

Heavy metals in vegetables and potential risk for human health

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ABSTRACT: Ingestion of vegetables containing heavy metals is one of the main ways in which these elements enter the human body. Once entered, heavy metals are deposited in bone and fat tissues, overlapping noble minerals. Slowly released into the body, heavy metals can cause an array of diseases. This study aimed to investigate the concentrations of cadmium, nickel, lead, cobalt and chromium in the most frequently consumed foodstuff in the São Paulo State, Brazil and to compare the heavy metal contents with the permissible limits established by the Brazilian legislation. A value of intake of heavy metals in human diets was also calculated to estimate the risk to human health. Vegetable samples were collected at the São Paulo General Warehousing and Centers Company, and the heavy metal content was determined by atomic absorption spectrophotometry. All sampled vegetables presented average concentrations of Cd and Ni lower than the permissible limits established by the Brazilian legislation. Pb and Cr exceeded the limits in 44 % of the analyzed samples. The Brazilian legislation does not establish a permissible limit for Co contents. Regarding the consumption habit of the population in the São Paulo State, the daily ingestion of heavy metals was below the oral dose of reference, therefore, consumption of these vegetables can be considered safe and without risk to human health.

Keywords: contamination, food chain, potentially toxic element, daily intake, consumption habit

Introduction

Potentially harmful metal contents in soils may come not only from the bedrock itself, but also from anthropogenic sources like solid or liquid waste deposits, agricultural inputs, and fallout of industrial and urban emissions (Wilson and Pyatt, 2007). Excessive accumulation in agricultural soils may result not only in soil contamination, but has also consequences for food quality and safety. So, it is essential to monitor food quality, given that plant uptake is one of the main pathways through which heavy metals (HMs) enter the food chain (Antonious and Kochhar, 2009).

Vegetables take up HMs and accumulate them in their edible and non-edible parts at quantities high enough to cause clinical problems to both animals and human beings. As an example, the consumption of contaminated food can seriously deplete some essential nutrients in the body causing a decrease of immunological defenses, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Oliver, 1997). Lăcătușu et al. (1996) reported that soil and vegetables contaminated with Pb and Cd in Copsa Mica and Baia Mare, Romania, significantly contributed to decrease human life expectancy (9-10 years) within the affected areas.

Cadmium and Pb are the most toxic elements for man (Volpe et al., 2009). In terms of environmental concentration, Pb is the HM closest to the level in which toxic signs manifest than any other substance (Baird, 2002). Others elements such as Cr, Co and Ni, although essential for men, at concentrations higher than those recommended, may cause metabolic disorders. Moreover, an increasing awareness in terms of the importance

of vegetables and fruits to human diet suggests that the monitoring of HMs in food crops should be carried out frequently, however information concerning this issue is scarce, especially in Brazil. Most major cities have been concerned with HM contents in vegetables (Ferré-Huguet et al., 2008). São Paulo city, one of the largest metropolitan regions in the world, with over 39 million inhabitants, does not have this kind of monitoring and concern. Therefore, this study aimed to investigate the concentrations of Cd, Pb, Ni, Co and Cr in the most frequently consumed vegetables of the São Paulo State, Brazil to compare then with the permissible limits established by Brazilian legislation. Also a value of intake of HMs in human diets was calculated to estimate the risk to human health.

Materials and Methods

Vegetable samples were collected in August 2009 at the São Paulo General Warehousing and Centers Company (CEAGESP), located in São Paulo/Brazil (23°32'14"S and 46°44'14"W; 723 m asl), which holds the largest public warehouses of the state and a complex of 13 central wholesalers, ensuring the supply of fruits and vegetables to the population. CEAGESP runs the Latin America's largest wholesale of fruits and vegetables, the world's third largest in volume of trade. In 2008, trades amounted 3.114 million tons of fruits, vegetables, flowers, fish and others. Samples were classified as fruits, green vegetables, leafy vegetables and general (tubers, bulbs, bean and rice), according to CEAGESP. In each group, only the most commonly traded vegetables in 2008 were collected, comprising 223 samples of 83 plant species.

Samples were identified and packed into polyethylene bags. In the laboratory, they were washed following the same procedures as for food preparation to remove any surface deposits (Chary et al., 2008). Then, the vegetables were rewashed with deionized water and separated into edible and non-edible parts. The edible parts were air-dried at 65°C, weighed and finely ground in a Willey mill.

A portion of 1 g of each sample was digested in a digestion block, with a nitric acid and perchloric acid (3:1) solution in Pyrex tubes (Miyazawa et al., 2009). After cooling they were diluted to a final volume of 25 mL with deionized water. All analyses were processed in triplicate. HM contents (Cd, Pb, Ni, Co and Cr) were determined in this solution by atomic absorption spectrophotometry (Varian-SpectrAA 140). The accuracy of the method was verified by analyzing the standard reference material 1515 - Apple Leaves (NIST).

For the total daily intake evaluation, the average metal content in each vegetable was calculated and multiplied by the respective consumption rate (IBGE, 2004). The consumption rate for children (0-6 years old) was established as 1/3 of that for adults. The descriptive statistics (mean and coefficient of variation) were conducted using the Excel software.

Risk to human health by the intake of metal-contaminated vegetables was characterized using a hazard quotient (HQ) (US EPA, 1989). HQ is the ratio between exposure and the reference oral dose (R_fD). If the ratio is lower than one (1), there will be no obvious risk. An estimate of the potential hazard to human health (HQ) through consumption of vegetables grown in metal-contaminated soil is described in Eq. (1):

$$HQ = (Div) \times (C_{metal}) / R_fD \times Bo \quad (1)$$

Where (*Div*) is the daily intake of vegetables (kg per day), (C_{metal}) is the concentration of metal in the vegetable ($mg\ kg^{-1}$), R_fD is the oral reference dose for the metal ($mg\ kg^{-1}$ of body weight per day), and *Bo* is the human body mass (kg). R_fD is an estimate of a daily oral exposure for the human population, which does not cause deleterious effects during a lifetime, generally used in EPA's noncancer health assessments.

Values of R_fD for Cd (0.001 $mg\ kg^{-1}$ per day), Ni (0.02 $mg\ kg^{-1}$ per day) and Cr (1.5 $mg\ kg^{-1}$ per day) were taken from Integrated Risk Information System (US EPA, 2010). The value of R_fD for Pb (0.0035 $mg\ kg^{-1}$ per day) was taken from WHO (1993). There is no consensus about the R_fD for Co, then the oral reference dose for Co was estimated as 0.043 $mg\ kg^{-1}$ per day (Food and Nutrition Board, 2004). The average *Bo* was taken as 70 kg for adults (WHO, 1993), and 19.25 kg for children 0-6 years old (IBGE, 2006).

To evaluate the potential risk to human health through more than one HM, the hazard index (HI) has been developed (US EPA, 1989). The hazard index is the sum of the hazard quotients, as described in Eq. (2). It

assumes that the magnitude of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ.

$$HI = \sum HQ = HQ_{Cd} + HQ_{Ni} + HQ_{Pb} + HQ_{Co} + HQ_{Cr} \quad (2)$$

Results and Discussion

The results of the quality control are summarized in Table 1. Concentrations of Cd, Ni, Pb, Co and Cr in vegetables (fresh weight) are shown in Tables 2, 3, 4 and 5. The concentrations of HMs in edible parts of vegetables were compared with the corresponding permissible limits established by National Agency for Sanitary Vigilance (Anvisa) (1965). According to Anvisa, the permissible limits for Cd, Ni, Pb and Cr are 1.0, 5.0, 0.5 and 0.1 $mg\ kg^{-1}$, respectively (fresh weight), with no recommendation for Co.

The Cd concentrations ranged between 0.01 and 0.18 $mg\ kg^{-1}$, and did not exceed the Brazilian limit for all vegetables. No value of Ni, which ranged between 0.01 and 0.74 $mg\ kg^{-1}$, exceeded the permissible limit. The Pb concentrations ranged between 0.02 and 2.50 $mg\ kg^{-1}$, meaning that 44.6 % of the samples exceeded the permissible limit. Leafy vegetables tend to accumulate high amounts of Cd, Ni, and Pb due not only to their large leaf area and high transpiration rate, but also to the fast growth rate of these plants as observed by Itanna (2002). However, concentrations of Pb, that exceeded the established permissible limit of 0.5 $mg\ kg^{-1}$, were not observed for a specific class of vegetables in this study.

Cr concentrations ranged between 0.01 and 0.60 $mg\ kg^{-1}$ and 44.2 % of the samples were found above the permissible limit, mainly for green vegetables accounting for 59.9 % of the total (Table 4). The highest concentrations of Pb and Cr exceeded the permissible limits by approximately 5 (national garlic) and 6 (Japanese pumpkin) times, respectively.

It is important to observe the permissible limits for HM contents established by International Agencies for Brazilian agricultural export goods, since they could be a barrier for international trades. Besides concentrate orange juice, the main Brazilian agricultural export products in Table 2 are mango, melon, papaya, grape, lemon, apple, pineapple, banana and guava. The permissible limit established by FAO/WHO (Codex Alimentarius

Table 1 – Certified Reference Material (SRM NIST 1515) data for checking the analytical accuracy.

Element	Certified Value	Observed Value	Recovery %
	mg kg ⁻¹		
Cd	0.013 ± 0.002	0.016 ± 0.006	123
Co ⁽¹⁾	0.09	0.073 ± 0.003	81
Cr ⁽¹⁾	0.3	0.225 ± 0.048	75
Ni	0.91 ± 0.12	0.728 ± 0.067	80
Pb	0.470 ± 0.024	0.366 ± 0.042	78

⁽¹⁾Noncertified value

Table 2 – Concentrations of heavy metals in fruits (fresh weight) consumed in the São Paulo State.

Vegetables	Scientific Name	State of Origin	n ^a	Consumption	Cd	Ni	Pb	Co	Cr
Avocado	<i>Persea americana</i>	SP	3	0.34	0.04 (0.11) ^b	0.40 (0.05)	0.04 (0.13)	0.23 (0.14)	0.21 (0.13)
'Hawai' Pineapple	<i>Ananas comosus</i> (L.)	SP	3	0.44	0.02 (0.13)	0.15 (0.13)	0.29 (0.19)	0.16 (0.13)	0.04 (0.02)
'Pérola' Pineapple	<i>Ananas comosus</i> (L.)	BA	3	0.44	0.03 (0.19)	0.16 (0.06)	0.57 (0.19)	0.12 (0.09)	0.03 (0.11)
'Nanica' Banana	<i>Musa ssp.</i>	SP	5	1.48	0.06 (0.11)	0.27 (0.05)	0.68 (0.13)	0.10 (0.11)	0.34 (0.11)
'Prata' Banana	<i>Musa sapientum</i> Schum.	SP	5	2.91	0.06 (0.10)	0.15 (0.06)	0.72 (0.12)	0.11 (0.12)	0.17 (0.07)
Red Guava	<i>Psidium guajava</i> L.	SP	4	0.21	0.01 (0.09)	0.13 (0.18)	0.42 (0.06)	0.17 (0.01)	0.08 (0.07)
'Bergamota' Orange	<i>Citrus reticulata</i>	SP	4	0.97	0.02 (0.16)	0.12 (0.05)	0.34 (0.14)	0.14 (0.05)	0.09 (0.14)
'Lima' Orange	<i>Citrus sinenses</i> (L.) Osbeck	SP	4	0.45	0.02 (0.07)	0.22 (0.11)	1.10 (0.11)	0.26 (0.06)	0.08 (0.02)
'Mexerica' Orange	<i>Citrus reticulata</i>	MG	4	0.97	0.06 (0.002)	0.16 (0.03)	0.55 (0.03)	0.15 (0.02)	0.01 (0.11)
'Pêra' Orange	<i>Citrus sinenses</i>	SP	4	2.91	0.05 (0.01)	0.15 (0.08)	0.85 (0.17)	0.15 (0.15)	0.09 (0.05)
'Tahiti' Lemon	<i>Citrus aurantifolia</i> (Christm.) Swingle	SP	4	0.81	0.03 (0.09)	0.22 (0.05)	0.47 (0.10)	0.13 (0.11)	0.30 (0.10)
'Fuji' Apple	<i>Malus silvestris</i> Mill.	SC	3	0.53	0.02 (0.16)	0.05 (0.11)	nd ^c	0.08 (0.17)	nd
'Gala' 1 Apple ^d	<i>Malus silvestris</i> Mill.	SC	3	0.53	0.03 (0.07)	0.04 (0.16)	0.29 (0.09)	0.06 (0.10)	nd
'Gala' 2 Apple ^d	<i>Malus silvestris</i> Mill.	PR	3	0.53	0.04 (0.08) ^b	nd ^c	nd	0.02 (0.15)	0.02 (0.13)
'Granny Smith' Apple	<i>Malus silvestris</i> Mill.	SC	3	0.53	0.05 (0.12)	0.08 (0.16)	0.02 (0.13)	0.04 (0.15)	0.05 (0.13)
'Formosa' Papaya	<i>Carica papaya</i> L.	ES	3	1.20	0.02 (0.09)	0.05 (0.10)	0.39 (0.14)	0.08 (0.15)	0.02 (0.07)
Papaya	<i>Carica papaya</i> L.	- ^e	3	1.20	0.01 (0.23)	0.40 (0.06)	0.58 (0.15)	0.15 (0.08)	0.09 (0.14)
Mango	<i>Mangifera indica</i> L.	PE	3	0.97	0.01 (0.12)	0.11 (0.08)	0.45 (0.14)	0.11 (0.12)	0.03 (0.13)
Yellow passion-fruit	<i>Passiflora edulis</i> f. <i>flavicarpa</i>	BA	4	0.36	0.03 (0.10)	0.23 (0.11)	0.91 (0.17)	0.20 (0.08)	0.10 (0.12)
Watermelon	<i>Citrullus lanatus</i>	GO	1	2.13	0.02	0.05	0.17	0.05	0.01
Melon	<i>Cucumis melo</i> L.	PE	3	0.39	0.02 (0.02)	0.20 (0.01)	0.54 (0.16)	0.32 (0.16)	0.02 (0.12)
Strawberry	<i>Fragaria X ananassa</i> Duch..	SP	5	0.12	0.03 (0.04)	0.10 (0.08)	0.40 (0.05)	0.14 (0.13)	0.04 (0.01)
'Benitaca' Grape	<i>Vitis vinifera</i> L.	PE	3	0.16	0.07 (0.04)	0.03 (0.15)	0.39 (0.12)	0.12 (0.06)	nd
'Itália' Grape	<i>Vitis vinifera</i> L.	PE	3	0.16	0.07 (0.19)	0.08 (0.01)	0.75 (0.17)	0.05 (0.12)	nd
'Niágara' Grape	<i>Vitis vinifera</i> L.	MG	3	0.16	0.09 (0.15)	0.09 (0.09)	0.64 (0.19)	0.13 (0.08)	0.01 (0.13)
'Rubi' Grape	<i>Vitis vinifera</i> L.	MG	3	0.16	0.09 (0.14)	0.08 (0.11)	0.49 (0.18)	0.11 (0.13)	nd

^a n = number of collected samples amount. ^b mean value and coefficient of variation. ^c nd = below detection limit, Ni < 0.09 mg L⁻¹, Pb < 0.09 mg L⁻¹ and Cr < 0.06 mg L⁻¹. ^d apple 1 and 2 = distinct precedence. ^e unknown origin.

Table 3 – Concentrations of heavy metals in leafy vegetables (fresh weight) consumed in the São Paulo State.

Vegetables	Scientific Name	State of Origin	n ^a	Consumption	Cd	Ni	Pb	Co	Cr
Chard	<i>Beta vulgaris</i> L. var. <i>cicla</i>	SP	2	0.12	0.05 ^b	0.21	0.58	0.17	0.07
Watercress	<i>Nasturtium officinale</i> sp.	SP	2	0.19	0.10	0.34	0.86	0.30	0.34
Iceberg Lettuce	<i>Lactuca sativa</i> L.	SP	2	0.22	0.03	0.13	0.41	0.07	0.20
Crisphead Lettuce	<i>Lactuca sativa</i> L.	SP	2	0.22	0.08	0.33	0.48	0.15	0.20
Smooth Lettuce	<i>Lactuca sativa</i> L.	SP	2	0.22	0.07	0.13	0.44	0.16	0.09
Coriander	<i>Coriandrum sativum</i> L.	SP	2	0.02	0.16	0.57	1.24	0.50	0.02
Cabbage	<i>Brassica oleracea</i> var. <i>acephala</i> D.C.	SP	2	0.09	0.12	0.54	1.66	0.51	0.39
Broccoli	<i>Brassica oleracea</i> L.	SP	2	0.20	0.08	0.26	0.93	0.25	0.48
Cauliflower	<i>Brassica oleracea</i> var. <i>botrytis</i>	SP	2	0.29	0.08	0.24	0.36	0.15	nd ^c
Endive	<i>Cichorium endivia</i> L.	SP	2	0.02	0.07	0.31	0.49	0.19	0.10
Spinach	<i>Tetragonia expansa</i>	SP	2	0.02	0.13	0.48	1.05	0.46	0.27
Sweetcorn	<i>Zea Mays</i> L.	SP	2	0.34	0.08	0.07	0.16	0.32	nd
White Cabbage	<i>Brassica oleracea</i> L. var. <i>capitata</i>	- ^d	2	0.50	0.04	0.21	0.60	0.23	0.06
Purple Cabbage	<i>Brassica oleracea</i> L.	-	2	0.50	0.06	0.32	0.63	0.34	0.43
Rocket	<i>Eruca sativa</i> L.	SP	2	0.02	0.07	0.29	0.76	0.21	0.23
Parsley	<i>Petroselinum crispum</i> (Mill.) Nym.	SP	2	0.02	0.18	0.70	1.02	0.47	0.03
Celery	<i>Apium graveolens</i> L.	SP	2	0.02	0.05	0.19	0.47	0.17	0.14

^a n = number of collected samples amount. ^b mean value. ^c nd = below detection limit, Cr < 0.06 mg L⁻¹. ^d unknown origin.

Table 4 – Concentrations of heavy metals in green vegetables (fresh weight) consumed in the São Paulo State.

Vegetables	Scientific Name	State of Origin	n ^a	Consumption kg per year	Cd	Ni	Pb	Co	Cr
'Seca' Squash	<i>Cucurbita moschata</i>	SP	2	0.25	0.04 ^b	0.19	0.25	0.19	0.13
'Italian' Pumpkin	<i>Cucurbita pepo</i> L.	SP	2	0.25	0.04	0.20	0.30	0.18	0.08
'Japanese' Pumpkin	<i>Cucurbita pepo</i> L.	BA	2	0.25	0.16	0.24	0.90	0.62	0.60
'Moranga' Pumpkin	<i>Cucurbita maxima</i> Duch	SP	2	0.25	0.06	0.28	0.56	0.30	0.13
'Paulista' Squash	<i>Cucurbita moschata</i>	SP	2	0.25	0.05	0.21	0.50	0.24	0.13
'Italian' Zucchini	<i>Cucurbita pepo</i> L.	- ^e	2	0.31	0.04	0.25	0.51	0.16	0.08
'Paulista' Zucchini	<i>Cucurbita moschata</i>	SP	2	0.31	0.04	0.23	0.59	0.19	0.15
Yellow Sweet Potato	<i>Ipomoea batatas</i> L.	SP	3	0.13	0.11 (0.10)	0.15 (0.04)	0.43 (0.10)	0.49 (0.10)	nd ^c
White Sweet Potato	<i>Ipomoea batatas</i> L.	SE	3	0.13	0.14 (0.002)	0.18 (0.04)	0.46 (0.001)	0.62 (0.15)	0.04 (0.02)
Pink Sweet Potato	<i>Ipomoea batatas</i> L.	SP	5	0.13	0.12 (0.08)	0.26 (0.04)	0.52 (0.15)	0.55 (0.02)	nd
Eggplant	<i>Solanum melongena</i> L.	SP	2	0.38	0.04	0.13	0.44	0.20	0.16
Beet	<i>Beta vulgaris</i> L.	SP	3	0.44	0.09 (0.02)	0.26 (0.10)	0.58 (0.12)	0.33 (0.06)	0.21 (0.10)
Yam	<i>Dioscorea</i> spp.	SP	2	0.11	0.06	0.20	0.72	0.16	0.20
Carrot	<i>Daucus corota</i> L.	MG	3	2.09	0.03 (0.01)	0.11 (0.12)	0.38 (0.09)	0.15 (0.05)	0.09 (0.10)
Chayote	<i>Sechium edule</i> Sw.	ES	2	1.07	0.09	0.74	0.31	0.55	0.29
Jilo	<i>Solanum gilo</i> Raddi	SP	2	0.33	0.06	0.15	0.28	0.22	0.26
Cassava 1 ^d	<i>Manihot esculenta</i> Crantz.	SP	2	0.82	0.13	0.11	nd	0.62	0.28
Cassava 2	<i>Manihot esculenta</i> Crantz.	SP	2	0.82	0.10	0.63	0.98	0.80	0.11
Arracacha	<i>Arracacia xanthorrhiza</i> Banc.	SP	4	0.65	0.09 (0.05)	0.24 (0.15)	1.18 (0.12)	0.19 (0.05)	0.41 (0.13)
'Caipira' Cucumber	<i>Cucumis sativus</i> L.	-	2	0.16	0.02	0.25	0.30	0.09	0.05
'Comum' Cucumber	<i>Cucumis sativus</i> L.	GO	2	0.16	0.02	0.16	0.25	0.08	0.02
'Japanese' Cucumber	<i>Cucumis sativus</i> L.	SP	2	0.16	0.02	0.12	0.37	0.09	0.12
Green Pepper	<i>Capsicum annuum</i> L.	SP	2	0.28	0.04	0.12	0.29	0.10	0.12
Red Pepper	<i>Capsicum annuum</i> L.	SP	2	0.28	0.05	0.16	0.25	0.21	0.20
Okra	<i>Abelmoschus esculentus</i> (L.) Moench.	SP	5	0.50	0.08 (0.12)	0.48 (0.03)	1.31 (0.07)	0.36 (0.05)	0.21 (0.06)
'Andrea' Tomato cv.	<i>Lycopersicon esculentum</i> Mill.	MG	2	1.38	0.03	0.20	0.17	0.15	nd
'Caqui' Tomato cv.	<i>Lycopersicon esculentum</i> Mill.	SP	2	1.38	0.04	0.14	0.20	0.11	0.01
'Long Life' Tomato cv.	<i>Lycopersicon esculentum</i> Mill.	MG	2	1.38	0.03	0.11	0.21	0.12	0.04
'Rasteiro' Tomato cv.	<i>Lycopersicon esculentum</i> Mill.	SP	2	1.38	0.02	0.10	0.19	0.10	0.02
Green Bean	<i>Phaseolus vulgaris</i> L.	SP	4	0.33	0.07 (0.06)	0.19 (0.13)	0.75 (0.11)	0.30 (0.08)	0.15 (0.11)

^a n = number of collected samples. ^b mean value and coefficient of variation. ^c nd = below detection limit, Pb < 0.09 mg L⁻¹ and Cr < 0.06 mg L⁻¹. ^d cassava 1 and 2 = distinct precedence. ^e unknown origin.

Table 5 – Concentrations of heavy metals in "general" vegetables (fresh weight) consumed in the São Paulo State.

Vegetables	Scientific Name	State of Origin	n ^a	Consumption kg per year	Cd	Ni	Pb	Co	Cr
Chinese Garlic	<i>Allium sativum</i> L.	- ^e	3	0.30	0.12 (0.12) ^b	0.47 (0.10)	1.94 (0.08)	0.44 (0.04)	0.26 (0.07)
National Garlic	<i>Allium sativum</i> L.	MG	3	0.30	0.09 (0.09)	0.42 (0.01)	2.50 (0.14)	0.37 (0.16)	0.29 (0.04)
Rice	<i>Oryza sativa</i>	RS	3	27.27	nd ^c	0.20 (0.11)	0.23 (0.14)	0.18 (0.16)	nd
'Ágata' Potato cv.	<i>Solanum tuberosum</i> ssp.	SP	2	1.52	0.09	0.22	0.99	0.13	0.27
'Asterix' Potato cv.	<i>Solanum tuberosum</i> ssp.	SP	2	1.52	0.09	0.37	1.02	0.28	0.13
'Caesar' Potato cv.	<i>Solanum tuberosum</i> ssp.	SP	2	1.52	0.11	0.27	0.48	0.16	nd
'Monalisa' Potato cv.	<i>Solanum tuberosum</i> ssp.	SP	2	1.52	0.12	0.22	0.77	0.21	0.03
Onion	<i>Allium cepa</i> L.	MG	3	3.47	0.02 (0.04)	0.06 (0.13)	0.49 (0.12)	0.09 (0.10)	0.07 (0.11)
Bean 1 ^d	<i>Phaseolus vulgaris</i>	-	3	3.04	0.01 (0.09)	0.44 (0.01)	0.26 (0.12)	0.08 (0.09)	0.002 (0.16)
Bean 2	<i>Phaseolus vulgaris</i>	-	3	3.04	0.002 (0.11)	0.16 (0.07)	0.70 (0.08)	0.13 (0.12)	nd

^an = number of collected samples amount. ^bmean value and coefficient of variation. ^cnd = below detection limit, Cd < 0.01 mg L⁻¹ and Cr < 0.06 mg L⁻¹. ^dbean 1 and 2 = distinct precedence. ^eunknown origin.

Commission, 1995) for Cd ranges between 0.05 and 0.4 mg kg⁻¹ and for Pb between 0.1 and 0.3 mg kg⁻¹, depending on food type. The fruits previously described had Cd concentrations below the codex limit. Pb concentrations exceeded the codex limit, except for apples and 'hawai' pineapple. So, in general, Brazilian agricultural export products do not show problems on HM content. There are no reference values for other HMs in the codex.

Daily intake of heavy metals

Cadmium is a dangerous element because it can be absorbed via the alimentary tract, penetrate through placenta during pregnancy, and damage membranes and DNA. Once in the human body, it may remain in the metabolism from 16 to 33 years and is connected to several health problems, such as renal damages and abnormal urinary excretion of proteins. Decrease in bone calcium concentrations and increase of urinary excretion of calcium have also been attributed to exposure to Cd, eventually causing death. It also affects reproduction and endocrine systems of women (WHO, 2004). Vegetables may contribute to about 70 % of Cd intake by humans, varying according to the level of consumption (Wagner, 1993).

The daily intake of Cd was estimated as 0.008 mg, which represents approximately 10.7 % of the R_iD, established to 0.001 mg kg⁻¹ of body weight per day, equivalent to 0.07 mg per day for a 70 kg adult (WHO, 1993). The daily intake was lower than the tolerable daily intake (TDI). The content of Cd intake found was lower than that reported in literature, which ranged between 0.0018 and 0.052 mg per day (Santos et al., 2004; Tripathi et al., 1997). The greatest contribution for Cd intake came from the 'monalisa' potato (0.00049 mg per day), followed by the 'prata' banana (0.00046 mg per day), 'pêra' orange (0.00041 mg per day) and carrot (0.00032 mg per day), accounting for 22.6 % of the total daily intake.

Ni does not have a specific function in humans; however, it is a co-factor for some microbial intestine enzymes. Ni content in the adult human body should remain below 0.1 mg per day, and excess may cause damages to DNA and cell structures (Kohlmeier, 2003). The daily intake of Ni was estimated as 0.046 mg, which represents approximately 3.3 % of R_iD established in 0.02 mg kg⁻¹ per day, equivalent to 1.4 mg per day for a 70 kg adult (WHO, 1993). The daily intake was below the TDI of 1.4 mg per day and lower than that reported in literature (0.089 and 0.231 mg per day) (Biego et al., 1998; Santos et al., 2004). The greatest contribution for the Ni intake came from rice (0.015 mg per day) and bean 1 (0.0036 mg per day), accounting for 40.7 % of the total daily intake.

The toxic effects of Pb focus on several organs, such as liver, kidneys, spleen and lung, causing a variety of biochemical defects. The nervous system of infants and children is particularly affected by the toxicity of this HM. Adults exposed occupationally or accidentally

to excessive levels of Pb exhibit neuropathology. There is association between Pb in human body and the increase of blood pressure in adults (Maihara and Fávoro, 2006). Although Pb effects are more relevant for children, calculations for risk assessment were made for adults and children. The daily intake of Pb was estimated to 0.102 mg, which is 41.7 % of R_iD value of 0.245 mg per day for a 70 kg adult. Although Pb concentrations were high in some vegetables, low consumption of vegetables results in low intake of this element. This value was below those reported in literature (0.025 and 0.521 mg per day) (Santos et al., 2004; Tripathi et al., 1997). Rice contributed with approximately 16.84 % for the Pb intake, followed by the 'pêra' orange (6.68 %), bean 2 (5.73 %), 'prata' banana (5.52 %) and onion (4.59 %).

Co is a constituent of the B₁₂ vitamin. The estimated daily intake of Co was 0.040 mg, which represents 1.3 % of the R_iD established as 3 mg per day (Food and Nutrition Board, 2004). There are few data on Co intake. Data in the literature range between 0.012 and 1.2 mg per day (Biego et al., 1998; Chary et al., 2008), and the Co intake estimated in this work remained below these values. Rice was the major contributor for Co intake with 0.013 mg per day (33.98 %) followed by cassava 2 with 0.0018 mg per day (4.54 %).

Cr is an important element for the insulin activity and DNA transcription. However an intake below 0.02 mg per day could reduce cellular responses to insulin (Kohlmeier, 2003). The daily intake, estimated as 0.016 mg, was lower than the R_iD established at 1.5 mg kg⁻¹ per day (equivalent to 105 mg per day) (US EPA, 2010). This value was also lower than that recommended by the US National Council (NRC, 1989) for Cr³⁺: from 0.05 to 0.2 mg. The daily intake of Cr estimated in this work was under than that reported in literature, which ranges between 0.013 and 0.098 mg per day (Biego et al., 1998; Santos et al., 2004). The greatest contribution for Cr intake came from 'prata' banana (0.0014 mg per day), followed by 'nanica' banana (0.0013 mg per day) and carrot (0.0012 mg per day), accounting for 24.7 % of the total daily intake.

Although the HQ-based risk assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of the risk level due to exposure to pollutants (Chary et al., 2008). Many researchers consider the risk estimation method reliable (Chary et al., 2008; Khan et al., 2008; Wang et al., 2005) and it has been proven to be valid and useful. However, this HQ method considers only exposure to HMs via consumption of vegetables, without taking into account other vias like dermal contact, soil ingestion, and other factors such as the presence of agrochemicals and herbicide molecules.

HQs for individual vegetables were all below 1.0 for all HMs, regardless the population type. HQ for Cd, Ni, Pb and Co ranged from 0 to 0.0071; from 0 to 0.0107; from 0 to 0.0701 and from 0 to 0.0045 for adults and

Table 6 – Values of Hazard Quotients (HQ) and Hazard Index (HI).

Population	Cd	Ni	Pb	Co	Cr	HI
Adults	0.107	0.033	0.417	0.013	0.00015	0.57
Children	0.130	0.039	0.499	0.016	0.00018	0.68

from 0 to 0.0075; from 0 to 0.0114; from 0 to 0.0747 and from 0 to 0.0048 for children. Cr values were lower than 0.00001 for children and adults. When the consumption habit of adults and children in the São Paulo State were analyzed, HQs for all HMs were below 1.0 (Table 6). So, the consumption of these vegetables can be considered safe with no risk to human health.

The sequence of HQ for adults and children followed the decrescent order $Pb > Cd > Ni > Co > Cr$ (Table 6). The sequence was the same for both; however the HQ for children was higher than that for adults, as verified by other researchers (Huang et al., 2008).

High Pb concentrations observed in many vegetables, although they do not pose a risk to human health, may be attributed to crops located near roads of heavy traffic. The main sources of this element to humans are inhalation of airborne Pb from vehicle emissions and from direct atmospheric deposition on soils, water, and crops, constituting the gateway into the food chain (Baird, 2002).

When the hazard index exceeds 1.0, there is concern for potential health effects (Huang et al., 2008). Even though there was no apparent risk when each metal was analyzed individually, the potential risk could be multiplied when considering all HMs. HI for adults and children were 0.57 and 0.68, respectively (Table 6). Although HI was higher for children, neither population suffered from ingestion of vegetables contaminated with HMs.

The relative contributions of Cd, Ni, Pb and Co to the aggregated risk were 18.8 %, 5.7 %, 73.1 % and 2.3 % for adults and 19.1 %, 5.7 %, 73.4 % and 2.4 % for children, respectively. Cr content was minimal, only 0.03 %, which may be related to the high R_fD of Cr (1.5 mg kg⁻¹ per day). Huang et al. (2008), and Wang et al. (2005) also found minimal contribution of Cr to aggregated risks via consumption of wheat grains and vegetables in Kunshan and Tianjin, China.

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