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Heavy Metal Levels in Leafy Vegetables from Selected Markets in Guyana

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Abstract Leafy vegetables (*Brassica oleracea*, *Brassica chinensis*, *Basella alba*, and *Lactuca sativa*) from market sites of Regions 3 and 4 in Guyana were tested for Cd, Pb, Cu, Zn, Co, Ni, Mn, and Fe. Results indicate significant differences in elemental concentrations among the vegetables analyzed, with Cd, Co, and Mn exceeding the safe limits in all vegetables, and Pb and Fe exceeding this limit in some vegetables. Cu and Zn did not exceed the safe limits in any of the vegetables. *B. chinensis* contained higher levels of Cd, Pb, and Co than the other vegetables while *B. alba* contained the lowest levels of these elements. Concentrations of the other elements varied in the vegetables so that no specific trend was established. A high concentration of some metals in the vegetables analyzed may be attributable to their concentrations in the soils irrigated with mineral-rich water and fertilized with metal-rich compounds. A risk factor exists for consumption of the leafy vegetables.

Keywords: heavy metals, leafy vegetables, safe limit, risk factor, irrigation, fertilizer, metal-rich compounds

Introduction

With increasing use of agrochemicals to "enhance" crop yield, there is a paradoxical decline in the quality of food produced. Of particular concern is the heavy metal ingredients of these chemicals and the risks associated with consumption of foods contaminated by them. This is because these metals are non-biodegradable (Farooq *et al*., 2008), non-thermodegradable (Sharma *et al*., 2007b), have long biological half-lives (Arora *et al*., 2008) and therefore readily accumulate to toxic levels in organisms. They are ubiquitous in the environment as a result of both natural and anthropogenic activities. In agriculture, use of pesticides, fertilizers, and contaminated irrigation water are major sources of enrichment (Abdu, 2010; Alam *et al*., 2003).

Heavy metals may follow one of several routes into the human body: through inhalation of dust, consumption of contaminated drinking water, or

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direct consumption of plants grown in metal-contaminated soils (Chary *et al*., 2008, Ul Islam *et al*., 2007). The result is varying degrees of illness, depending on the duration of exposure to these metals and influx within the duration (Farooq *et al*., 2008).

Heavy metals have several debilitating effects even at very low concentrations. They have been reported to be mutagenic, neurotoxic, teratogenic, and carcinogenic. Cardiovascular diseases, renal dysfunction, bone disorders, digestion disorders, immune impairment, reproductive injury, and nervous disorders have all been attributed to metal toxicity in humans. Additionally, there have been reports of severe depletion of some essential nutrients in the body following consumption of heavy metal-contaminated foods (Al Jassir *et al*., 2005; Türkdoğan *et al*., 2002).

Pb and Cd are two of the most commonly occurring and commonly studied heavy metal contaminants because of their toxicity to human health and other organisms. However, metals such as Zn, Cu, Fe, Mn, and Co are essential for the growth and development of plants and other organisms, including humans. (Sharma *et al*., 2009; Zheng *et al*., 2007). Plants are actually the primary source of metals in human diet. This is because they are able to bioaccumulate the cationic and anionic forms of noxious heavy metals from the soil through hyperaccumulation, which is as a result of vacuolar compartmentalization and chelation, simultaneously during the process of accumulating necessary nutrients such as N, P, and K.

Hyperaccumulaters contain an internal hypertolerance mechanism to resist the cytotoxic burden of accumulated metals as well as a powerful scavenging mechanism for the efficient uptake of these potentially toxic elements from the soil, via the xylem, to the leaves. However, metals may also be accumulated by plants through their foliar surfaces (Farooq *et al*., 2008). They may deposit on the surface of the plant (adsorption) or penetrate plant cells (absorption). Following uptake, heavy metals are usually partitioned, with accumulation of greater concentrations in leaves and roots than in fruits (Sharma *et al*., 2008). Despite these mechanisms, metal uptake and bioaccumulation by plants depend on several factors including climate, atmospheric

depositions, plant species, degree of maturity of plant species, concentration of metals in the soil, solubility of metals, soil pH, presence of fertilizers or pesticides in the soil, other soil conditions (Sharma *et al*., 2007a; Cobb *et al*., 1999).

Nutritionists often encourage and recommend consumption of more fruits and vegetables in the diet due to their immense health benefits. They are rich sources of nutrients as well as suppliers of important functional food components such as carbohydrates, proteins, vitamins, and minerals (Chary *et al*., 2007). They also act as buffering agents for acid substances obtained during the digestion process. According to Cobb *et al*. (1999), a recommended consumption of vegetables is 3-5 servings per day. However, this may be risky if the potential toxicity, persistent nature, and cumulative behaviour of heavy metals are taken into consideration.

Leafy vegetables are popular in Guyanese cuisine; they are a constituent of at least one daily meal. In spite of this, investigations concerning the quality assurance of this food group are practically negligible, especially as it relates to heavy metal contaminants. Such investigations are therefore much needed as the extensive use of agrochemicals is a popular practice in Guyana;

subsequent analyses of results will help to ensure that the level of any ensuing metal contaminant meets agreed international limits. This study presents data on the levels of Pb, Cd, Zn, Cu, Ni, Co, Mn, and Fe in selected leafy vegetables sold at two local markets in Guyana.

Materials and methods

Collection of samples: Sharma *et al*., 2008 reported that leafy vegetables accumulate heavy metals in greater concentrations than other vegetables. As such, samples were primarily leafy vegetables (*Lactuca sativa* [lettuce], *Brassica oleracea* [cabbage], *Basella alba* [thick-leaf calalloo], and *Brassica chinensis* [pak-choi]). These were randomly collected, in triplicates, from different vendors at each location (Bourda Market and Stabroek Market in Region 4, and Parika Market in Region 3) during 2012.

Pretreatment

Washing of samples: The collected vegetable samples were washed with distilled water to remove dust particles and to simulate the human intake conditions.

***Drying of samples*:** The leaves of the vegetable samples were removed from the stems and air-dried for one day. They were then chopped into small pieces using a knife and further dried in an oven at 70 ℃ for 3-4 days.

Grinding of samples: Dried samples of the vegetables were ground into a fine powder using a mortar and pestle. Powdered samples were stored in polyethylene bags (Ziploc bags), until used for acid digestion.

Heavy Metal Analysis

***Preparation of samples / wet digestion*:** Samples were transported to the Central Laboratory of the Guyana Sugar Corporation (GuySuCo), at LBI, East Coast Demerara. 1g of dried vegetable sample was weighed and placed in a digestion flask containing a mixture of 20mL concentrated HNO_3 and 20mL concentrated H_2SO_4. A blank sample was prepared applying the same acid mixture (without the vegetable sample) into an empty digestion flask. The mixture was allowed to boil at 80-90 ℃ initially and then to 150 ℃, until a clean solution was obtained. After cooling, the solution was filtered with Whatman No.42 filter paper. 5mL of each the resulting solution was then transferred to a 100mL volumetric flask. This was repeated for each replicate of each dried vegetable sample.

An Atomic Absorption Spectrophotometer (AAS, model 220) was used to determine levels of Pb, Cd, Cu, Zn, Co, Ni, Mn, and Fe in the digested solutions. Working standard solutions of each element were prepared from stock standard solutions. This was done to ensure accuracy and that the analytical values were within the range of certified value.

Statistical Analysis: Three samples of each vegetable were assayed and analyzed individually. Data was reported as mean $\pm$ SD. Two-Way ANOVA with Replication was used to determine significant difference between groups, considering a level of significance of less than 5% (p<0.05). Least Significant.

Difference (LSD) Pairwise Comparison was used to compare individual means within each group. Additionally, mean levels of metals were correlated with each other for the different vegetables. All the statistical analyses were computed with Stastix 8 software and Microsoft Excel.

Results and discussion

Farming is the main livelihood for persons in Region 3. Farmers engage in agricultural practices such as crop rotation, which usually require longer periods of time in order for the desired effects to manifest. Farmers in Region 4, on the other hand, engage in more haphazard crop sessions because farming is not their main livelihood. This is due to many factors including limited land space/availability for farming, unsuitable soil pH, and the scope for employment in more demanding professions, among others. As such, farmers tend to use a lot of fertilizers on their crops to stimulate faster growth rates over a short period of time in order to reap the benefits of their venture before engaging themselves in other viable activities.

Heavy metals are included in many fertilizers and pesticides as micronutritional or biocidal components; they may be otherwise present as naturally occurring contaminants. Phosphate fertilizer is the main source of soil pollution by heavy metals, especially Cd. This is because Cd is naturally found as an impurity in phosphate rocks (Alam *et al*., 2003). However, Cd is a non-essential element, in both plants and animals. Its accumulation in plants alters mineral nutrients uptake, inhibits stomatal opening, disturbs the Calvin cycle enzymes, photosynthesis, carbohydrate metabolism, changes the antioxidant metabolism, and lowers crop productivity (Nazar *et al*., 2012). In humans, Cd irreversibly accumulates in the liver, kidneys, and lungs (Sobukula *et al*., 2010). The liver and kidneys synthesize metallothionein, a Cd-inducible protein that protects the cells by tightly binding the toxic Cd ion. However, long-term intake of this metal may cause renal, prostate, and ovarian cancers (Türkdoğan *et al*., 2002).

As it relates to the more toxic metals (Pb and Cd), *B.chinensis* was found to contain greater concentrations than the other vegetables (Table 1). Additionally, a consistent trend of relative abundance of these metals was seen in vegetables from both locations. Keilig and Ludwig-Müller (2009) suggested that such occurrences may be due to quercetin, a type of phenolic compound that exhibits metal-binding activities. Studies (Cartea *et al.*, 2011, Olajire and Azeez, 2011, Farooq *et al*., 2008) have shown that *B.oleracea* has more (7 of 17) quercetin compounds than *B.chinensis* while *L.sativa* and *B.alba* have more of these metal-binding compounds than *B.oleracea*.

No consistent trend was found for the other metals, probably because these are all essential microelements whose concentrations are affected by a variety of abiotic and biotic factors. For instance, plants use these elements by converting them to other forms; hence this affects their levels within the plant. One-time sampling may thus inaccurately reflect their levels in a given species, especially since different species have different nutrient requirements.

Additionally, the more toxic elements have the tendency to compete with the microelements for uptake by plants. However, in high enough concentrations, these microelements have the tendency to outcompete uptake of toxic metals.

Two-way factor ANOVA with replication comparisons revealed that differences between levels of metals in the different vegetables sampled, as well as between levels of each metal, are significantly different (Table 2). Further LSD pairwise comparison (Tables 3 and 4) shows specific significant differences between sampled vegetables, particularly for levels of Cd, Pb, and Co.

Cd concentration exceeded the safe limit, as given by FAO/WHO (2007), in all of the vegetables from both regions. Al Jassir *et al*. (2005) reported that leafy vegetables accumulate Cd from soil much more efficiently than any other heavy metals. However, vegetables from Region 4 generally had higher mean levels of Cd, Pb, Zn, Cu, and Co. Pb in the soil comes from deposition of atmospheric Pb (from automobile emissions and industrial exhaust), Pb-containing pesticides and fertilizers, and sewage sludge (Al Jassir *et al*., 2005). Exposure to Pb is of concern mainly because of its possible detrimental effects on intelligence, reproduction, gastrointestines, and immunity (Alam *et al*., 2003).

Pb levels in *B.oleracea* and *B.chinensis* collected from both regions exceeded the safe limit but this was not the case in *L.sativa* and *B.alba*. This may be attributed to the quercetin content of each vegetable and, in particular, the lack of these compounds in *B.chinensis* hence the high Pb levels. Hu and Ding (2009) reported that *B.chinensis* accumulates more Pb than *B.alba*. Additionally, the Pb levels in *B.oleracea* do not significantly exceed the safe limit. Sharma and Dubey (2005) found that when Pb is taken up from the soil by plants, it remains mostly in the root region since it cannot effectively pass through endodermis of roots. Leaves, however, absorb great quantities of Pb from the atmosphere. It is possible that *B.oleracea* and *B.chinensis* are able to effectively accumulate Pb from the atmosphere through their leaves as well as from the soil (Pb is able to move up the plant via the symplastic route in some instances) while the roots and leaves of *L.sativa* and *B.alba* offer a little more resistance to its uptake.

Pb and Cd are among the most abundant, particularly toxic, heavy metals in the environment (Radwan and Salama, 2006) and are also the most significant heavy metals affecting vegetable crops (Kachenko and Singh, 2000). Excess Pb in plants causes stunted growth, chlorosis, and blackening of the root system (Sharma and Dubey, 2005) while excess Cd results in substitution for Zn and Fe in cellular metabolism (Verbruggen *et al*., 2009).

Among all heavy metals, Zn is the least toxic and is important for normal growth and development in both plants and animals. It is an essential component of many enzymes. Cu is also an essential micronutrient which functions as a biocatalyst and is interrelated with the functions of Zn and Fe in organisms. In humans, it is required for body pigmentation, and helps to maintain a healthy central nervous system and prevent anaemia (Sobukola *et al*., 2010). Like Zn, Cu is essential for proper enzyme activity in plants. Although Zn and Cu are essential elements, slight increases in their levels (beyond the safe limits) may interfere with physiological processes (Al Jassir *et al*., 2005).

Zn levels in *B.alba* from Region 3 and all of the vegetables from Region 4, except *B.oleracea*, exceeded the safe limit. In Region 4, Zn levels in all of the samples were not significantly different from each other whereas in Region 3, levels in *B.alba* were significantly different from those in *B.oleracea* and *B.chinensis*. Zn levels in *L.sativa* were not significantly different from the other vegetables. The interaction between Zn and Cd may be biphasic, that is, antagonistic or synergistic (Kalavrouziotis *et al*., 2009). An antagonistic interaction between these two elements is evident from the correlation analyses for two of the vegetables (Table 5).

Cd and Zn have similar nuclear structure and ionic radius so that they affect nucleic acid metabolism in the same manner. However, Cd is easier to transfer from the soil to edible parts of plants because of its ability to immediately bind to enzymes upon entry into plant cells, unlike Zn (Zheng *et al*., 2007). Sufficient Zn is essential to neutralize the toxic effects of Cd (Radwan and Salama, 2006). However, excess Zn in plants may lead to Fe deficiency, severe stunting, and chlorosis. Türkdoğan *et al*. (2002) reported that elevated levels of Zn may result in damage to the pancreas, disruption of protein metabolism, and arteriosclerosis in humans.

Presence of excess amounts of Cu in plants can reduce Zn availability because absorption of both cations is through the same mechanism. However, most plants contain the amount of Cu which is inadequate for normal growth and which is usually ensured through the use of synthetic or organic fertilizers (Itanna, 2002). This is reflected in the results of this study since all of the vegetables had levels of Cu which were lower than the safe limit. *L.sativa* was generally richer in Cu while *B.oleracea* had low levels of this element. This is corroborated by Farooq *et al*. (2008). From Tables 3 and 4, it is evident that the differences in Cu levels in samples from Region 3 were not significant whereas in Region 4, Cu levels in *B.oleracea* were significantly different from *L.sativa* and *B chinensis* and the levels in *B.alba* were not very different from those in the other vegetables.

Co promotes many growth processes in plants, including stem, coleoptiles, and hypocotyl elongation,

leaf expansion, and bud development (Grover and Purves, 1976). It is an essential component of several enzymes and coenzymes. High level of Co induces Fe deficiency and suppresses Cd uptake by roots. It also interacts synergistically with Zn and antagonistically with Ni (Palit *et al*., 1994). According to Tables 3 and 4, Co levels exceeded the safe limit in all of the samples from both regions. However, this is of little concern in human nutrition since Co is an integral component of vitamin B_{12} and is involved in preventing and treating pernicious anaemia, red blood cell production, and the maintenance of normal nervous system functions (Sobukola *et al*., 2010).

Differences in Co levels among the vegetables were significant. Kalavrouziotis *et al*. (2009) reported that leafy vegetables may accumulate significant quantities of Co. Co has been found to interact with many elements. It has a biphasic relationship with Cd. Correlation analyses for three of the four vegetables suggest a synergistic interaction between these two elements:

Ni also plays some roles in body functions including enzyme functions (Sobukola *et al*., 2010). It is not an essential element in plant nutrition and was not detected in any of the samples in this study.

Mn and Fe are closely related from a chemical standpoint. Even though they have many properties in common and enter into many similar reactions, Mn cannot play Fe's role in chlorophyll production. Mn, which is found exclusively in the veins of plants, has been found to enhance growth; it enhances the oxygen-carrying power of oxidizing enzymes such as oxidases and peroxidases. In large amounts, it causes necrosis of root tips, chlorosis and subsequent inhibition of carbohydrate production (Kelley, 1912). In humans, Mn is associated with bone development, and with amino acid, lipid, and carbohydrate metabolism but it is toxic in excess; it can cause manganism, which shares many common features with Parkinson's disease.

Fe is an essential element for all organisms because it is a cofactor for fundamental biochemical activities such as energy metabolism, oxygen transport, and DNA synthesis. Therefore, both a deficiency and an excess have strong impacts on growth. Fe is found in the mitochondria and chloroplasts of plant cells and is a crucial component of many of the compounds that are involved in the processes occurring in these two organelles. Even though Fe is present at high concentrations in soils, its bioavailability to plants is usually very low and therefore Fe deficiency is a common problem. A deficiency causes chlorosis and decreased root tip growth. Plants use various iron uptake mechanisms to combat this problem including the use of siderophores and release of protons to lower surrounding soil pH (thereby increasing Fe solubility).

In this study, Mn and Fe levels were well above the safe limits in all samples. Generally, *B.alba* and *B.chinensis* had extremely high levels of both elements while *L.sativa* and *B.oleracea* had lower levels (still above the safe limit). This difference is very evident and significant, in the case of Fe, in samples from Region 3. In Region 4, Fe levels in *B.oleracea* were significantly different from those of other vegetables; the levels in *L.sativa* were not very different from those of *B.alba* and *B.chinensis*.

In samples from Region 4, the difference in Mn levels among the samples were not significant while in Region 3, Mn levels in *B.alba* differed significantly from the other vegetables. The availability of Mn in the soil is directly related to pH, as with all of the other metals. The lower the pH, the higher the availability of Mn, as well as Fe. Soil pH of <5.0 causes toxicity conditions in sensitive crops. From this, it can be deduced that the soils in which the sample crops were grown are acidic in nature, particularly for *L.sativa* from Region 4 and *B.alba* from both regions (Tables 3 and 4).

Additionally, the tap water in Guyana, which is commonly used for irrigation of crops, contains high levels of minerals such as Mn and Fe.

Table 7 shows a synergistic interaction between Mn and Fe in *B.oleracea* and *B.chinensis*. Somers and Shive (1942) reported that Mn determines the state of oxidation of Fe in plants and that optimum growth of plants is obtained when a ratio of approximately 1:2.5 (Fe to Mn) occurs. Symptoms manifested with excess Fe are identical with those manifested when Mn is deficient and vice versa. Mn, in high concentration, oxidizes ferrous Fe (Fe^{2+}) to ferric Fe (Fe^{3+}), which is the inactive form. This is reflected in *L.sativa*, which shows an antagonistic interaction between the two elements.

In humans, Fe is required for protein synthesis and development. It is a major component of Fe-haeme proteins such as haemoglobin, Fe-sulphur enzymes such as fumarate reductase, proteins for Fe storage and transport (such as transferrin and ferritin), and other Fe-containing or Fe-activated enzymes such as NADH dehydrogenase and succinate. One of the most serious forms of Fe overload is acute Fe poisoning (Fraga, 2005).

Mn, on the other hand, also plays many essential roles in human nutrition. It is largely located in the mitochondria, where it helps to activate enzymes needed for the use of biotin, thiamin, and vitamin C, and is a constituent of some enzymes. It is also important for the formation of thyroxine and is essential to proper digestion and metabolization of proteins, reproduction, normal bone structure, and normal functioning of the central nervous system. The highest concentrations of Mn are found in the liver, thyroid, pituitary, pancreas, kidneys, and bone (Watts, 1990).

Table 1. Relative abundance of metals in leafy vegetables

Metals	Relative Abundance	
	Region 3	Region 4
Pb	PAK>CAB>LET>POI	PAK>CAB>LET>POI
Cd	PAK>CAB>LET>POI	PAK>CAB>LET>POI
Zn	POI>LET>CAB>PAK	LET>PAK>POI>CAB
Cu	LET>POI>PAK>CAB	LET>PAK>POI>CAB
Co	PAK>CAB>LET>POI	PAK>CAB>LET>POI
Mn	POI>PAK>CAB>LET	LET>POI>PAK>CAB
Fe	POI>PAK>LET>CAB	PAK>POI>LET>CAB

PAK = *B. chinensis* LET = *L. sativa*
CAB = *B. oleracea* POI = *B. alba*

Table 2. Two-Way ANOVA with Replication comparison of metals in leafy vegetables (p<0.05)

Comparison	*F* Value	*P Value*	*F* Crit	Significance

Between leafy vegetables of Region 3	15.571	$1.75E^{-07}$	2.769	Significant
Between different metals in samples (Region 3)	65.913	$1.4E^{-23}$	2.266	Significant
Between leafy vegetables of Region 4	4.314	0.008	2.769	Significant
Between different metals in samples (Region 4)	14.993	$3.87E^{-10}$	2.266	Significant

Table 3. Mean levels (ppm) and LSD Pairwise Comparison of Pb, Cd, Zn, Cu, Co, Ni, Mn, and Fe of selected leafy vegetables from Region 3

Vegetables	% H_2O Content	Pb	Cd	Zn	Cu	Co	Ni	Mn	Fe
B.oleracea	92.5	5.54 ± 0.41 ^{A}	4.16 ± 0.19 ^{B}	40.08 ± 19.58 ^{B}	18.31 ± 2.31 ^{A}	13.00 ± 0.49 ^{B}	ND *	25.93 ± 11.40 ^{B}	42.82 ± 14.51 ^{B}
B. alba	92.7	0.81 ± 1.08 ^{C}	1.67 ± 0.39 ^{D}	67.13 ± 6.21 ^{A}	27.61 ± 3.80 ^{A}	8.69 ± 0.68 ^{D}	ND *	91.35 ± 38.05 ^{A}	291.24 ± 113.02 ^{A}
L.sativa	94.0	2.76 ± 0.46 ^{B}	3.15 ± 0.19 ^{C}	46.64 ± 2.71 ^{AB}	28.44 ± 20.73 ^{A}	11.77 ± 0.33 ^{C}	ND *	22.06 ± 11.62 ^{B}	108.15 ± 12.03 ^{B}
B.chinensis	89.0	6.83 ± 1.19 ^{A}	5.25 ± 0.21 ^{A}	36.11 ± 5.93 ^{B}	20.72 ± 1.46 ^{A}	15.86 ± 0.48 ^{A}	ND *	42.73 ± 13.07 ^{B}	238.40 ± 39.43 ^{A}
Safe Limit ^{a}		5.00	0.20	60.00	40.00	0.50	–	6.70	5.00

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually
Mean values in the same column followed by the same superscript letters are not significantly different (p>0.05), Pb = Lead; Cd = Cadmium; Zn = Zinc; Cu = Copper; Co = Cobalt; Ni = Nickel Mn = Manganese; Fe = Iron, ^{a}FAO/WHO standard (2007); Joint Codex Alimentarius Commission; FAO/WHO has not set a safe limit for Ni., Not detected; levels were below the detection limit

Table 4. Mean levels (ppm) and LSD Pairwise Comparison of Pb, Cd, Zn, Cu, Co, Ni, Mn, and Fe of selected leafy vegetables from Region 4

Vegetables	% H_2O Content	Pb	Cd	Zn	Cu	Co	Ni	Mn	Fe
B.oleracea	90.8	5.09 ± 0.92 ^{B}	4.66 ± 0.16 ^{B}	49.75 ± 23.95 ^{A}	13.71 ± 3.11 ^{B}	14.45 ± 0.66 ^{B}	ND *	21.07 ± 9.25 ^{A}	42.49 ± 9.00 ^{D}
B. alba	92.3	2.01 ± 0.67 ^{C}	2.53 ± 0.21 ^{D}	77.64 ± 53.37 ^{A}	20.78 ± 1.42 ^{AB}	9.80 ± 0.47 ^{D}	ND *	152.49 ± 83.14 ^{A}	176.94 ± 73.84 ^{B}
L.sativa	94.3	3.17 ± 1.16 ^{BC}	3.84 ± 0.25 ^{C}	103.85 ± 98.34 ^{A}	29.63 ± 7.49 ^{A}	13.02 ± 0.35 ^{C}	ND *	294.18 ± 242.10 ^{A}	115.85 ± 34.10 ^{BC}
B.chinensis	90.8	7.92 ± 1.32 ^{A}	5.89 ± 0.11 ^{A}	95.05 ± 52.33 ^{A}	27.07 ± 6.55 ^{A}	17.39 ± 0.38 ^{A}	ND *	30.21 ± 18.01 ^{A}	298.91 ± 34.64 ^{A}
Safe Limit ^{a}		5.00	0.20	60.00	40.00	0.50	–	6.70	5.00

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually
Mean values in the same column followed by the same superscript letters are not significantly different (p>0.05), Pb = Lead; Cd = Cadmium; Zn = Zinc; Cu = Copper; Co = Cobalt; Ni = Nickel Mn =

Manganese; Fe = Iron, [a] FAO/WHO standard (2007); Joint Codex Alimentarius Commission; FAO/WHO has not set a safe limit for Ni., [*]Not detected; levels were below the detection limit

Table 5. Correlation of Cd * Zn

Vegetable	**Region 3**	**Region 4**
B. alba	-0.999	-0.787
B. chinensis	-0.935	-0.756

Table 6. Correlation of Co * Cd

Vegetable	Region 3	Region 4
B. alba	0.988	0.952
B. oleracea	0.992	0.879
L. sativa	0.760	0.648
B. chinensis	0.990	-0.554

Table 7. Correlation of Mn * Fe

Vegetable	Region 3	Region 4
B. alba	0.763	-0.912
B. oleracea	0.763	0.912
L. sativa	-0.661	-0.974
B. chinensis	1.000	0.557

Conclusion

The results from this study suggest that significant differences exist in the elemental concentrations among leafy vegetables analyzed and this may be due, in part, to the two different geographical locations from which samples were obtained, and also the concentration of phenolic compounds within leaves of each sampled species of plant. Cd, Co, and Mn levels exceeded the daily intake limits, as well as Pb and Fe in most instances, while Zn and Cu levels were below the respective limits. As such, there exists a risk factor for consumption of the leafy vegetables which were sampled in this investigation.

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References

Al Jassir, M. S., Shaker, A. and Khaliq, M. A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabia. Bulletin of Environmental Contamination and Toxicology 75:1020-1027.

Alam, M. G. M., Snow, E. T. and Tanaka, A. (2003). Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. The Science of the Total Environment 308:83-96.

Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chemistry 111:811-815.

Cartea, M., Francisco, M., Soengas, P. and Velasco, P. (2011). Phenolic compounds in *Brassica* vegetables. Molecules 16:251-280.

Chary, S., Kamala, C., Samuel, D. and Raj, S. (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicology and Environmental Safety 69:513-524.

Cobb, G., Sands, K., Waters, M., Wixson, B. and Dorward-King, E. (2000). Accumulation of heavy metals by vegetables grown in mine wastes. Environmental Toxicology & Chemistry 19:600-607.

Farooq, M., Anwar, F. and Rashid, U. (2008). Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrial area. Pakistan Journal of Botany 40:2099-2106.

Fraga, C. (2005). Relevance, essentiality and toxicity of trace elements in human health. Molecular Aspects of Medicine 26:235-244.

Grover, S. and Purves, W. (1976). Cobalt and plant development, Plant Physiology 57:886-889.

Harmanescu M, Alda L, Bordean D, Gogoasa I, Gergen I. (2011). Heavy metals risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. Chemistry Central Journal 5:1-10.

Hu, X. and Ding, Z. (2009). Lead/cadmium contamination and lead isotopic ratios in vegetables grown in peri-urban and mining/smelting contaminated sites in Nanjing, China. Bulletin of Environmental Contamination and Toxicology 82:80-84.

Itanna, F. (2002). Metals in leafy vegetables grown in Addis Ababa and toxicological implications. Ethiopian Journal of Health Development 16:295-302.

Kachenko, G. and Singh, B. (2006). Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. Water, Air, and Soil Pollution 169:101-123.

Kalavrouziotis, I. K., Koukoulakis, P. H., Manouris, G. and Papadopoulos, A. H. (2009). Interactions between cadmium, lead, cobalt, and nickel in broccoli, irrigated with treated municipal wastewater. European Water 25-26:13-23.

Keilig, K. and Ludwig-Müller, J. (2009). Effect of flavonoids on heavy metal tolerance in *Arabidopsis thaliana* seedlings. Botanical Studies 50:311-318.

Kelley, W. (1912). The function and distribution of manganese in plants and soils. Hawaii Agricultural Experiment Station, Honolulu. Bulletin No. 26:7-41.

Nazar, R., Iqbal, N., Masood, A., Iqbal, M., Khan, R., Syeed, S. and Khan, N. (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. American Journal of Plant Sciences 3:1476-1489.

Olajire, A. A. and Azeez, L. (2011). Total antioxidant activity, phenolic, flavonoid, and ascorbic acid contents of Nigerian vegetables. African Journal of Food Science and Technology 2:22-29.

Palit, S., Sharma, A. and Talukdar, G. (1994). Effects of cobalt on plants. The Botanical Review 60:149-181.

Radwan, M. and Salama, A. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology 44:1273-1278.

Sharma, P. and Dubey, R. (2005). Lead toxicity in plants. Brazilian Journal of Plant Physiology 17:35-52.

Sharma, R., Agrawal, M. and Marshall, F. (2007a). Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology 47:583-591.

Sharma, R., Agrawal, M. and Marshall, F. (2007b). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicology and Environmental Safety 66:258-266.

Sharma, R., Agrawal, M. and Marshall, F. (2008). Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. Environmental Pollution 154:254-263.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A. and Kajihausa, O. E. (2010). Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. African Journal of Food Science 4:389-392.

Somers, I. and Shive, J. (1942). The iron-manganese relation in plant metabolism. Plant Physiology 17:582-602.

Türkdoğan, M. K., Kilicel, F., Kara, K., Tuncer, I. and Uygan, I. (2002). Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environmental Toxicology and Pharmacology 13:175-179.

Ul Islam, E., Yang, X., He, Z. and Mahmood, Q. (2007). Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. Journals of Zhejiang University-Science B. 8:1-13.

Verbruggen, N., Hermans, C. and Schat, H. (2009). Mechanisms to cope with arsenic or cadmium excess in plants. Current Opinion in Plant Biology 12:1-9.

Watts, D. (1990). The nutritional relationships of manganese. Journal of Orthomolecular Medicine 5:219-222.

Zheng, N., Wang, Q. and Zheng, D. (2007). Health risk of Hg, Pb, Cd, Zn, and Cu to the inhabitants around Huludao zinc plant in China via consumption of vegetables. The Science of the Total Environment 383:81-89.

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