: Análise de consumo alimentar no Brasil, Rio de Janeiro. <https://portaldeboaspraticas.iff.fiocruz.br/wp-content/uploads/2019/07/liv50063.pdf>. Accessed 10 Jun 2022
29. IBGE (2019) Instituto Brasileiro de Geografia e Estatística. Tábua completa de mortalidade para o Brasil – 2018. Breve análise da evolução da mortalidade no Brasil. https://biblioteca.ibge.gov.br/visualizacao/periodicos/3097/tcmb_2018.pdf. Accessed 07 April 2022
30. IBGE (2020) Instituto Brasileiro de Geografia e Estatística – Pesquisa de orçamentos familiares 2017–2018: análise de consumo alimentar pessoal no Brasil. Rio de Janeiro, Brasil
31. Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press: Washington D.C, USA
32. Itho J, Saitho Y, Futatsugawa S, Sera K (2006) Elemental analysis of vegetables on the market - Comparison with Wild Plants. *Int J PIXE* 16(3–4):209–219. <https://doi.org/10.1142/S0129083506000988>
33. Kabata-Pendias (2010) A Trace elements in soil and plant. CRC Press, EUA. <https://doi.org/10.1201/b10158>

34. Kano C, Cardoso AI, Villas Boas RL (2010) Influência de dose de potássio nos teores de macronutrientes em plantas e sementes de alface. *Hortic Bras* 28(3):287–291. <https://doi.org/10.1590/S0102-05362010000300008>
35. Kawashima LM, Soares LMV (2003) Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. *J Food Compos Anal* 16(5):605–611. [https://doi.org/10.1016/S0889-1575\(03\)00057-7](https://doi.org/10.1016/S0889-1575(03)00057-7)
36. Khan ZI, Ahmad K, Ashraf M, Shoaib N, Parveen R, Bibi Z, Mustafa I, Noorka IR, Tahir HM, Akram NA, Ullah MF, Yaqoob R, Tufarelli V, Fracchiolla M, Cazzato E (2016) Assessment of toxicological health risk of trace metals in vegetables mostly consumed in Punjab, Pakistan. *Environ Earth Sci* 75:433. <https://doi.org/10.1007/s12665-016-5392-0>.
37. Kim MJ, Moon Y, Tou JC, Mou B, Waterland NL (2016) Nutritional value, bioactive compounds, and health benefits of lettuce (*Lactuca sativa* L.). *J Food Compos Anal* 49:19–34. <https://doi.org/10.1016/j.jfca.2016.03.004>
38. Kraemer C, Adami FS, Roselen MD, Souza CFV, Marmitt LG, Oliveira EC (2020) Nitrato nitrito cálcio potássio e sódio em vegetais folhosos orgânicos hidropônicos e convencionais. *Uningá Review* 35:1–12. <http://revista.uninga.br/index.php/uningareviews/article/view/2933>. Accessed 13 May 2022
39. Latif A, Bilal M, Asghar W, Azeem M, Ahmad MI, Abbas A, Ahmad MZ, Shahzad T (2018) Heavy Metal Accumulation in Vegetables and Assessment of their Potential Health Risk. *J Environ Anal Chem* 5:1. <https://doi.org/10.4172/2380-2391.1000234>
40. León-Cañedo JA, Alrcón-Silvas SG, Fierro-Sañudo JF, de Oca GARM, Partida-Ruval L, Díaz-Valdés T, Páez-Osuna F (2019) Mercury and other trace metals in lettuce (*Lactuca sativa*) grown with two low-salinity shrimp effluents: Accumulation and human health risk assessment. *Sci Total Environ* 650(2):2535–2544. <https://doi.org/10.1016/j.scitotenv.2018.10.003>
41. Liang G, Gong W, Li B, Zuo J, Pan L, Liu X (2019) Analysis of heavy metals in foodstuffs and an assessment of the health risks to the general public via consumption in Beijing, China. *Int J Environ Res Public Health* 16(6):909. <https://doi.org/10.3390/ijerph16060909>
42. Liu T, Yang M, Han Z, Ow DW (2016) Rooftop production of leafy vegetables can be profitable and less contaminated than farm-grown vegetables. *Agron Sustain Dev* 36:41. <https://doi.org/10.1007/s13593-016-0378-6>
43. Luc JAM (2006) Growing better cities: Urban agriculture for sustainable development, International Development Research Center (IDRC). Ottawa, Canada
44. Mancarella S, Pennisi G, Gasperi D, Marchetti L, Loges V, Orsini F, Gianquinto G, Vianello G, Antisari LV (2016) Antimony accumulation risk in lettuce grown in Brazilian urban gardens. *Environ quality* 20:35–47. <https://doi.org/10.6092/issn.2281-4485/6306>
45. Manta DS, Angelone M, Bellanca A, Neri R, Sprovieri M (2002) Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. *Sci Total Environ* 300(1–3):229–243. [https://doi.org/10.1016/S0048-9697\(02\)00273-5](https://doi.org/10.1016/S0048-9697(02)00273-5)
46. Massaquoi LD, Ma H, Liu XH, Han PY, Zuo SM, Hua ZX, Liu DW (2015) Heavy metal accumulation in soils plants and hair samples: an assessment of heavy metal exposure risks from the consumption of vegetables grown on soils previously irrigated with wastewater. *Environ Sci Pollut Res* 22:18456–18468. <https://doi.org/10.1007/s11356-015-5131-1>
47. Muniz AS, Carvalho GAD, Raices RSL, Souza SLQ (2022) Organic vs conventional agriculture: evaluation of cadmium in two of the most consumed vegetables in Brazil. *Food Sci Technol* 42:106721. <https://doi.org/10.1590/fst.106721>
48. Ngigi AN, Muraguri BM (2019) ICP-OES determination of essential and non-essential elements in *Moringa oleifera*, *Salvia hispanica*, and *Linum usitatissimum*. *Sci Afr* 6:00165. <https://doi.org/10.1016/j.sciaf.2019.e00165>
49. Pančevski Z, Stafilov T, Bačeva K (2014) Distribution of Heavy Metals in Lettuce and Carrot Grown in the Vicinity of Lead and Zinc Smelter Plant. *Int Res J Pure Appl Chem* 9(1–2):17–26.

50. Parr R, Crawley H, Abdulla M, Iyengar GV, Kumpulainen J (1992) Human dietary intakes of trace elements. Report: IAEA Vienna, Austria
51. Payandeh K, Nazarpour A, Velayatzadeh M (2021) Human Health Risk of Some Heavy Metals Accumulated in Tomatoe Cucumber, Potato, and Onion Grown in Dezful and Shushtar. *Arch Hyg Sci* 10(4):299–314. <http://dx.doi.org/10.32598/AHS.10.4.314>. 7
52. Sala FC, Costa CP (2012) Retrospectiva e tendência da alfacicultura brasileira. *Hort Bras* 30(2):87–194, <https://doi.org/10.1590/S0102-05362012000200002>
53. Säumel I, Kotsyuk I, Hölscher M, Lenkerei C, Weber F, Kowarik I (2012) How healthy is urban horticulture in high traffic areas? Trace metal concentrations in vegetable crops from plantings within inner city neighborhoods in Berlin Germany. *Environ Pollut* 165:124–132. <https://doi.org/10.1016/j.envpol.2012.02.019>
54. Sevik H, Ozel HB, Cetin M, Özel HU, Erdem T (2019) Determination of changes in heavy metal accumulation depending on plant species plant organism and traffic density in some landscape plants. *Air Qual Atmos Health* 12:189–195. <https://doi.org/10.1007/s11869-018-0641-x>
55. Sharma A, Katnoria JK, Nagpal AK (2016) Heavy metals in vegetables: screening health risks involved in cultivation along wastewater drain and irrigation with wastewater. *SpringerPlus* 5:488. <https://doi.org/10.1186/s40064-016-2129-1>
56. Silva PSC, Zahn GS, Souza FA (2022) Contribuições do Reator IEA-R1 para a Pesquisa Nuclear: II Workshop Anual do Reator de Pesquisas – WARP 2. Blucher, Brazil
57. Solgi E, Alipour H, Majnooni F (2019) Investigation of the concentration of metal in two economically important fish species from the Caspian Sea and assessment of potential risk to human health. *Ocean Sci J* 54:503–514. <https://doi.org/10.1007/s12601-019-0024-8>
58. USEPA (2005) U.S. Environmental Protection Agency. Toxicological review of barium and compounds in support of summary information on the Integrated Risk Information System (IRIS). https://iris.epa.gov/static/pdfs/0010_summary.pdf. Accessed 16 May 2022
59. Wang L, Tao W, Smardon RC, Xu X, Lu X (2018) Speciation sources and risk assessment of heavy metals in suburban vegetable garden soil in Xianyang City, Northwest China. *Front Earth Sci* 12:397–407. <https://doi.org/10.1007/s11707-017-0658-8>
60. WHO (2016) World Health Organization. Barium in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO Press, Geneva

Figures



Figure 1

(a) Lettuce cultivated on the rooftop and (b) experimental urban garden overview on the rooftop at Agronomy College



Figure 2

Google Maps Traffic near the study site (yellow star) in São Paulo for a typical Thursday rush hour (4:25 PM). Low traffic intensity (green color line); medium traffic intensity (orange color line); and high traffic intensity (red color line).

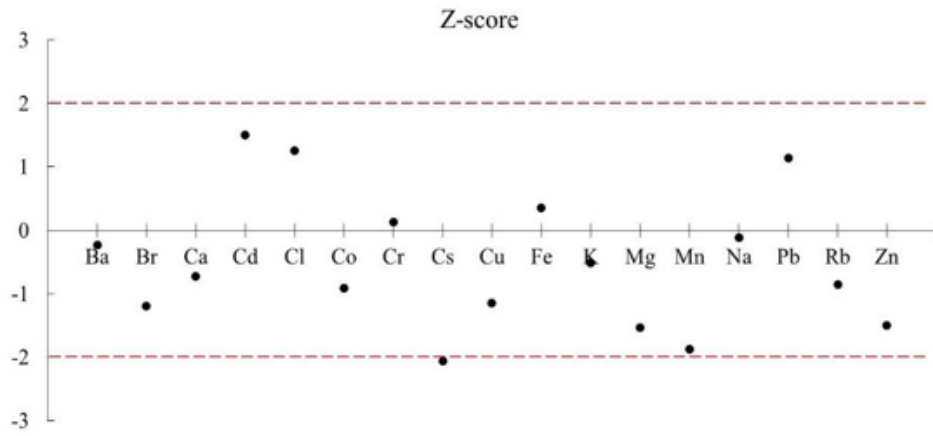


Figure 3

Z-score values obtained for the elements determined in reference materials (n=4)

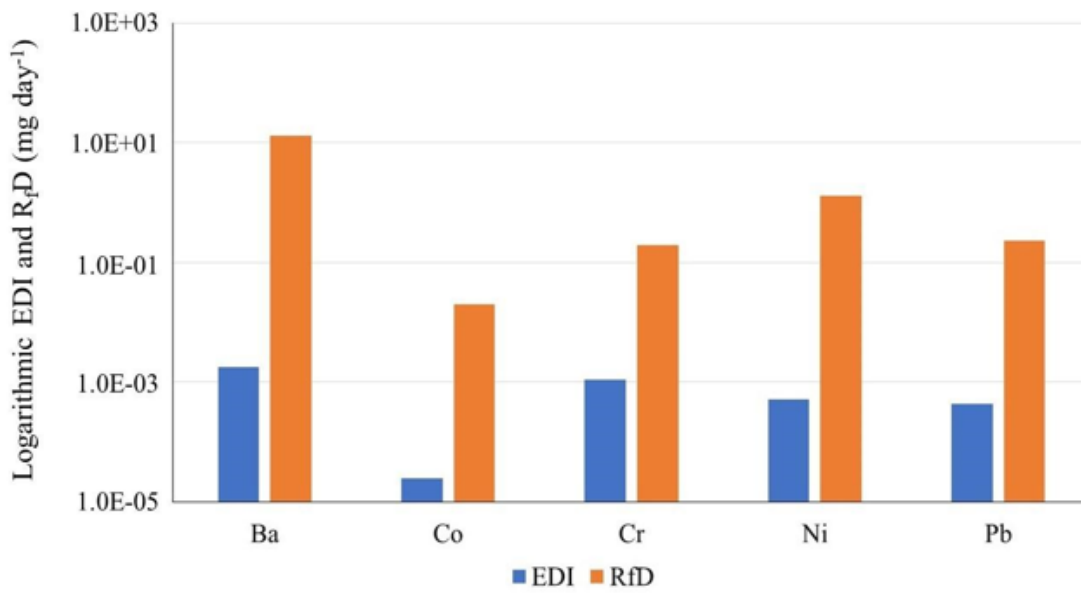


Figure 4

Estimated daily intake and R_fD for edible parts of lettuce

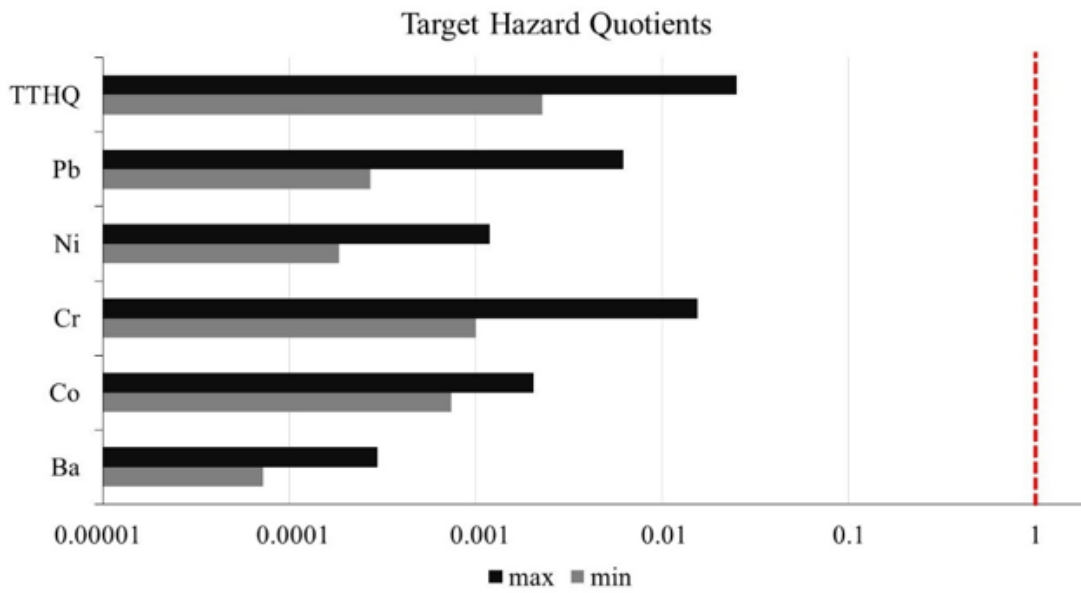


Figure 5

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

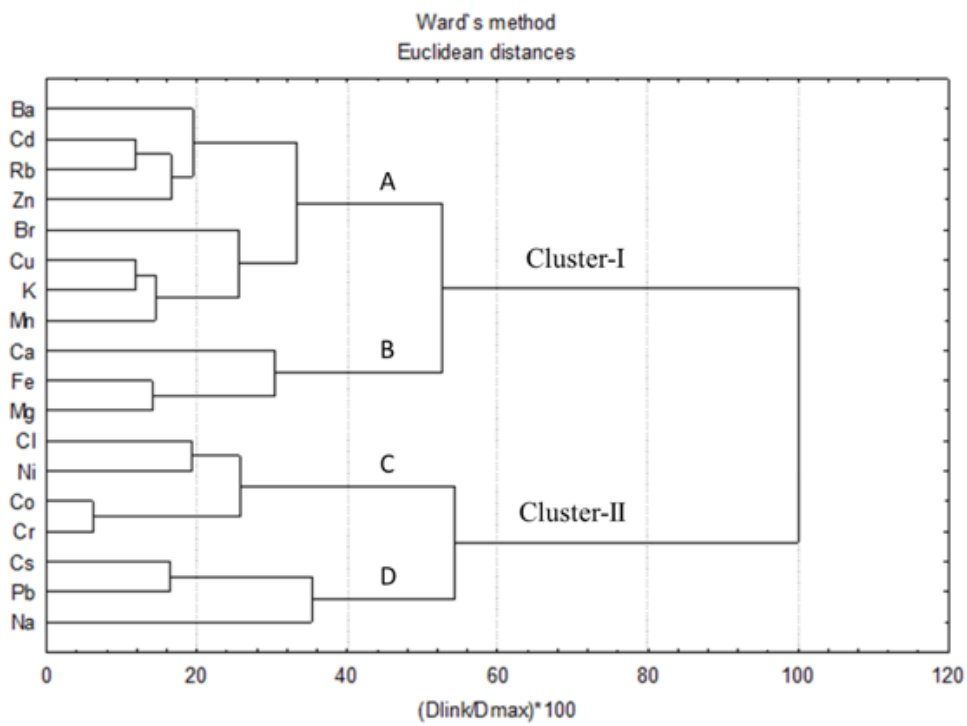


Figure 6

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

