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Assessment of toxic metals in vegetables with the health implications in Bangladesh

Md. S. Islam[*1, 2] Md. K. Ahmed[3], Ram Proshad[1] and Saad Ahmed[1]

[1]*Department of Soil Science, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh*
[2]*Graduate School of Environment and Information Sciences, Yokohama National University, Japan*
[3]*Department of Oceanography, Dhaka University, Dhaka-1000, Bangladesh*

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Abstract. This study was conducted to investigate the levels of heavy metals in twelve species of vegetables and assessment of health risk. Samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS). The ranges of Cr, Ni, Cu, As, Cd and Pb in vegetables species were 0.37-5.4, 0.03-17, 0.35-45, 0.01-2.6, 0.001-2.2, and 0.04-8.8 [mg/kg, fresh weight (fw)], respectively. The concentrations of As, Cd and Pb in most vegetable species exceeded the maximum permissible levels, indicating unsafe for human consumption. Health risks associated with the intake of these metals were evaluated in terms of estimated daily intake (EDI), and carcinogenic and non-carcinogenic risks by target hazard quotient (THQ). Total THQ of the studied metals from most of the vegetables species were higher than 1, indicated that these types of vegetables might pose health risk due to metal exposure. The target carcinogenic risk (TR) for As ranged from 0.03 to 0.48 and 0.0004 to 0.025 for Pb which were higher than the USEPA acceptable risk limit (0.000001) indicating that the inhabitants consuming these vegetables are exposed to As and Pb with a lifetime cancer risk. The findings of this study reveal the health risks associated with the consumption of heavy metals through the intake of selected vegetables in adult population of Bangladesh.

Keywords: toxic metals; vegetables species; health risk; Bangladesh

1. Introduction

The human body requires at least 20 elements for optimal health (Broadley and White 2010). Many of the elements/minerals are essential in physiological and biochemical processes such as water absorption, enzyme catalysis, hormone functions (Gutzeit *et al*. 2008). Element deficiencies may result in major debilitating effects including reduced defense systems, reduced physical and mental development and acuity. Fortunately, consumption of appropriate foods especially different types of vegetables for optimal health can supply the needed micro-/macronutrients. Contaminations from toxic heavy metals in a number of foods pose serious health problems (Nachman *et al*. 2013) that range from shortness of breath to several types of cancers (Dogan *et al*.

∗Corresponding author, Ph.D., E-mail: msaifulpstu@yahoo.com or islam-md.saiful-nj@ynu.jp

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2005, Iqbal and Shah 2013). The environmental safety of vegetables against pollution is especially crucial to human health. A continuous determination of total amounts enables evaluation of possible routes through which food elements are ingested. Thus, elemental concentration assessments in vegetables are very important to risk assessment studies. Vegetables can uptake heavy metals and accumulate them in their edible and inedible parts (Rahman *et al*. 2013) in quantities high enough to cause clinical problems both to animals and humans consuming these metal-rich plants (Islam *et al*. 2014a). Therefore, heavy metal contamination in vegetables cannot be underestimated as these foodstuffs are important components of human diet. Vegetables are rich sources of vitamins, minerals and fibers and also have beneficial antioxidative effects (Ali and Al-Qahtani 2012). However, intake of metal-contaminated vegetables may pose a risk to the human health. Heavy metals such as Cr, Cu, Cd, Pb and metalloids like As have been considered the most toxic elements in the environment and included in the US Environment Protection Agency (EPA) list of priority pollutants (Cameron 1992, Lei *et al*. 2010, Wuana *et al*. 2016). Chromium and Ni are known to cause a variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema, and tumors (Forti *et al*. 2011), while high intake of Cu and Cd can cause adverse health problems such as liver and kidney damage (WHO 1995, Tuzen 2009, Islam *et al*. 2015a). Lead has been associated with pathological changes in organs and the central nervous system, leading to decrements in intelligence quotients (IQs) in children. Cadmium is toxic to the cardiovascular system, kidneys and bones (Fang *et al*. 2014) while inorganic As, a human carcinogen, is the most toxic form of arsenic (Anawar *et al*. 2002, EU 2002, Banerjee *et al*. 2011). Sufficient amount of Zn is very important for normal body functions and its deficiency can cause anorexia, diarrhoea, dermatitis and depression, immune dysfunction and poor wound healing, nevertheless, its toxicity can causes a sideroblastic anemia (Muhammad *et al*. 2011; Khan *et al*. 2013). Health risks have been evaluated by numerous methods but most commonly, risk to the human health is computed in terms of target hazard quotients (THQs) which is based on the concentration of the metal in edible parts in comparison with the reference dose of the metal and intake/body weight of the consumers, while carcinogenic health risk is assessed by calculating the target cancer risk (TR) (USEPA 2006, 2010, Yang *et al*. 2011).

Concern over the environmental pollutants particularly the toxic heavy metals has increased immensely in Bangladesh during the last few decades in the wake of population explosion, industrialization, urbanization and other human activities (Islam *et al*. 2015b, 2016). Studied vegetables are being used by the local inhabitants on regular basis since long time, but to our knowledge, no systematic investigation has been carried out to find the health risks associated with metal concentration in these vegetables. The present investigation was, therefore, aimed to assess the concentrations and sources of heavy metals in commonly consumed vegetables of some extensive cultivated agricultural fields located at the southern part of Bangladesh and to evaluate the potential non-carcinogenic and carcinogenic health risks for the inhabitants in the study area.

2. Materials and methods

2.1 Study area and sampling

The agriculture fields selected besides the Paira River located at the southern part of Bangladesh (Fig. 1). The area of Patuakhali district about 3204.58 km^2 and population density is 451 person per square km (Islam *et al*. 2014c). The study area is located between latitudes

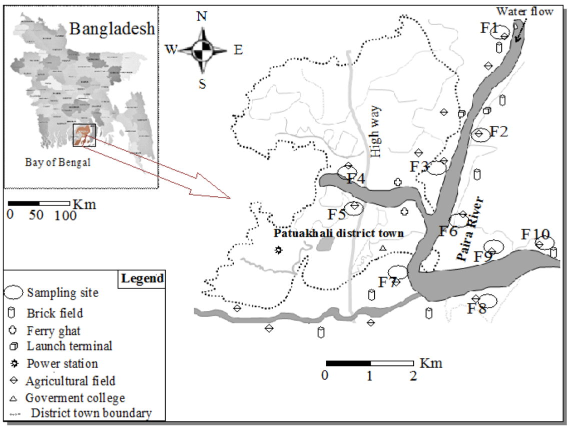

Fig. 1 Map of the study area of Paira Riverside fields of Patuakhali district, Bangladesh

22º20′49.87″and 22º27′27.18″N and longitudes 90º23′58.24″and 90º26′54.68″E. Several acres of agricultural lands have been irrigated by river water and farmers cultivate various types of vegetable crops for their economic importance. Therefore, the fields of this riverside area have been chosen for the present study. The sampling was conducted in August−September, 2013 and April-May, 2015. Twelve different vegetable species i.e., Brinjal (*Solanum melongena*), Bottle gourd (*Lagenaria siceraria*), Bean (*Phaseolus vulgaris*), Chili (*Capsicum annuum* L), Carrot (*Daucus carota*), Green amaranth (*Amaranthus hybridus*), Lentil (*Lens culinaris*), Onion (*Allium cepa*), Pumpkin (*Cucurbita maxima*), Potato (*Solanum tuberosum*), Red amaranth (*Amaranthus gangeticus*) and Tomato (*Solanum lycopersicum*) were collected from ten agricultural fields of tidal floodplain soils. At each sampling site, three to five replicate vegetable samples were randomly collected. Each vegetable sample was carefully washed with distilled water and the edible part of vegetables were cut into small pieces and then oven dried at 70-80 ℃ to attain constant weight (Islam *et al*. 2014a). The moisture contents in vegetables were calculated by recording the fresh and dry weights. The dried samples were crumbled and pulverized with a porcelain mortar and pestle and stored in airtight clean zip lock bag in freezer condition up to chemical analysis was carried out.

2.2 Sample analysis

All chemicals were analytical grade reagents and Milli-Q (Elix UV5 and MilliQ, Millipore, USA) water was used for solution preparation. For vegetable, 0.5 g of dried powdered sample was treated with 6 mL 69% HNO_3 (Kanto Chemical Co, Tokyo, Japan) and 2 mL 30% H_2O_2 (Wako Chemical Co, Japan) in a microwave digestion system (Berghof Speedwave® Germany). The digested samples were then transferred into a Teflon beaker and total volume was made up to 50 mL with Milli-Q water. The digested solution was then filtered by using syringe filter (DISMIC®– 25HP PTFE, pore size = 0.45 µm) Toyo Roshi Kaisha, Ltd., Tokyo, Japan and stored in 50 mL polypropylene tubes (Nalgene, New York).

2.3 Instrumental analysis and quality assurance

For measuring heavy metals, samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7700). Multi-element Standard XSTC-13 (Spex CertiPrep® USA) solutions was used to prepare calibration curve. The calibration curves with $R^2 > 0.999$ were accepted for concentration calculation. Internal calibration standard solutions containing 1.0 mg/L of Indium, Yttrium, Beryllium, Tellurium, Cobalt and Thallium were purchased from Spex CertiPrep® USA. All test batches were evaluated using an internal quality approach and validated if they satisfied the defined Internal Quality Controls (IQCs). Before starting the sequence, relative standard deviation (RSD < 5%) was checked by using tuning solution purchased from the Agilent Technologies.

2.4 Data calculation

2.4.1 Estimated daily intake (EDI) of metal

Estimated daily intakes (EDIs) of heavy metals (mg/day) were calculated using their respective average concentration in food samples by the weight of food items consumed by an individual (body weight 60 kg for an adult in Bangladesh) (FAO 2006), which was obtained from the household income and expenditure survey (HIES 2011, Islam *et al*. 2014a, Shaheen *et al*. 2016) and calculated by the following formula

$$EDI = \frac{FIR \times C}{BW} \tag{1}$$

where FIR is the vegetable intake rate (brinjal-130, bottle gourd-80, bean-130, chili-10.5, carrot-130, green amaranth-50, lentil-30, onion-22, pumpkin-100, potato-70.3, red amaranth-50, and tomato-130 g/person/day), C is the concentration of heavy metals in vegetables [mg/kg, fresh weight (fw)], BW is the body weight assuming 60 kg for adult residents in the present study.

2.4.2 Non-carcinogenic and carcinogenic risk assessment

The methodology for non-carcinogenic risks estimation was applied in accordance with that provided in the U.S. Environmental Protection Agency (USEPA) Region III's Risk-based Concentration Table (USEPA 2010). The non-carcinogenic risk for individual metal through vegetables consumption were assessed by the target hazard quotient (THQ) (USEPA 1989), which is ''the ratio of a single substance exposure level over a specified time period (e.g., sub-chronic) to a reference dose (RfD) for that substance derived from a similar exposure period''. The equation used for estimating the target hazard quotient is as follows

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times BW \times AT} \tag{2}$$

$$\text{TTHQ (Individual vegetable)} = \text{THQ}_{\text{toxicant 1}} + \text{THQ}_{\text{toxicant 2}} + \ldots\ldots + \text{THQ}_{\text{toxicant n}} \tag{3}$$

Letter (T) in the expression "TTHO" means total. In order to assess the overall potential for non-carcinogenic effects from more than one heavy metal, a hazard index (HI) has been formulated based on the Guidelines for Health Risk assessment of Chemical Mixtures of USEPA (1989) as follows

$$\text{HI} = \sum \text{TTHQ} = \text{TTHQ}_{\text{vegetable 1}} + \text{TTHQ}_{\text{vegetable 2}} + \ldots\ldots + \text{TTHQ}_{\text{vegetable n}} \tag{4}$$

where *THQ* is the target hazard quotient; *EFr* is the exposure frequency (365 days/year); *ED* is the exposure duration (70 years); *FIR* is the vegetables intake rate (Table 4); *C* is the metal concentration in vegetables (mg/kg fw); *RfD* is the oral reference dose (mg/kg/day); *AT* is the averaging time for non-carcinogens (365 days/year × number of exposure years). The oral reference doses were based on 1.5, 0.02, 0.04, 0.0003, 0.0005 and 0.004 mg/kg/day for Cr, Ni, Cu, As, Cd and Pb, respectively (USEPA 2010; Islam *et al*. 2014c). If the THQ is less than 1, the exposed population is unlikely to experience obvious adverse effects. If the THQ is equal to or higher than 1, there is a potential health risk and related interventions and protective measurements should be taken.

The equation used for estimating the target carcinogenic risk factor (lifetime cancer risk) (USEPA 1989) is as follows

$$TR = \frac{EFr \times ED \times FIR \times C \times CSFo}{BW \times AT} \times 10^{-3} \quad (5)$$

where TR represents target cancer risk or the risk of cancer over a lifetime; *AT* is the averaging time for carcinogens (365 days/year × *ED*); CSFo is the oral carcinogenic slope factor from the Integrated Risk Information System USEPA (2010) database. It is 1.5 and 8.5× 10^{-3} $(mg/kg/day)^{-1}$ for As and Pb, respectively.

2.4.3 Statistical analysis

The data were statistically analyzed using the statistical package software, SPSS 16.0 (SPSS, USA). A multivariate method in terms of principal component analysis (PCA) and cluster analysis (CA) were used to obtain the distribution of heavy metals by detecting similarities or differences among samples. The PCA was performed using Varimax normalized rotation on the data-set while the CA was applied to the standardized matrix using Ward's Method and the results are reported in the form of dendrogram to discover a system of organizing variables where each group shares properties in common.

3. Results and discussion

3.1 Metal contaminations in vegetables

The concentrations of heavy metals (mg/kg fw) in vegetables are summarized in Table 1. The concentration of metals varied among vegetable species. The average concentration of heavy metals in all vegetables were in the decreasing order of Cu > Ni > Cr > Pb > As > Cd. The mean concentration of Cr ranged from 0.88 (*S. lycopersicum*) to 2.1 mg/kg (*A. Gangeticus*) (Table 1). A recent study by Rahman *et al*. (2013) showed that the mean Cr concentration in leafy and non-leafy vegetables from Bangladesh were 1.12 mg/kg (range: 0.35-4.50 mg/kg) and 0.64 mg/kg (range: 0.18–1.91 mg/kg), respectively. Chromium concentration in vegetables of the present study was slightly higher than the other studies in Bangladesh and comparable to the other studies from other countries (Table 2). In Bangladesh, the main sources of Cr in agricultural soils where the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Islam *et al*. 2009, Bhuiyan *et al*. 2011, Islam *et al*. 2015b). Average concentration Ni ranged from 0.92 (*A. cepa*) to 5.1 mg/kg (*A. gangeticus*) (Table 1). The mean concentration of Cu in vegetables ranged from 2.1 mg/kg (*D. carota*) to 6.9 mg/kg (*C. annuum*) (Table 1). Alam *et al*. (2003) reported Cu concentration of 8.50 and 15.50

mg/kg in leafy and non-leafy vegetables, respectively, from Samta village in Bangladesh which was higher than the present study. However, Cu concentration in vegetables of the present study was comparable to the study in Varanasi, India (Sharma *et al*. 2007), where the mean Cu concentration was 36.4 mg/kg (range: 20.5-71.2 mg/kg) (Table 2).

The mean concentration of As in vegetable species ranged from 0.06 mg/kg (*S. melongena*) to 0.30 mg/kg (*C. maxima*, *S. lycopersicum*) (Table 1). Rmalli *et al*. (2005) reported that As concentration in vegetables collected from Bangladesh ranged from 0.005 to 0.50 mg/kg, with a mean of 0.06 mg/kg. The range of As levels in home-grown vegetables from Samta village in Bangladesh was 0.02-0.49 mg/kg (Alam *et al*. 2003). The range of As was 0.07-3.9 mg/kg in

Table 1 Metal concentration (mg/kg fw) in vegetables collected from tidal floodplain soils Bangladesh

Common name	Scientific name		Cr	Ni	Cu	As	Cd	Pb
Brinjal	*S. melongena*	Mean±SD	1.2±0.62	3.9±3.2	3.9±3.0	0.06±0.06	0.16±0.15	0.39±0.57
		Range	0.62-2.7	0.18-8.7	0.38-8.6	0.01-0.21	0.003-0.39	0.06-1.9
Bottle gourd	*L. siceraria*	Mean±SD	0.94±0.37	4.4±3.1	4.4±2.0	0.15±0.32	0.13±0.13	0.58±1.0
		Range	0.57-1.6	0.13-11	0.90-7.4	0.02-1.1	0.004-0.39	0.06-3.5
Bean	*P. vulgaris*	Mean±SD	1.2±0.43	1.2±1.4	2.9±2.3	0.15±0.08	0.11±0.17	1.3±2.6
		Range	0.59-1.8	0.03-4.0	0.76-6.9	0.03-0.28	0.004-0.54	0.05-8.8
Chili	*C. annuum*	Mean±SD	0.92±0.42	1.0±1.5	6.9±14	0.25±0.28	0.13±0.20	0.24±0.22
		Range	0.37-1.7	0.07-4.4	1.0-45	0.02-0.68	0.002-0.54	0.04-0.73
Carrot	*D. carota*	Mean±SD	1.2±0.45	2.1±3.9	2.1±1.2	0.19±0.13	0.09±0.13	0.73±1.0
		Range	0.38-1.7	0.11-12	0.35-4.4	0.04-0.39	0.001-0.34	0.07-3.1
Green amaranth	*A. hybridus*	Mean±SD	1.8±0.93	4.5±5.6	4.1±2.1	0.21±0.15	0.45±0.65	1.7±1.8
		Range	0.69-3.4	0.48-17	1.2-8.2	0.02-0.55	0.02-2.2	0.09-6.3
Lentil	*L. culinaris*	Mean±SD	1.0±0.24	2.4±3.2	2.7±2.5	0.07±0.08	0.04±0.06	0.44±0.44
		Range	0.67-1.5	0.08-9.8	0.56-8.5	0.02-0.28	0.003-0.18	0.06-1.2
Onion	*A. cepa*	Mean±SD	1.1±0.33	0.92±1.6	2.4±2.2	0.14±0.08	0.22±0.48	0.52±0.49
		Range	0.76-1.7	0.07-5.5	0.57-7.0	0.03-0.29	0.003-1.6	0.14-1.8
Pumpkin	*C. maxima*	Mean±SD	0.94±0.21	2.9±2.5	3.8±2.6	0.30±0.81	0.08±0.10	0.28±0.22
		Range	0.65-1.3	0.16-6.8	0.68-8.2	0.02-2.6	0.01-0.26	0.06-0.80
Potato	*S. tuberosum*	Mean±SD	0.95±0.52	1.8±1.9	3.4±2.5	0.10±010	0.14±0.31	0.60±0.94
		Range	0.48-2.2	0.10-6.3	0.35-8.2	0.02-0.31	0.002-0.99	0.06-2.8
Red amaranth	*A. Gangeticus*	Mean±SD	2.1±1.8	5.1±4.1	3.6±1.5	0.17±0.16	0.35±0.45	1.4±1.3
		Range	0.57-5.4	0.17-12	1.5-6.3	0.02-0.45	0.004-1.4	0.06-4.2
Tomato	*S. lycopersicum*	Mean±SD	0.88±0.28	1.1±0.94	2.3±1.1	0.30±0.71	0.10±0.09	0.29±0.25
		Range	0.46-1.2	0.12-2.9	0.64-3.3	0.02-2.3	0.002-0.23	0.04-0.87
Chinese standard in vegetables (Li *et al*. 2012)			0.5	0.3	10	NA	0.05	0.1
Permissible levels as per (FAO and WHO 2011)			2.3	10	40	0.1	0.05	0.1

Table 2 Comparison of heavy metals concentration (mg/kg fw) in vegetables with the reported values in the literatures

Study area	Cr	Ni	Cu	As	Cd	Pb	References
Patuakhali (Bangladesh)	1.2 (0.37-5.4)	2.6 (0.03-17)	3.6 (0.35-45)	0.18 (0.01-2.6)	0.17 (0.001-2.2)	0.71 (0.04-8.8)	This study
Noakhali (Bangladesh	0.64 (0.18-1.91)	1.44 (0.32-4.67)	20.6 (2.1-86.3)	0.05 (0.011-0.145)	0.058 (0.006-0.265)	3.7 (0.67-16.5)	Rahman *et al.* 2013
Dhaka (Bangladesh)	0.69 (0.25-1.4)	3.2 (0.52-10)	12 (4.5-22)	0.08 (0.01-0.45)	0.15 (0.003-0.85)	0.84 (0.08-3.7)	Islam *et al*. 2014b
Dhaka (Bangladesh)	1.660	3.0	3.9	NA	0.62	3.9	Ahmad and Gani 2010
Varanasi (India)	NA	NA	36.4 (20.5-71.2)	NA	2.08 (1.1-4.5)	1.42 (0.9-2.2)	Sharma *et al.* 2007
New South Wales (Australia)	NA	NA	1.9	NA	0.2	3.1	Kachenko and Singh 2006
Pearl River Estuary (China)	0.17	0.22	0.7	NA	0.063	0.064	Li *et al*. 2012

vegetables from Chandpur and Jamalpur districts (Das *et al*. 2004) and <0.04-1.9 mg/kg in vegetables from Sathkhira, Rajshahi and Comilla districts (Williams *et al*. 2006). The range of As levels in this study was comparable with the results from other studies (Alam *et al*. 2003, Rahman *et al*. 2013, Islam *et al*. 2014b). In the study area, As contaminated ground water (Polizzotto *et al*. 2013, Neumann *et al*. 2010) is being used for irrigation along with various As-enriched fertilizers and pesticides for the cultivation of vegetables (Bhuiyan *et al*. 2011, Alam *et al*. 2003). Moreover, the substance containing As might be transformed by the addition of carbon and hydrogen as a methyl group (CH_3) resulting in methylarsines which is much more toxic to living things than the unmethylated forms (Bai *et al*. 2011). In vegetables, mean concentration of Cd ranged from 0.04 (*L. culinaris*) to 0.45 mg/kg (*A. hybridus*) (Table 1). The Cd concentration in vegetables collected from Samta village varied between 0.01 and 0.22 mg/kg (Alam *et al*. 2003). The mean Cd concentration of vegetables from Matlab in Bangladesh was 0.03 mg/kg (Khan *et al*. 2010) which was much lower than that found in our study. The range of Cd in vegetables of the present study was comparable with the results from other studies (Alam *et al*. 2003, Rahman *et al*. 2013, Islam *et al*., 2014b, Ahmad and Gani 2010, Sharma *et al*. 2007, Kachenko and Singh 2006, Li *et al*. 2012) (Table 2). The highest mean concentration of Pb was observed in *A. hybridus* (1.7 mg/kg) followed by *A. Gangeticus* (1.4 mg/kg) (Table 1). In the present study, the concentration of Pb in vegetables were in line with the study in Samta Bangladesh (Alam *et al*. 2003), where the range of Pb concentration in all vegetables was 0.14-1.7 mg/kg. According to the FAO/WHO guideline values, the concentration of Cr, Ni, Cu, As, Cd, and Pb about 5.0, 4.2, 0.83, 42, 47 and 82 % of vegetable samples, respectively, exceeded the maximum level of the tolerance limit of contaminants in vegetables (Table 1).

PCA has commonly been used for investigating metal sources, anthropogenic activities, or soil parent materials (Cai *et al*. 2012). In the present study, three principal components (PC) were extracted from the values of heavy metal concentrations in vegetables (Fig. 2). In the PCA analysis, first three PCs were computed and the variance explained by them was 33.8%, 19.4% and 17.1% in vegetables (Table 3). Among three groups, one group revealed similar loadings of Cr, Ni, Cd and Pb in vegetables, indicated that these were mostly contributed by anthropogenic activities

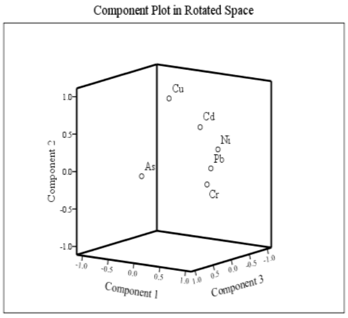

Fig. 2 Principal component analysis of heavy metals in vegetables collected from agricultural fields of tidal floodplain area in Bangladesh. Considering the highest component loading, first PC exhibited elevated loadings of Cr, Ni, Cd and Pb, second PC exhibited elevated loadings of As and third PC exhibited elevated loadings of Cu and As

Table 3 Total variance explained and component matrices for the heavy metals in vegetables collected from tidal floodplain soils of Bangladesh

	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.0	33.8	33.8	2.0	33.8	33.8	1.8	30.7	30.7
2	1.2	19.4	53.1	1.2	19.4	53.1	1.3	21.6	52.3
3	1.0	17.1	70.3	1.0	17.1	70.3	1.1	17.9	70.3
4	0.8	13.3	83.6						
5	0.6	9.3	92.9						
6	0.43	7.09	100						

Elements	Component matrix			Rotated Component Matrix		
	PC1	PC2	PC3	PC1	PC2	PC3
Component Matrix						
Cr	0.67	0.49	-0.10	0.80	-0.11	0.23
Ni	0.70	-0.14	-0.27	0.65	0.27	-0.29
Cu	0.35	-0.67	0.54	-0.07	0.93	0.04
As	0.08	0.58	0.78	0.05	0.04	0.97
Cd	0.70	-0.27	0.15	0.49	0.59	-0.03
Pb	0.69	0.22	-0.15	0.73	0.07	0.02

Extraction Method: Principal Component Analysis

such as fertilizers, pesticides, sewage sludge, organic manures and composts, mining, smelting and industries (Renner 2004, Manzoor *et al*. 2006, Shah and Shaheen 2007). The depositions of atmospheric particulates released by automobile emissions like coal and fuel combustion, vehicle emissions and municipal waste disposal were believed to contribute these metals in the urban areas, from where the vegetable samples were collected (Cui *et al*. 2004, Manzoor *et al*. 2006, Pandey *et al*. 2012). Considering the highest component loading, first PC exhibited elevated loadings of Cr, Ni, Cd and Pb, second PC exhibited elevated loadings of As and third PC exhibited elevated loadings of Cu and As. PCA analysis revealed that the apportionment of same kind of heavy metals in vegetables were not similar, which might be due to the emission of heavy metals to the environment and accumulation by the plants. Furthermore, using the overall heavy metals concentration in vegetables species, cluster analysis (CA) with dendrogram using Ward's Method was adopted to divide the vegetables species in to several groups as shown in Fig. 3. Different clusters were formed between different selected vegetables species, the vegetables in each group were of similar nature. Moreover, on the basis of heavy metal concentrations in some vegetables showed strong significant correlations by forming primary groups/clusters with each other (Fig. 3).

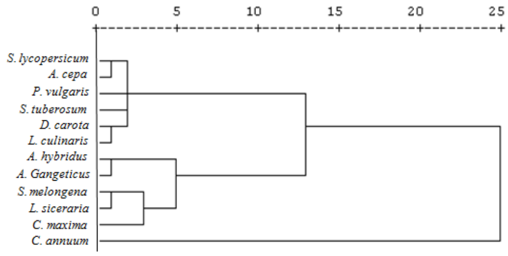

Fig. 3 Cluster analysis (CA) of the vegetables collected from agricultural fields of tidal floodplain area in Bangladesh

Table 4 Estimated daily intakes of heavy metals (mg/day) from consumption of vegetable species by Bangladeshi adult population

Vegetables species	Consumption rate (g/day/person)	Cr	Ni	Cu	As	Cd	Pb
S. melongena	130	0.15	0.51	0.51	0.01	0.02	0.05
A. hybridus	50	0.09	0.22	0.20	0.01	0.02	0.09
A. gangeticus	50	0.11	0.25	0.18	0.01	0.02	0.07
L. siceraria	80	0.08	0.35	0.35	0.01	0.01	0.05
S. lycopersicum	130	0.11	0.15	0.30	0.04	0.01	0.04
C. maxima	100	0.09	0.29	0.38	0.03	0.01	0.03
C. annuum	10.5	0.01	0.01	0.07	0.00	0.00	0.00
D. carota	130	0.15	0.27	0.28	0.02	0.01	0.10
P. vulgaris	130	0.15	0.16	0.38	0.02	0.01	0.17
A. cepa	22	0.02	0.02	0.05	0.00	0.00	0.01
S. tuberosum	70.3	0.07	0.13	0.24	0.01	0.01	0.04
L. culinaris	30	0.03	0.07	0.08	0.00	0.00	0.01
Total intake from vegetables		1.06	2.44	3.03	0.17	0.13	0.66
Maximum tolerable daily intake (MTDI)		0.20[a]	0.30[b]	30[c]	0.126[c]	0.046[c]	0.21[c]

[a]RDA 1989
[b]WHO 1996
[c]JECFA 2000

Table 5 Target hazard quotient and target carcinogenic risks due to heavy metals exposure from vegetables species

Species	Target hazard quotients (THQs)							Target carcinogenic	
	Cr	Ni	Cu	As	Cd	Pb	Total	As*	Pb
S. melongena	0.002±0.001	0.43±0.35	0.21±0.16	0.40±0.43	0.68±0.66	0.21±0.31	1.9	0.09±0.10	0.007±0.010
A. hybridus	0.001±0.001	0.20±0.25	0.09±0.05	0.59±0.44	0.79±1.3	0.35±0.40	2.0	0.13±0.09	0.012±0.013
A. gangeticus	0.001±0.001	0.21±0.17	0.07±0.03	0.48±0.44	0.58±0.76	0.28±0.26	1.6	0.11±0.10	0.010±0.009
L. siceraria	0.001±0.000	0.30±0.21	0.15±0.07	0.71±1.5	0.35±0.35	0.19±0.35	1.7	0.16±0.35	0.007±0.012
S.	0.001±0.000	0.12±0.10	0.12±0.06	2.1±5.1	0.43±0.40	0.16±0.13	3.0	0.48±1.2	0.005±0.005
C. maxima	0.001±0.000	0.24±0.21	0.16±0.11	1.7±4.5	0.28±0.33	0.12±0.09	2.5	0.38±1.0	0.004±0.003
C. annuum	0.0001±0.00	0.01±0.01	0.03±0.06	0.15±0.16	0.05±0.07	0.01±0.01	0.2	0.03±0.04	0.0004±0.00
D. carota	0.002±0.001	0.22±0.42	0.12±0.08	1.4±0.93	0.39±0.56	0.40±0.54	2.5	0.31±0.21	0.014±0.018
P. vulgaris	0.002±0.001	013±0.15	0.16±0.12	1.1±0.60	0.47±0.76	0.72±1.4	2.6	0.25±0.13	0.025±0.049
A. cepa	0.0003±0.00	0.02±0.03	0.02±0.02	0.18±0.09	0.16±0.35	0.05±0.04	0.4	0.04±0.02	0.002±0.002
S. tuberosum	0.001±0.000	0.11±0.11	0.10±0.07	0.39±0.40	0.32±0.72	0.17±0.28	1.1	0.09±0.09	0.006±0.009
L. culinaris	0.0003±0.00	0.06±0.08	0.03±0.03	0.12±0.13	0.04±0.06	0.05±0.05	0.3	0.03±0.03	0.002±0.002

*Assuming 50% inorganic As present in vegetables for produce carcinogenic risk (Saha and Zaman 2013)

3.2 Health risk assessment

3.2.1 Estimated daily intake of heavy metals

The dietary exposure approach of heavy metals of vegetables consumption is a reliable tool for investigating a population's diet in terms of intake levels of nutrients, bioactive compounds and contaminants, providing important information about the potential nutritional deficiencies or exposure to food contaminants (WHO 1985). The EDI of Cr, Ni, Cu, Zn, As, Cd and Pb were evaluated according to the mean concentration of each metal in each species of vegetable and the respective consumption rate for each species of vegetable (Santos *et al*. 2004). The EDI of the studied metals from consumption of vegetables are shown in Table 4. In vegetable samples, mean values of EDI showed the same descending order of Cu > Ni > Cr > Pb > As > Cd. Total daily intake of Cr, Ni, Cu, As, Cd and Pb were 1.06, 2.44, 3.03, 0.17, 0.13 and 0.66 mg/day, respectively. The total EDI of the studied metals (except Cu) through consumption of vegetables were higher than the maximum tolerable daily intake (MTDI) (Table 4), indicated that these vegetables might pose risk to the consumers in the study area, Bangladesh.

3.2.2 Non-carcinogenic and carcinogenic risk

The target hazard quotient (THQ) for non-carcinogenic risk and target carcinogenic risk (TR) of the six studied metals from consuming vegetables for adults inhabitants are presented in Table 5. The THQ of each metal through consumption of vegetables decreased in the order of As > Cd > Pb > Ni > Cu > Cr. The THQ value for individual metal (except some species of As) in vegetable was less than unity, which is considered as safe for human consumption. Total THQ values of the studied metals (except Cr) from all vegetables were higher than 1, indicated that if people consume these types of vegetables in their diet, they might be at risk. As and Cd exhibited relatively higher THQ compared to all other metals in the study area. Among the selected vegetable species the highest total THQ was observed for *S. lycopersicum* (3.0) followed by the *P. vulgaris* (2.6) (Table

5) indicating potential non-carcinogenic risks. The total metal THQ value [(sum of individual metal THQ (HI)] due to consumption of vegetables in the study area was 19.8 (>1). Potential health risks from exposure to vegetables are therefore of great concern. The analysis of non-carcinogenic health hazards resulting from exposure to metals through vegetables intake indicated that the investigated species were not safe for human consumption (Table 5).

Due to the lack of oral slope factor of Cd, target carcinogenic risks (TR) derived from the intake of As and Pb through the consumption of different vegetables are listed in Table 5. The TR values for As ranged from 0.03 to 0.48 and 0.0004 to 0.025 for Pb which were higher than the acceptable risk limit (0.000001) (USEPA 2010) indicating that the inhabitants consuming these vegetables are exposed to As and Pb with a lifetime cancer risk. The percentage of inorganic As depend on the types of food. For instance, in fish, the percentage of inorganic As is only up to 11 %, whereas in food commodities other than fish and seafood, it is assumed to vary from 50 to 100 % of the total arsenic (EFSA 2006). If we assume 50 % of the total arsenic as inorganic As (Saha and Zaman 2013) then carcinogenic risk through the consumption of vegetable is reduced. In addition, there are also other sources of metal exposures, such as consumption of other foodstuffs and dust inhalation, which were not included in this study. If the whole intake of metals through dietary means (vegetables and other foods) would be taken into account, the potential health risks involved in the consumption of local food should not be ignored.

4. Conclusions

This study revealed the presence of heavy metal concentration in selected highly consumed vegetables grown in Bangladesh as well as EDI of metal from those foods by adult Bangladeshi people and their health risk implications from consuming those foods in terms of THQ and TR. Vegetables grown in the agricultural fields of Patuakhali district were contaminated by the toxic metals, especially As, Cd and Pb were higher than the maximum allowable concentration (MAC) which could be a potential health concern to the local residents. Most of the metals from dietary intake of vegetables were higher than the maximum tolerable daily intake (MTDI), suggesting a considerable risk. THQ revealed that the consumption of studied vegetables species can result in adverse non-carcinogenic health risks to the consumers. The results also elucidated that the concentrations of As and Pb in vegetables species might exert lifetime cancer risks. The findings of this study significantly contribute to the field of food safety, considering the health risk for Bangladeshi population as it represents the composite samples of highly consumed vegetables, grown and consumed in the country. To figure out the As poisoning in human body through vegetables consumption, intensive investigation on the As speciation is recommended.

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