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COMPOSITION AND DIETARY EXPOSURE OF HEAVY METALS THROUGH VEGETABLES COMMONLY CONSUMED BY DHAKA CITY RESIDENTS

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Abstract:

Estimation of heavy metals intake is necessary for risk evaluation, and possibly to determine relationships between adverse effects observed in humans and dietary exposure to particular substances. Currently, heavy metal pollution is a serious concern to the environment. To determine the levels of heavy metals in major vegetables consumed by the residents of Dhaka city and to assess the dietary risk exposure to heavy metals 25 vegetable samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). A total of six heavy metals— lead, chromium, cadmium, mercury, arsenic and antimony were estimated in this study. The concentration of heavy metals varied widely among different vegetable samples. The average concentrations of heavy metals in vegetables fall within the safe limit established by regulatory organizations except for Pb (131.9 µg/100g) and Cr (15.55 µg/100g) which exceeded the safe limits. Colocasia/taro was the highest source of Pb (1043 µg/100g) and Cd (19.02 µg/100g) whereas papaya (green) contained the highest amount of Cr (57.97 µg/100g). Among other vegetables long bean (355.1 µg/100g), pointed gourd (293.8 µg/100g), white gourd (197.1 µg/100g) and banana (green) (168.5 µg/100g) were mostly contaminated with Pb while coriander leaf (55.00 µg/100g) and water spinach (39.01 µg/100g) were mostly contaminated with Cr. The concentration of Cd, Hg, As and Sb in all vegetables were below the safe limits. The daily intake of heavy metals was determined using the total weight of vegetables consumed each day multiplied by the concentrations of heavy metals in vegetables. The daily intake of Cd (8.84 µg), Hg (2.1 µg), As (2.8 µg) and Sb (2.77 µg) through vegetables were below the risk level except for Pb (317.9 µg) and Cr (37.48 µg). As the main meal of average Bangladeshi people is boiled rice served with some sorts of vegetables, our findings indicate that the residents of Dhaka city are at risk from Pb and Cr contamination.

Introduction:

Heavy metals are potential environmental contaminants with the capability of causing human health problems if present in excess in the food we eat. They are given special attention throughout the world due to their toxic effects even at very low concentrations. The effect of heavy metal contamination of vegetables cannot be underestimated as these foods are important components of human diet. Vegetables are rich sources of vitamins, minerals, and fibers and also have beneficial anti-oxidative effects. However, the intake of heavy metal-contaminated vegetables may pose a risk to human health; hence the heavy metal contamination of food is one of the most important aspects of food quality assurance [1-3]. Heavy metals, in general, are not biodegradable, have long biological half-lives, and have the potential for accumulation in different body organs, leading to unwanted side effects [4, 5]. Plants take up heavy metals by absorbing them from air borne deposits on the parts of the plants exposed to the air from the polluted environments as well as from contaminated soils through root systems. Also, the heavy metal contamination of vegetables may occur due to their irrigation with contaminated water [6].

The estimation of dietary exposure is crucial for risk evaluation and to determine the relationships between adverse effects observed in humans and dietary exposure to particular heavy metals. Risk assessment is a continually evolving process as new information on contaminants, their implicated health effects, and their occurrence in food are all factors that should be continuously studied and monitored [7]. The need for monitoring chemicals in the food supply is essential as consumers are unable to know what toxic chemicals and nutritional imbalances are posed by the foods they consume. When present at unacceptable amounts, chemicals are known or suspected to be responsible for a range of human health problems.

Numerous studies investigating dietary exposure to heavy metals have been carried out by several countries using the total diet study (TDS) approach [8-11], which consists of purchasing, at retail locations foods commonly consumed by the population, processing them for usual consumption, often combining them into food composites or aggregates, homogenizing them and analyzing them for toxic chemicals and certain nutrients. This approach takes into account the effects of kitchen preparation on the amounts of contaminants and nutrients in foods and provides dietary intake data for use by regulatory agencies and the

public. In Bangladesh, there is little or no monitoring of chemical contamination of the food supply. A survey of literature has shown that studies related to determination of heavy metal status of different food available in Bangladesh are very limited, particularly in Dhaka city. Due to almost nonexistence of such data, this study was necessary for determining their amount with an emphasis on the physiological aspects.

Bangladesh is blessed with many horticultural crops. Different kinds of vegetables are grown during the year in tropical Bangladesh, but very little is known about the heavy metals content of local vegetables. In an average Bengali home, the main meal would consist of boiled rice served with vegetables. So, the evaluation of dietary exposure of heavy metals through vegetables may be helpful for risk assessment of Bangladeshi population. In our study, we collected vegetables from four wholesale markets of Dhaka city. The vegetables that are mostly consumed were selected and analyzed for concentrations of heavy metals (Pb, Cr, Cd, Hg, AS and Sb) by inductively coupled plasma mass spectrometry (ICP-MS). The daily intake of heavy metals was determined using the total weight of vegetables consumed each day multiplied by the concentration of heavy metals in vegetables. The existing vegetable consumption patterns as provided by the Household Income & Expenditure Survey (HIES) [12] was used to calculate the dietary exposure.

The purpose of this study was to update the food composition database of selected vegetables commonly consumed by the residents of Dhaka city and to estimate the health risks from heavy metals in vegetables. This has been assessed by comparing the level of heavy metals in vegetables with the safe limit set by WHO Codex Alimentarius Commission [13] and USDA GAIN Report [14].

Methods and Materials:

Sampling protocol and collection

A sampling plan is the predetermined procedure for selection, collection, preservation, transportation and preparation of the analytical portion to be used from a lot as samples. A sampling plan should be a well organized document for program objectives [15]. The primary objective in food sampling is to collect representatives of food sample and to ensure that changes in nutrient composition do not occur between collection and analysis [16].

In the present study, in order to minimize the geographical variation, the selected vegetables samples (n=25) were purchased and collected from commercial settings of wholesale markets located at different locations of Dhaka city, where consumable food items are brought from all over the country. It thus ensured that representative consumable food items were obtained from all geographical locations. Each food sample was purchased from five different sites (shops) of each selected location (market). Also to avoid sampling error, a large portion (approximately 0.5 kg) of replicate samples for every item was collected from each collection point. However, sample size was limited by time, cost, sampling methods and logistics of sample handling, analysis and data processing.

In total 25 vegetables items-five subsamples-four locations = 500 vegetables samples were collected. Vegetable samples were collected in clean dark plastic poly bags and then transported to lab within the shortest time span. Since there was limited scope to study the seasonal variation in nutrient composition in the food composition database, food items, particularly the vegetables, fruits and fishes were collected during their peak availability period. Samples were collected very early morning from the collection points, taken to the lab and immediately processed for analysis.

Preparation of vegetable samples

Based on general guidelines provided by the WHO [8] and on sampling schemes described in the literature [11], a composite sampling approach was applied in the preparation of vegetable samples. The samples were prepared 'as consumed' before compositing and analysis.

For preparation of samples, five sub-samples collected from each market were washed with tap water followed by distilled and deionized water, gently swabbed with tissue paper and dried in air. Then equal portion of each sub-sample (peeled where needed) was weighed and cut into small pieces using a cleaned stainless knife on a cleaned plastic cutting surface. Then diced/portioned food samples were taken in a stainless steel bowl and mixed. Hand gloves were used throughout the process. Food composites were dried in an oven at 105°C until a fixed weight was obtained. Dried samples were ground into fine powder with a food blender (equipped with a stainless-steel blade). For each food sample, 3.0 g of blended powder from four sub-samples were combined to make a single composite sample. These composite samples were stored at -20°C until further analysis. In accordance with good laboratory practice, the equipment used for preparing and homogenizing the samples was thoroughly washed between each preparation (e.g. cleaning with a laboratory-grade detergent, rinsing thoroughly with hot tap water, rinsing or soaking with acid solution,

rinsing thoroughly with deionized water) to avoid the risk of cross-contamination. A total of 25 final composite samples were prepared for chemical analysis.

Digestion of composite Samples

The dried powdered samples (approximately 1 g) were digested in Teflon vessels with 9 ml nitric acid (70% v/v, supra pure) and 1 ml hydrogen peroxide (50% v/v), using a closed microwave digestion system. Blank digestion was also performed to quantify possible contamination during sample preparation and analysis. After digestion each sample was filtered into a 50 ml volumetric flask and filled to the mark with deionized water. The samples were further diluted as required and analyzed by ICP-MS. For quality control and validation of the analytical techniques, a Certified Reference Material (CRM) was also taken as a check on the accuracy of the analysis. Teflon vessels and all glass ware used were previously treated for 24 h in diluted nitric acid and rinsed with double distilled deionized water prior to use.

Analysis of heavy metals profile

The analytical quantification of heavy metals in composite samples such as Pb, Cr, Cd, Hg, As and Sb was determined by inductively coupled plasma mass spectrometry (ICP-MS). Performance of the instrument was checked by analyzing the certified reference material (NIES, Japan) concurrently to check the precision of the instrument. The results were reported on a wet weight basis.

Calculation of dietary intake

To calculate the distribution of exposure amounts, the existing vegetable consumption pattern as provided by the Household Income and Expenditure Survey (HIES) of Bangladesh Bureau of Statistics (BBS) [12] was combined with the average concentration for each heavy metals under study. The risk characterization was performed for average and high intake amounts and for percentage of individuals exceeding toxicological reference values (PMTDI) or not meeting the RDAs. Following equation was used for the calculation of dietary intake.

$$\begin{aligned} &\text{Daily intake of heavy metals } (\mu\text{g/day}) \\ &= [\text{Daily vegetable consumption (g)} \times \text{vegetable heavy metal concentration } (\mu\text{g/g})]. \end{aligned}$$

Results and Discussion:

The aim of this study was to monitor the presence of heavy metals in vegetables collected from different wholesale markets of Dhaka city. Five hundred vegetable samples were collected and analyzed for determination of heavy metals. A total of six heavy metals— lead, chromium, cadmium, mercury, arsenic and antimony were estimated. The food consumption data of HIES [12], 2010 were used to calculate the daily intake of heavy metals through vegetables by the residents of Dhaka city.

The analytical quality of the work was checked by analysis of certified reference materials (CRMs 10-b, NIES, Japan). The recovery percentages based on CRMs showed good agreement with the certified reference values for all elements. Corrections based on recovery percentages were not performed. The results of present reports have been presented as fresh weight basis of food.

Heavy metals analysis

Results of heavy metal analysis are presented in Table 1 and Table 2 and in Figure 1. The observed concentrations of heavy metals in the vegetables were compared with the recommended limit as established by WHO Codex Alimentarius Commission¹³ to assess the levels of food contamination.

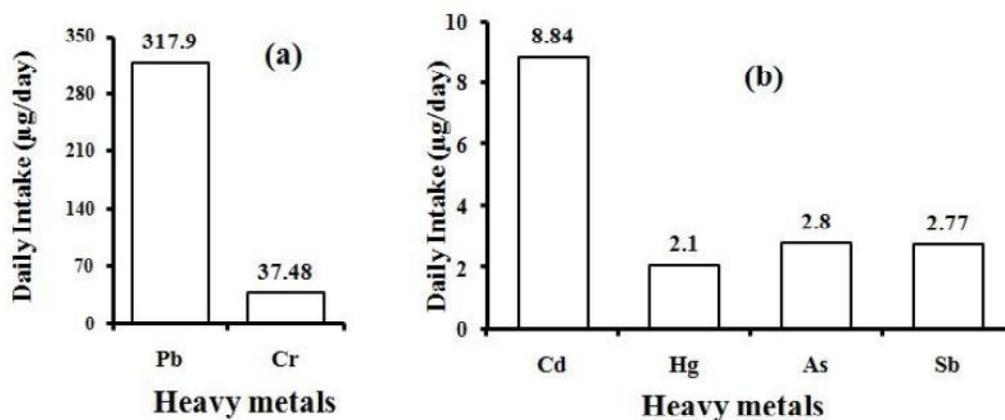
Table 1. Concentration ($\mu\text{g}/100\text{g}$) of heavy metals in vegetables.

English Name	Scientific Name	Pb	Cr	Cd	Hg	As	Sb
Banana (green)	<i>Musa paradisiaca</i>	168.5	14.52	0.29	0.22	nd	nd
Bitter gourd	<i>Momordicacharantia</i>	15.83	9.22	0.72	0.34	nd	nd
Brinjal	<i>Solanummelongema</i>	87.9	9.28	6.94	1.12	0.6	nd
Cabbage	<i>Brassica oleracea</i>	95.09	5.21	0.85	0.13	nd	nd
Cauliflower	<i>Brassica oleracea</i> var. <i>Botrytis</i>	66.91	14.51	2.35	0.82	1.04	nd
Carrot	<i>Daucuscarota</i>	108.9	14.89	2.87	0.37	nd	0.30
Colocasia/Taro	<i>Colocasiaesculenta</i>	1043	28.29	19.02	0.65	nd	nd
Coriander leaf	<i>Coriandrum</i> <i>sativum</i>	18.68	55.00	4.94	0.38	1.27	nd
Cucumber	<i>Cucumissativus</i>	45.01	3.07	0.46	0.10	0.18	1.15
Green chili	<i>Capsicum annum</i>	159.9	24.37	3.19	0.66	0.69	nd
Indian spinach	<i>Basella alba</i>	7.32	10.05	2.75	7.83	0.44	nd
Lady's finger	<i>Abelmoschus</i> <i>esculentus</i>	16.71	17.83	4.9	4.93	nd	nd
Long bean	<i>Vigna</i> <i>anguiculata</i> sub. <i>sesquipedalis</i>	355.1	9.94	7.20	0.22	nd	nd
Papaya(green)	<i>Carica papaya</i>	58.74	57.97	3.43	0.33	nd	nd
Pointed gourd	<i>Trichosanthes</i> <i>dioica</i>	293.8	8.80	0.8	0.77	4.40	3.90
Potato	<i>Solanum</i> <i>tuberosum</i>	65.84	14.58	11.71	0.45	nd	nd
Pumpkin	<i>Cucurbita</i> <i>maxima</i>	31.26	12.84	1.67	0.3	nd	nd
Radish	<i>Raphanus</i> <i>sativus</i>	71.47	4.03	0.86	0.10	1.14	nd
Ribbed gourd	<i>Luffa</i> <i>acutangula</i>	2.79	3.96	1.3	0.48	nd	nd
Snake gourd	<i>Trichosanthes</i> <i>anguina</i>	39.41	6.33	0.60	0.19	1.10	1.76
Spiny gourd	<i>Momordica</i> <i>dioica</i>	37.62	7.70	0.33	0.67	0.58	nd
Tomato	<i>Solanum</i> <i>lycopersicum</i>	152.4	5.96	1.39	0.11	nd	nd
Water gourd	<i>Lagenaria</i> <i>siceraria</i>	134.1	5.36	9.42	0.10	nd	nd
Water spinach	<i>Ipomoea</i> <i>aquatica</i>	24.49	39.01	1.80	0.50	1.27	nd
White gourd	<i>Benincasa</i> <i>hispida</i>	197.1	6.04	2.00	0.10	nd	nd

Table 2. Range ($\mu\text{g}/100\text{g}$) and mean concentrations ($\mu\text{g}/100\text{g}$) of heavy metals in vegetables.

	Pb	Cr	Cd	Hg	As	Sb
Range	2.79-1043	3.07-57.97	0.29-19.02	0.10-7.83	0.18-4.4	0.3-3.9
Mean	131.9	15.55	3.67	0.87	1.16	1.15
Safe level	30.0	5.0	10.0	3.0*	10.0	-----

Safe level set by the Codex; Alimentarius Commission (FAO/WHO, 2001).*USDA GAIN Report [14]

**Figure 1:** Per capita daily intake of heavy metals: (a) Pb& Cr and (b) Cd, Hg, As and Sb.

Lead: Exposure to lead (Pb) is of concern mainly because of its possible detrimental effects on intelligence. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders [17]. In the present study, colocasia/taro (1043 µg/100g) contained high amounts of Pb while long bean (355.1 µg/100g) and pointed gourd (293.8 µg/100g) had medium and Ribbed gourd (2.79 µg/100g) low amounts of Pb (Table 1). As shown in Table 2, the amount of Pb in vegetables ranged from 2.79 to 1043 µg/100g which was consistent with the result (0.0-1044 µg/100g) found by Szymczak et al. [18]. Rahman et al. [19] assessed the median concentrations of Pb in Bangladeshi vegetables and found it to be 2100 µg/100g. Mean concentration of Pb (131.9 µg/100g) (Table 2) in our study was higher than the allowable amounts of Pb as set by WHO Codex Alimentarius Commission [13]. Lead limit set by the Codex was 30 µg/100g fresh vegetables. Yang et al. [20] also reported that the concentrations of Pb in vegetables available in Chinese market exceeded the safety limits given by FAO/WHO and Chinese regulations, indicating serious contamination. The dietary exposure of Pb through vegetables by Dhaka city residents was 317.9 µg/day (Figure 1a). Rahman et al. [19] reported 56.4 µg daily consumption of Pb from vegetables and drinking water in a severely As-contaminated area of Bangladesh. On the other hand, Alam et al. [21] estimated the average daily intake of Pb 74.7 µg through vegetables in Samta village of Jessore district, Bangladesh.

Chromium: Although water insoluble chromium (III) compounds have health benefits, Cr(VI) compounds, which are powerful oxidizing agents and thus tend to be irritating and corrosive and can cause DNA damage [22, 23]. In the present study, papaya (green) (57.97 µg/100g) and coriander leaf (55.00 µg/100g) were found to contain high amounts and colocasia/taro (28.29 µg/100g) moderate amount of Cr. On the other hand, radish (4.03 µg/100g), ribbed gourd (3.96 µg/100g) and cucumber (3.07 µg/100g) contained low amounts of Cr (Table 2). In the present study, the range and mean of Cr in the vegetable group were found to be 3.07-57.97 µg/100g and 15.55 µg/100g (Table 2), respectively whereas Leblanc et al. [24] reported a mean 5.00 µg/100g concentration of Cr in French vegetables. Rahman et al. [19] reported very low median concentrations of Cr (80.0 µg/100g dry weight) in vegetables of Bangladesh. In this study, the mean concentration of Cr in vegetables was higher than the safe limit recommended by WHO Codex Alimentarius Commission [13]. As shown in Figure 1a, we found that the per capita daily intake of Cr through vegetables was 37.48 µg for residents of Dhaka city. On the other hand, Rahman et al. [19] reported a lower daily intake of Cr (20.8 µg) from vegetables and drinking water for Bangladeshi people.

Cadmium: Cadmium is an extremely toxic metal commonly found in industrial workplaces and is easily taken up and accumulated by plants and crops through the root systems. Cadmium acts as a catalyst in forming reactive oxygen species [25]. It increases lipid peroxidation, depletes antioxidants, glutathione and protein-bound sulfhydryl groups, and promotes the production of inflammatory cytokines [25]. In the present study, the highest concentration of Cd was found in colocasia/taro (19.02 µg/100g) whereas cucumber (0.46 µg/100g), spiny gourd (0.33 µg/100g) and banana (green) (0.29 µg/100g) were the lowest source of Cd among the vegetables. Potato (11.71 µg/100g) and water gourd (9.42 µg/100g) contained moderate amount of Cd (Table 1). The range and mean of Cd in vegetables were 0.29-19.02 µg/100g and 3.67 µg/100g (Table 2), which was lower than the range 12-113 µg/100g reported by Weldegbriel et al. [26] in Ethiopia. Rahman et al. [19] reported the median concentrations of Cd 11.1 µg/100g in vegetables of Bangladesh. The allowable amount of Cd, as set by the WHO Codex Alimentarius Commission [13] is 10.0 µg/100g of vegetables. Thus, the amounts of Cd in the vegetables of our study were within the safe limit. Our study also indicates that the average Cd amounts measured in vegetables from Dhaka city were higher than in vegetables of Bombay, India, which exhibited Cd amounts of 0.12 µg/100g to 2.46 µg/100g [27]. Similar results were also reported by Alam et al. [21] in vegetables collected from Samta village of Jessore district, Bangladesh. Al-Rmalli [28] found that the Cd concentration ranged from 0.33 to 30.3 µg/100g in leafy vegetables collected from Sylhet district of Bangladesh.

The daily exposure of Cd per person in our recent study was 8.84 µg (Figure 1b) through vegetables. On the other hand, Rahman et al. [19] reported 2.9 µg daily consumption of Cd from vegetables and drinking water by the average Bangladeshi adult. On the other hand, in Samta village of Jessore district, Bangladesh, Alam et al. [21] estimated the average daily intake of Cd to be 9.45 µg through vegetables. Al-Rmalli et al. [29] reported daily intake of 12.03 µg Cd from vegetables by Bangladeshi residents living in UK.

Mercury: Mercury (Hg) is a heavy metal occurring in several forms, all of which can produce toxic effects in high doses. Toxic effects include damage to the brain, kidney, and lungs [30]. All vegetables had a very low amount of Hg (0.10-4.93 µg/100g) whereas Indian spinach had the highest amount of Hg (7.83 µg/100g) (Table 1). In this study, the range and mean of Hg in vegetables were found to be 0.10-7.83 µg/100g and 0.87 µg/100g (Table 2), which was within the limit of safe level of Hg in vegetables (3.0 µg/100g, USDA GAIN Report) [14]. Leblanc et al. [24] found 0.5 µg/100g of Hg in vegetables of France. The dietary exposure of Hg through vegetables was 2.1 µg/day per person by residents of Dhaka city (Figure 1b).

Arsenic: Arsenic and many of its compounds are especially potent poisons. They can cause hydrogen peroxide production which may form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure [31]. The majority of vegetables contained very low amounts of As (0.18-1.27 µg/100g) whereas pointed gourd contained the highest amount of As (4.40 µg/100g) (Table 1). Saha and Zaman [32] investigated the concentrations of heavy metals in vegetables collected from Shaheb Bazar of Rajshahi City, Bangladesh and also reported that pointed gourd contains the highest concentrations of As in vegetables.

In this study, the range and mean of As in vegetables were found to be 0.18-4.4 µg/100g and 1.16 µg/100g (Table 2). Whereas, Leblanc et al. [24] found 1.1 µg/100g of As in vegetables. Rahman et al. [19] reported 9.0 µg/100 g median concentrations of As in Bangladeshi vegetables. Alam et al. [21] reported that the mean and range of the total As concentrations in all the vegetables from the Dhaka markets were 11.4 and 0.10–29.3 µg/100g (dry-weight basis), respectively. On the other hand, Williams et al. [33] reported that the As concentrations in leafy vegetables of Bangladesh vary from 13.0 to 79.0 µg/100 g (dry-weight basis). The safe level of As in vegetables is set at 10.0 µg/100g for vegetables by WHO Codex Alimentarius Commission [13]. As shown in Figure 1b, the per capita daily intake of As from vegetables was 2.8 µg. Rahman et al. [19] reported a very high amount of daily intake of As (839 µg) by Bangladeshi people from vegetables and drinking water in a severely As-contaminated area of Bangladesh. Alam et al. [21] estimated the average daily intake of As 27.78 µg through vegetables in Samta village of Jessore district, Bangladesh.

Antimony: Antimony toxicity occurs either due to occupational exposure or during therapy. Occupational exposure may cause respiratory irritation, pneumoconiosis, antimony spots on the skin and gastrointestinal symptoms. In addition antimony trioxide is possibly carcinogenic to humans³⁴. Besides its harmful effects, as a therapeutic agent, antimony has been mostly used for the treatment of leishmaniasis and schistosomiasis³⁴. Except a few exceptions, most of the vegetables contain undetectable levels of Sb while pointed gourd (3.90 µg/100g) contained the highest amount of Sb (Table 1). The range and mean of Sb in vegetables were 0.3-3.9 µg/100g and 1.15 µg/100g (Table 2). The dietary exposure of Sb through vegetables was 2.77 µg/day per person (Figure 1b).

The data presented in the present study indicate that the average concentration of the studied heavy metals through vegetables fall within the range of values reported by others and below the safe limits, except for Pb and Cr. (Table 1). As colocasia/taro (1043 µg/100g), long bean (355.1 µg/100g), pointed gourd (293.8 µg/100g) and white gourd (197.1 µg/100g) contained high amounts of Pb, avoiding of these vegetables may reduce Pb exposure. Papaya (green) (57.97 µg/100g), coriander leaf (55.00 µg/100g) and colocasia/taro (28.29 µg/100g) contained high amounts of Cr. By consumption of less of such vegetables, one may reduce exposure to Cr. Among other heavy metals, Cd exposure was also high, though it was below the safe limit. Only colocasia/taro (19.02 µg/100g) and potato (11.71 µg/100g) contained amounts of Cd greater than that of the safe limit. So according to our present study consumption of vegetables with less amounts of colocasia/taro, long bean, pointed gourd, white gourd, Papaya (green), coriander leaf and potato by Dhaka city residents may reduce the heavy metal toxicity.

In Bangladesh, soil is contaminated with heavy metals and pesticides residues through the application of chemical fertilizers, and pesticides (SRDI) [35]. Heavy metals occur in many fertilizers and in some pesticides, purposefully included as micro nutritional or biopesticidal components or present as naturally occurring contaminants. The main sources of soil pollution by heavy metals are phosphate fertilizers. Therefore, indiscriminate use of fertilizer and pesticides needs to be controlled to reduce heavy metal contamination.

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