HEAVY METAL CONTAMINATION IN GREEN LEAFY VEGETABLES COLLECTED FROM DIFFERENT MARKET SITES OF KATHMANDU AND THEIR ASSOCIATED HEALTH RISKS

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Abstract: The present study was carried out to assess contamination of five heavy metals (As, Cd, Cr, Pb and Zn) in five different types of green leafy vegetables *viz.*, mustard (*Brassica campestris*), garden crees (*Lepidium sativum*), fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*), and spinach (*Spinacea oleracea*) collected from different market sites of Kathmandu. Atomic absorption spectrometer was used to determine the concentrations of these metals in a total of 45 test vegetables. Results showed a substantial accumulation of heavy metals in roots and leafy shoots of the vegetables. The range of various metals was 0.9-12.0, 2.7-12.5 and 16.8-158.7 mg kg^{-1} in roots and 3.2-19.1, 5.3-32.9 and 23.9-223.1 mg kg^{-1} in leafy shoots of the test vegetables for Cd, Pb and Zn respectively. However, the levels of As and Cr were found below the detection limits. Results revealed that the average concentrations of all metals (both leafy shoots and roots together) in the vegetables were found in order of their abundance as Zn>Pb>Cd. It was also found that Pb and Cd levels exceeded the maximum permissible limits set by FAO/WHO for human consumption. Daily intake of heavy metals through consumption of the vegetables was also investigated. From the present study, we conclude that a monitoring plan and a health risk assessment are necessary to evaluate the levels of metal concentration in vegetables in order to develop the proper measures for reducing excessive build-up of these metals in the food chain.

Keywords: Heavy metals; Vegetables; Daily intake; Kathmandu.

INTRODUCTION

Vegetables are common diet taken by populations throughout the world, being sources of essential nutrients, antioxidants and metabolites.[1] They also act as buffering agents for acid substance obtained during the digestion process. However, both essential and toxic elements are present in vegetables over a wide range of concentrations as they are said to be good absorber of metals from the soil.[2,3] Reports have shown that, vegetables grown in heavy metal rich soils are also contaminated.[4,5] Vegetables absorb these metals from contaminated soils as well as from polluted environmental deposits through the roots and incorporate them into the edible part of plant tissues or deposit on the surface of vegetables.[6,7] Some heavy metals such as Cr, Mn, Ni, Zn, Cu, and Fe are considered essential components for biological activities in the body; however, their presence in elevated levels is reported to cause problem to human.[2] On the other hand, Pb, Cd, Hg and As are non essential and play toxic role to living organism and hence are considered as toxic elements.

A number of factors influence the concentration of heavy metals on and within plants. These factors include climate, atmospheric deposition, the nature of soil on which the plant is grown, application of fertilizers and irrigation with wastewater.[8,9] The water of rivers can be polluted by heavy metals such as Pb, Cu, Zn, Fe, Cr, Cd, Hg etc. The major sources of heavy metals are industrial effluents and indiscriminate disposal of domestic or sewage drainage directed to the rivers untreated or partially treated.[10] Vegetables are contaminated with heavy metals as reports by Bahemuka and Mubofu[11] revealed metal contamination from samples collected in regions within the proximity of River Msimbazi.

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Othman[12] analyzed vegetables, from Ukonga, Kiwalani, Tabata, Buguruni and Sinza in Dares Salaam, which were cultivated along highways. In both studies concentrations of Cd, Pb, Zn, Cr and Ni were above the permissible limits. Sharma and Chettri[13] reported contamination of heavy metals in agricultural soils and vegetables grown along the riverside of Kathmandu valley. In suburban areas, the use of industrial or municipal wastewater is common practice in many parts of the world[5] and Kathmandu city is no exception. Most of the vegetable farms are situated either along the bank of polluted rivers, roadsides or highways which might be receiving deposits of metals from vehicle emissions. Besides, due to the population increasing as well as rapid development of transport infrastructure, agriculture and industry, heavy meals such as Cu, Zn, Cd, Pb, Cr, As and Hg are emitted into environment in large quantities through atmospheric deposition, solid waste emissions and wastewater irrigation. It is therefore anticipated that the most consumed green leafy vegetables such as mustard, garden crees, coriander, fennel and spinach, marketed in Kathmandu grown either in the valley itself or neighbouring areas are contaminated with heavy metals. It is also because vegetable farming includes application of manure and chemical fertilizers, which are reported to be a source of metals in food.[14,15] Hence, the aim of the present study was to determine the concentrations of heavy metals in selected green leafy vegetables collected from different market sites of Kathmandu and to estimate the daily intake of the metals.

MATERIALS AND METHODS

Study sites and vegetable sampling

Five green leafy vegetables like mustard (*Brassica campestris*), garden crees (*Lepidium sativum*), fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*) and spinach (*Spinacea oleracea*) were sampled from nine different vegetable markets *viz.,* Kalimati (S_1), Ason (S_2), Kasthamandap (S_3), Balkhu (S_4), Gongabu (S_5), Chabahil (S_6), Tinkune (S_7), Baneshwor (S_8) and Thapathali (S_9) in Kathmandu; the details of the test vegetables are given in Table 1. A total of 45 samples (each five vegetable types from the nine different market sites) were collected over a period of four months during the dry season (September-December). All the collected samples were separately packed up in polythene bags and brought to the laboratory for sample treatment and analysis.

Sample treatment

The samples thus collected were washed with clean tap water to remove the soil particles adhered to the surface of the vegetables. Then, the root and leafy shoot stalks of each sample were separated and both of these portions were sliced into pieces and dried separately on a sheet of paper to eliminate excess of moisture contents. Once air dried, each sample was weighed and further oven dried at 60 °C till constant weight. The dried samples were grinded and passed through a sieve of 2 mm size. Finally, the samples were stored at room temperature in clean and dry polyethylene bottles with screw caps for further analysis. Prior to sample storage, all bottles were pre-washed with nitric acid, rinsed with deionized water, dried and tested for contamination by leaching with 5% nitric acid.

Table 1: Vegetable samples collected from the market sites of Kathmandu

S.No.	Commonname	Vernacular name	Botanicalname	*Market sites
1	Mustard	Tori	*Brassica campestris*	S_1-S_9
2	Garden crees	Chamsur	*Lepidium sativum*	S_1-S_9
3	Fennel	Saunf	*Foeniculum vulgare*	S_1-S_9
4	Coriander	Dhania	*Coriandrum sativum*	S_1-S_9
5	Spinach	Palung	*Spinacea oleracea*	S_1-S_9

* Kalimati (S_1), Ason (S_2), Kasthamandap (S_3), Balkhu (S_4), Gongabu (S_5), Chabahil (S_6), Tinkune (S_7), Baneshwor (S_8) and Thapathali (S_9)

Sample digestion and analysis of heavy metals

Vegetable samples (1 g) were digested after adding 15 ml of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80 °C until a transparent solution was obtained.[16] After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. The samples were analyzed by atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA) using a nitrous oxide-acetylene flame for As and an air-acetylene flame for Cd, Cr, Pb and Zn respectively. The instrument was fitted with specific lamp of particular metal. The instrument was calibrated using manually prepared standard solution of respective heavy metals as well as drift blanks. Standard stock solutions of 1000 ppm for all the metals were obtained from Sisco Research Laboratories Pvt. Ltd., India. These solutions were diluted for desired concentrations to calibrate the instrument.

Precision and accuracy of analysis was assured through repeated analysis of samples against National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the heavy metals. The results were found within ±2% of the certified value. Quality control measures were taken to assess contamination and reliability of data. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after five determination to calibrate the instrument. The coefficients of variation of replicate analysis were determined for different determinations for precision of analysis and variations below 10% were considered correct. All the concentration of the metals is expressed in mg kg^{-1} in dry weight.

Daily intake of metal (DIM)

The daily intake of heavy metals through the consumption of the vegetables tested was calculated according to the given equation.[17]

Daily intake of metal (µg day^{-1}) = C_{metal} x $D_{food\ intke}$ / $B_{average\ weight}$

Where, C_{metal} = heavy metal concentration in plants

$D_{food\ intake}$ = daily intake of vegetable

$B_{average\ weight}$ = average body weight

The average daily vegetable intake rate was calculated by conducting a survey where 100 people having average body weight of 60 kg were asked for their daily intake of particular vegetable from the experimental area in each period of sampling.[18]

Statistical analysis

All statistical analyses were performed on an IBM-PC computer using the Microsoft EXCEL (version 2003). Analysis of Kruskal-Wallis test were employed to examine statistical significance of difference in the mean concentrations of metals between group of families of vegetables using Statistical Package for Social Sciences (SPSS) program, version 11.5. Similarly, the significance of differences between the concentrations of heavy metals in vegetables was shown by using Student's t-test. A probability level of $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Levels of heavy metals in the vegetables

Heavy metal concentrations (mean, ranges and standard deviation) in leafy shoots and roots of different types of green leafy vegetables marketed in Kathmandu are presented in Table 2. Results revealed variable metal levels in different types and parts of the vegetable samples under investigation. The levels of Pb in all commodities were ranged between 2.7 and 12.5 mg kg^{-1} in roots of spinach and garden crees while the metal ranged between 5.3 and 32.9 mg kg^{-1} in leafy shoots of spinach and mustard respectively. Cd contents varied from 0.9 mg kg^{-1} in roots of spinach to 12.0 mg kg^{-1} in roots of mustard and from 3.2 to 19.1 mg kg^{-1} in leafy shoots of fennel respectively. Similarly, Zn levels were ranged from 16.8 to 158.7 mg kg^{-1} in roots and from 23.9 to 223.1 mg kg^{-1} in leafy shoots of garden crees and mustard respectively. Among the vegetables, the highest mean levels of Cd (8.9 mg kg^{-1}), Pb (19.2 mg kg^{-1}) and Zn (107.6 mg kg^{-1}) were detected in leafy shoots of mustard and so with the roots (5.6 mg kg^{-1} for Cd and 71.7 mg kg^{-1} for Zn) except for garden crees roots (9.5 mg kg^{-1} for Pb). The contents of As and Cr, however were found below the detection limits in all the test vegetables (Table 2). Based on the results, the average concentrations of all metals (both leafy shoots and roots together) in the vegetables can be ranked by abundance in the order as Zn>Pb>Cd (Fig. 1). Among all the heavy metals, Zn showed maximum and Cd minimum levels in all the vegetables. Sharma and Chettri[13] also reported highest accumulation of Zn in spinach grown in production sites of Kathmandu valley. Similarly, Sharma *et al.*[19] also found highest concentration of Zn as compared to Cu, Cd and Pb in the vegetables collected from market as well as production sites of Varanasi city, India. Similar trends were also reported by Dogheim *et al.*,[20] and Radwan and Slama.[21] Uptake of heavy metals by plants is often influenced by plant species, growth stage, soil type and metal species.[6] Availability of heavy metal ions are influenced by various factors including soil pH, physical and chemical soil properties, clay content and Mn oxide concentration.[22] Besides, they are also altered by innumerable environmental and human factors and nature of the plant.[5]

Samarghandi *et al.*[23] determined the concentrations of Pb, Ni, Cd and Cr in vegetables which were irrigated with polluted water in Hamadan city, Iran. They reported that Pb concentration in vegetables was more than the permissible limitation for human foods. The concentrations of other heavy metals were lower than permissible limitation for human foods. When the present concentrations of metals were compared with permissible limits given by FAO/WHO,[24] Pb and Cd levels were found to exceed the maximum permissible limits for human consumption (Table 2). Zn level was however found within the permissible limit. Zhuang *et al.*[25] have also found higher than the maximum permissible levels of Cd and Pb concentrations in vegetables collected from six sampling sites around Dabaoshan mine located at Shaoguan city, Guangdong, southern China. It was found that most of the vegetable types marketed in Kathmandu were grown along the bank of rivers, roadsides and highways irrigated with wastewater as well as from vegetable farms with possible use of fertilizers and pesticides in excessive amount (source: personal communication with vegetable grosser). Continuous irrigation of agricultural land with sewage and wastewater may cause heavy metal accumulation in the soil and vegetables.[26] Besides, pesticides and fertilizers are known to be the main sources of heavy metal pollution in agricultural areas.[27] Moreover, the transportation and marketing systems of vegetables play a significant role in elevating the contaminant levels of heavy metals which may pose a threat to the quality of the vegetables with consequences for the health of the consumers of locally produced foodstuffs.[23]

Table 2. Concentration levels of heavy metals (mg kg^{-1} dry weight) in the test vegetables marketed in Kathmandu (mean ± SD, n = 9)

Vegetable	Parts	As	Cd	Cr	Pb	Zn
	Leafy	ND	8.9 ± 2.3	ND	19.2 ± 6.8	107.6±34.6
Mustard	Shoots		(5.7-14.4)		(12.4-32.9)	(72.4-223.1)
	Roots	ND	5.6 ± 1.9	ND	7.1 ± 2.6	71.7 ± 17.5
			(4.9-12.0)		(4.1-11.1)	(43.6-158.7)
	Leafy	ND	6.6 ± 1.6	ND	11.1 ± 3.5	31.6 ± 7.3
Garden crees	shoots		(5.7-8.6)		(9.2-12.5)	(23.9-39.8)
	Roots	ND	3.3 ± 0.9	ND	9.5 ± 3.1	24.6 ± 5.9
			(2.6-4.7)		(8.5-15.5)	(16.8-29.9)
	Leafy	ND	8.3 ± 3.7	ND	10.2 ± 2.9	72.1 ± 12.6
Fennel	shoots		(3.2-19.1)		(7.5-12.0)	(59.5-101.2)
	Roots	ND	5.1 ± 1.5	ND	7.2 ± 2.5	56.9 ± 11.0
			(2.2-11.0)		(4.6-10.5)	(45.3-72.1)
	Leafy	ND	7.7 ± 3.2	ND	9.5 ± 2.4	101.2 ± 25.8
Coriander	shoots		(5.8-9.3)		(7.7-12.0)	(81.0-139.9)
	Roots	ND	2.9 ± 1.1	ND	5.5 ± 1.9	66.7 ± 9.3
			(1.9 -5.6)		(4.5-7.2)	(55.6-70.5)
	Leafy	ND	7.4 ± 2.4	ND	11.5 ± 3.1	85.8 ± 19.5
Spinach	shoots		(3.9-9.4)		(5.3-16.8)	(72.5-127.6)
	Roots	ND	3.7 ± 2.1	ND	4.9 ± 2.7	34.8 ± 12.5
			(0.9-7.5)		(2.7-7.6)	(31.5-57.0)
Recommended maximum limits for vegetables*		0.43	0.20	2.30	0.30	99.40

ND = Not detected, Levels were below the detection limit. Values in the parentheses are minimum and maximum concentration of each element.*Source: FAO/WHO (2001)

In the present study, significant differences were found in Pb concentrations between the test vegetables (P = 0.00, Kruskal-Wallis test) but not in Cd concentrations. Results also showed a significant variation in the levels of Zn between the test vegetables (p = 0.005, Kruskal-Wallis). Moreover, a positive correlation between Cd and Zn ($r = 0.783$, $p<0.05$), Pb and Zn ($r = 0.813$, $p<0.05$) and Cd and Pb ($r = 0.879$, $p<0.05$) could indicate a common source of the metals in the test vegetables marketed in Kathmandu. The common sources of such contamination could either be the farming sites irrigated with polluted water or during transportation and marketing of the vegetables.[23,26]

Estimation of daily intake of heavy metals (DIM)

The FAO/WHO[28] recommends a population dietary intake goal of more than 400 g day^{-1} for vegetables. Many advanced countries have campaigns for promoting consumption of vegetables, especially in the framework of the International Fruits and Vegetables Alliance.[28,29] The National Nutrition and Food Research Institute of Iran have estimated that the average consumption of edible vegetables is 218 g person^{-1} day^{-1}.[3] Formal interviews conducted in the present study showed that the average consumptions of fresh mustard, garden crees, fennel, coriander and spinach person^{-1} day^{-1} are 250 g each, respectively. If the levels of heavy metal contamination (Table 2) recorded in this study are representative of contaminant concentrations in the vegetables tested and consumed by the interview sample then the contribution of each of the heavy metals to the dietary intake would be 37.1 µg, 80.0 µg and 0.5 mg day^{-1} for mustard, 27.5 µg, 46.3 µg and 0.1 mg day^{-1} for garden crees, 34.6 µg, 42.5 µg and 0.3 mg day^{-1} for fennel, 32.1 µg, 39.6 µg and 0.4 mg day^{-1} for coriander and 30.8 µg, 47.9 µg and 0.4 mg day^{-1} for spinach respectively, for Cd, Pb and Zn (Table 3).

From the estimated daily intake of the studied heavy metals through the consumption of the test vegetables, it can be suggested that the consumption of average amounts of these contaminated vegetables does not pose a health risk for the consumers as the values obtained are below the provisional tolerable daily intakes (PTDIs) (Table 3) set by Joint FAO/WHO Expert Committee on Food Additives[30] for heavy metals intake based on the body weight of an average adult (60 kg body weight). However, the study showed remarkable contributions of these vegetables to daily intake of Cd, Pb and Zn. Debeca *et al*.[31] reported 54 μg day^{-1} as the dietary intake for Pb in adult while Dick *et al*.[32] reported 412 μg day^{-1}. Similarly, Bahemuka and Mubofu[11] reported that the daily intake for Cd, Cu, Pb and Zn in vegetables in Dares Sallam was found to be 21.6, 858.6, 426.6 and 3.7 mg day^{-1} respectively. The estimated dietary intake of metals in the present study was found to be inconsistent with the values reported in the above studies.

Table 3: Daily intake of heavy metals (DIM) through consumption of the test vegetables.

DIM			
Vegetables	**Cd** (μg **day^{-1}**)	**Pb** (μg **day^{-1}**)	**Zn** (mg **day^{-1}**)
Mustard	37.1	80.0	0.5
Garden crees	27.5	46.3	0.1
Fennel	34.6	42.5	0.3
Coriander	32.1	39.6	0.4
Spinach	30.8	47.9	0.4
*PTDI	60	214	60

**j*oint FAO/WHO Expert Committee on Food Additives, 1999

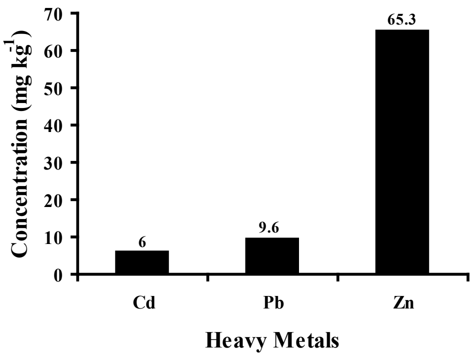

Fig. 1: Average concentration of heavy metals in green leafy vegetables

CONCLUSION

From the present study it can be concluded that Cd and Pb concentrations in all the vegetables were above the permissible limits set by FAO/WHO for human consumption. The levels of Zn, however fall within the maximum permissible limit. Although the test vegetables have significant accumulation of heavy metals in their root portions which are usually discarded for cooking purpose, the remaining metal levels accumulated in leafy shoots are still not safe for consumption. However, daily intake of metals suggests that the consumption of average amounts of these contaminated vegetables does not pose a health risk for the consumers as the values obtained are below the provisional tolerable daily intakes (PTDIs). Nevertheless, consumption of foodstuff with elevated levels of heavy metals for long term may lead to high level of accumulation in the body causing related health disorders. It is therefore suggested that regular monitoring of heavy metals in vegetables is essential in order to prevent excessive build-up of these metals in the human food chain. Besides, the metal contaminated areas should be discouraged for commercial farming of such leafy vegetables. Instead, those areas may be replaced by some other non-metal accumulating plants.

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