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Evaluation of Health Risk and Arsenic Levels in Vegetables Sold in Markets of Dhaka (Bangladesh) and Salamanca (Spain) by Hydride Generation Atomic Absorption Spectroscopy

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Abstract The main objective of this study was to determine arsenic (As) levels in vegetables collected from the markets of Dhaka, Bangladesh and for comparison from Salamanca, Spain by HGAAS under optimal conditions, and the potential health risk from consumption of these vegetables. The mean and range of the total As concentration in the vegetables from the markets of Dhaka, Bangladesh were 114 and 1.0–293 μg/kg, respectively. Total As concentration in 77 % of Bangladesh vegetables measured was higher than that recommended by the standard. The mean and range of As concentrations for vegetables grown in Spain were 65 and bdl–130 μg/kg, respectively, for Salamanca, 102 and bdl–423 μg/kg, respectively, for Almeria. The As content of the Bangladesh vegetables was approximately twofold to threefold higher than those observed for the vegetables from Almeria and Salamanca (Spain), but in some cases, were similar or less. Daily consumption of As-rich vegetables may result in an additional source of As in the diet, based on the provisional tolerable intake of As for adults recommended by WHO.

Keywords Arsenic · Vegetables · Food chain · Health risk

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Arsenic uptake by the edible parts of plants, especially from the bioavailable As of soils contribute a significant part of As into the human food chain. Arsenic exposure via consumption of field, garden and home-grown vegetables, crops, and fruits may constitute a potential health risk. As a result, it is the most common and priority interest contaminant at hazardous waste sites in the United States and the EU, and has been classified by the US Environmental Protection Agency (EPA 2001) as a group A human carcinogen to skin and lungs (WHO 1980). Although As is available mostly as inorganic form (arsenate and arsenite) in the water and soils, but biological samples such as vegetables, crops, fish etc contain both inorganic and organic forms (MMA, DMA and TMA) of As in different proportions. Long-term exposure to As via water, air, soils and food chain can cause significant health effects on the gastrointestinal tract, respiratory tract, skin, liver, cardiovascular system, hematopoietic system, nervous system, etc. As for example, many symptoms of As poisoning and arsenicosis are clinically observed in populations of a few countries, particularly in Bangladesh, India, Taiwan, Inner Mongolia, Chile, Argentina etc (Anawar et al. 2002).

Due to the low solubility and bioavailability of As in soils, As uptake by terrestrial plants is generally low and variable that rarely exceeds 1 mg/kg DW (dry weight basis) (Adriano 2001), and sometimes varies from less than 0.01 to about 5 mg/kg (Mandal and Suzuki 2002). A number of studies have reported the total As content of foodstuffs from different countries (Dabeka et al. 1993; Tsuda et al. 1995; Sapunar-Postruznik et al. 1996; Roychowdhury et al. 2002; Alam et al. 2003; Das et al. 2004). For example, Sapunar-Postruznik et al. (1996) reported low levels of As in fruits (0.2 μg/kg) and vegetables (0.4 μg/kg) in Croatia. The mean and range of As concentrations in food samples collected from different cities in Canada were 73.2 and

<0.1–4,830 µg/kg, respectively (Dabeka et al. 1993). Schoof et al. (1999) reported high concentrations of As in the sea fish (2,360 µg/kg) compared to 74 µg/kg of inorganic As in rice. However, the ranges of As in vegetables grown in an As-affected region of Bangladesh were 19–489 µg/kg (Alam et al. 2003) and 70–3,990 µg/kg (Das et al. 2004). Food composites (potato skin, leaves of vegetables, rice, wheat, cumin, turmeric powder, and cereals, etc.) collected from As-affected areas of the Murshidabad district, West Bengal, India contained 7–373 µg/kg of As (Roychowdhury et al. 2002).

A large number of analytical techniques are available to determine the concentrations of As in different environmental samples. Out of those, inductively coupled plasma mass spectrometry, inductively coupled plasma atomic emission spectrometry or inductively coupled plasma optical emission spectrometry, flame atomic absorption spectrometry, hydride generation and graphite furnace-atomic absorption spectrometry are widely used (Ajtony et al. 2008). Hydride generation atomic absorption spectrometry (HGAAS) is an easily affordable, well established and well-known technique for the determination of As and some other hydride-forming elements in environmental samples (Anawar 2012), because this method is the most sensitive technique with limits of detection (LOD) in the range from µg/L to ng/L. An important advantage is related to the analyte separation from the matrix components, offering considerable suppression of matrix effects (Dedina and Tsalev 1995). HGAAS can directly measure the high concentrations of As in the acid digest samples without any further modification for interferences.

The study by Alam et al. (2003), Das et al. (2004), Williams et al. (2006) and Kurosawa et al. (2008) focused on As levels in vegetables specifically collected from 2 to 3 limited regions of Bangladesh such as Samta and Marua village located in the Jessore district, that are well-known to contain very high levels of As in groundwater. However, their studies can not predict the general view of As intake by the populations living in Dhaka and other cities of Bangladesh, and their health risk. Therefore, the objective of this study was to estimate the total As concentration in different types of vegetables collected from the markets of Dhaka, whose origin of cultivation was unknown, by HGAAS, prior to microwave assisted acid digestion. For comparision, the similar types of vegetables were collected from the two regions of Spain, namely Salamanca and Almeria.

Materials and Methods

A total of 28 vegetable samples of bean, carrot, potato, tomato, brinjal, onion, khirai and pulse were collected from the markets of Dhaka in Bangladesh and 40 samples from Salamanca market in Spain. The vegetable samples in Salamanca market came from the agricultural fields of two localities: 17 samples of bean, carrot, potato, brinjal, onion, kalabachin, pimiento, and lettuce from Almeria (Spain) and 23 vegetable samples of carrot, potato, tomato, brinjal, onion, kalabachin, pimiento, and lettuce from Salamanca fields (Spain). Collected vegetable samples were cleaned with deionized water and air-dried at room temperature for several days. The air-dried vegetable samples were powdered homogenously and preserved in dessicator until analysis.

Powdered vegetable samples (0.5 g) were digested with water (2 mL), hydrogen peroxide (2 mL) and conc. HNO_3 (8 mL) using a CEM (MDS-2000) microwave oven at pressure of 9 atm (10 min) and at 12 atm (15 min). After cooling, the digests were passed through a pre-washed filter (Whatman no. 540), the digestion tubes were rinsed three times, passing washings through the filter and the digests were enriched with conc. H_2SO_4 (0.5 mL). The digests were then heated at 230°C for 2–3 h and concentrated by evaporation to approximately 0.5 mL.

The standard stock solutions for As(III) (1 mg/L) were prepared in 100 mL calibrated flask using commercial stock standard (Panreac Quimica SA). Standard solutions of As(III) were prepared by stepwise dilution of a 1 mg/L standard solution just before use. Diluted working solutions were prepared daily by serial dilutions of this stock solution.

Arsenic was measured by HGAAS. The operating conditions for As measurement are described in Table 1. The burner parameters and the burner position were optimized daily to have the As Hollow Cathode Lamp radiation at target point.

The HGAAS technique was used for the determination of As in certified and real samples of vegetables. The HGAAS technique is based on the reaction of $NaBH_4$ with the acidified sample and the separation of the analyte, as hydride from the matrix before measurement. It was one of the main advantages, as it significantly reduces the possibility of interferences (Anawar 2012). For pre-reduction of As(V) to As(III), 5 mL ascorbic acid (25 %), 5 mL potassium iodide (25 %) and hydrochloric acid (2M) were added to the digests up to the volume of 25 mL. Arsenic concentrations were measured in duplicate by using VARIAN spectra AA-220 and hydride generator VGA-76 atomic absorption spectrophotometer (AAS) with a commercial stock standard (Panreac Quimica SA).

The accuracy and precision of the analyses for As were checked against the Maize leaves material FD8, Commission of the European Communities, Joint Research Centre ISPRA. The results obtained were in excellent agreement with the certified values for the method of microwave digestion.

Table 1 Operational conditions employed in the determination of As by HG-FAAS

Parameter	As
Wavelength (nm)	193.7
Spectral bandwidth (nm)	0.4
Lamp	As Hollow Cathode Lamp
Lamp current	8 mA
Oxidant (air) L/min	17
Fuel (acetylene L/min)	2.2
Signal processing	Peak area (absorbance)
Replicate	3
Background correction mode	Deuterium
Atomization site	Quartz tube atomizer (QTA)
$NaBH_4$ solution concentration	0.5 % (m/v), stabilized with 0.1 % (m/v) NaOH
$NaBH_4$ and HCl solution flow rate	2.2 mL min^{-1}

All statistical analyses were performed using the Microsoft EXCEL (version 2003). Analysis of variance (ANOVA) was employed to examine the statistical significance of differences in the mean concentration of As between (or among) group of families of vegetables. All levels of As are reported as milligrams of total As per kilogram. The average value was achieved using replicate measurements of the several same type of samples taking into account the correction by the recovery value. In addition, the range and the standard deviation (S.D.) were determined.

Results and Discussion

The calibration curve fit (at least five standard concentrations) displayed R^2 values >0.98 in all cases. The method's recovery of As (0.79 ± 0.08 mg/kg) from a certified reference material was not significantly different from the certified value (0.77 ± 0.1 mg/kg). The percentage recoveries of total As were observed as 102.6 %. The mean As concentration in blank digests was 0.09 μg/L, and the method's detection limit (LODs) for As in vegetable tissue was 0.07 μg/L. The precision of the method was assessed by performing the analysis of reference sample ten times for a single sample. The precision of the techniques, expressed as RSD, was between 5 % and 10 %. A total of 31 different vegetable samples were collected from the markets of Dhaka, Bangladesh in order to determine their As content and health risk. For comparison purposes, vegetable samples were also collected from the local markets of Salamanca, Spain, that originated from Salamanca agricultural field (24 samples), and Almeria agricultural field (Spain) (23 samples) to determine their As content. The analytical results of total As concentration from all vegetable samples are summarized in Tables 2, 3 and 4.

Table 2 Concentration of As in vegetables collected from the market of Dhaka (μg/kg)

Sample	Sample no	Range	Mean	±SD
Bean	3	26–182	96	79
Brinjal	3	80–293	160	116
Carrot	6	99–227	174	48
Khirai	3	20–175	98	110
Onion	4	92–232	136	65
Potato	5	22–160	101	69
Pulse	2	75–85	80	7.1
Tomato	5	1–112	65	50

The vegetable samples of brinjal, carrot and onion in Bangladesh had higher concentrations of As in contrast to bean, khirai, potato, pulse and tomato. Potato had medium level of As and the brinjal had the highest As concentration. The mean and range of the total As concentrations in all the vegetables from the market of Dhaka, Bangladesh were 114 and 1.0–293 μg/kg (dry-weight basis), respectively (Table 2). The highest mean values of As were for the carrot, 174 μg/kg, followed by brinjal, 160 μg/kg and onion, 136 μg/kg. The mean accumulation ability of the vegetables decreased in the order of carrot > brinjal > onion > potato > khirai > bean > pulse > tomato in Bangladesh. The food safety standard for inorganic As in vegetables, adopted in China, which is the only one of its kind in the world, is 50 μg/kg (MOH and SAC 2005). Total As concentration (inorganic + organic) in majority of Bangladesh vegetables measured was higher than that recommended by the standard.

Williams et al. (2006) reported the As concentrations from 130 to 790 μg/kg (dry-weight basis) in leafy vegetables of Bangladesh. Alam et al. (2003) and Kurosawa et al. (2008) found As concentrations of 440 μg/kg, and 690–1,700 μg/kg, respectively, in taro leaves collected from the Marua and Samta village, two most well-known As contaminated areas, located in the Jessore District of Bangladesh. Das et al. (2004) found As concentrations ranging from 90 to 3,990 μg/kg in the leaf of arum tuber and from 70 to 1,360 μg/kg in potatoes. The mean and range of As contents in vegetables investigated in this study are lower than those from the previous studies. However, As contents for some specific vegetables and some samples of the same type are similar with the previous studies. For example, the As contents for potato are similar to those found by Das et al. (2004). The concentration of As in Bangladesh vegetables sold in UK market ranged from 5 to 540 µg/kg with a mean value of 54.5 µg/kg (Al Rmalli et al. 2005). The range of As content in vegetables, sold in the markets of Dhaka and measured in this study, is lower, but the mean As content is higher than the results reported by Al Rmalli et al. (2005). The mean As concentrations in vegetables (from Bangladesh) in our study are lower than those in potato skin (104–293), leaf of vegetables (212–295), and arum leaf (331–341), but similar to that in vegetables (92–123) from West Bengal, India (Roychowdhury et al. 2002).

The mean and range of As concentrations in Bangladesh vegetables in our study are many folds higher than those from the studies of Canada (7 µg/kg; Dabeka et al. 1993), UK (2/4.9 µg/kg, fresh weight; FSA 2003), and Croatia (0.4 µg/kg; Sapunar-Postruznik et al. 1996). In our case, we do not know if the vegetables investigated in our study came from an As-affected region of Bangladesh or not. Nevertheless, particularly high levels of As detected in majority of these vegetables suggest that they may originate from As-affected regions. This is highly plausible considering the scale of the As problem in Bangladesh, where 41 out of the 64 districts are known to contain high levels of As in their groundwater (BGS and DPHE 2001) that is used for irrigation on the agricultural fields.

The vegetable samples of brinjal, carrot, kalabachin, and lettuce had the higher As concentrations than bean, onion, potato, and pimiento from Almeria (Spain) (Table 3). The vegetables of brinjal, carrot, kalabachin, lettuce, onion, pimiento, potato, and tomato from Salamanca fields (Spain) had lower to medium concentrations of As (Table 4). The mean accumulation ability of the vegetables decreased in the order of brinjal > lettuce > carrot > kalabachin > onion > bean > potato > pimiento in Almeria, and lettuce > brinjal > onion > kalabachin > carrot > pimiento > tomato > potato in Salamanca. The mean and range of As concentrations for vegetables grown in Spain were 65 and bdl–130 µg/kg, respectively, for Salamanca (Table 4), 102 and bdl–423 µg/kg, respectively, for Almeria (Table 3). Highest As concentration was 130 µg/kg in lettuce from Salamanca, 423 µg/kg in brinjal

Table 3 Concentration of As in vegetables from Almeria (Spain) (µg/kg)

Sample	Sample no	Range	Mean	±SD
Bean	3	3–27	17	12
Brinjal	2	362–423	393	43
Carrot	3	33–196	93	90
Kalabachin	3	72–97	83	12.7
Lettuce	3	152–206	173	29
Onion	3	26–52	43	15
Pimiento	3	bdl-17	5.7	9.8
Potato	3	bdl-28	9.4	16.1

Table 4 Concentration of As in vegetables from Salamanca (Spain) (µg/kg)

Sample	Sample no	Range	Mean	±SD
Brinjal	2	78–99	89	15
Carrot	3	55–67	63	6.7
Kalabachin	3	54–80	64	14
Lettuce	3	98–130	110	17
Onion	3	48–103	65	26
Pimiento	2	55–64	60	6
Potato	5	bdl-27	24	3.8
Tomato	3	41–52	46	5.6

and 206 µg/kg in lettuce from Almeria. The average As content of the vegetables from Bangladesh was approximately twofold to threefold higher than those observed for vegetables from Almeria and Salamanca of Spain excluding brinjal and lettuce. Arsenic levels found in vegetables of Spain, in some cases, are similar to and /or higher than those recorded for vegetables of Bangladesh. As for example, lettuce and brinjal from Almeria had the highest As concentration than any vegetables collected from Dhaka, Almeria and Salamanca. The brinjal, carrot, kalabachin, lettuce and onion from Salamanca, and brinjal, carrot, kalabachin and lettuce from Almeria of Spain showed moderate to elevated levels of As. In both regions of Spain, brinjal and lettuce showed the highest As concentrations. This raises the possibility that the level of As intake via certain vegetables may be significant for the population of Spain and requires further investigations.

According to WHO (1981), 1.0 mg of inorganic As per day may give rise to skin lesions within a few years. The provisional maximum tolerable daily intake (PMTDI) of inorganic As in the UK is 120 µg. More than 60 % of the total As in foodstuffs exists in the more harmful inorganic form (Dabeka et al. 1993). The implication of our study is that a section of the Bangladesh and Spanish population may be unknowingly consuming As-contaminated vegetables. This could have serious long-term health problems for these people that need to be explored in detail. In Bangladesh, vegetables make up approximately 16 % of the total diet. The average per capita consumption of leafy and non-leafy vegetables is 130 g/person/day for males and females of all ages. This is considerably less than the recommended amount of 200 g/person/day from the nutritional point of view (Hassan and Ahmad 2000). The average intake of total As from leafy and non-leafy vegetables was estimated to be 14.82 µg/day that is about two times lower than the estimates (27.78 µg/day) of Alam et al. (2003) from Samta village of Bangladesh. Compared to the tolerable weekly intakes (PTWIs) and provisional maximum tolerable daily intakes (PMTDIs) recommended by the Joint Expert Committee on the Food Additives (JECFA), dietary exposures to As via vegetables in Dhaka is below the JECFA PTWI of 15 µg/kg body weight (equivalent to 130 µg/day for a 60 kg adult) for inorganic As (WHO 1989). Arsenic intake per day via consumption of vegetables in Dhaka is less than the total dietary As intake per day in Japan (160–280 µg; Tsuda et al. 1995), USA (88 µg; Gunderson 1991), the United Kingdom (65 µg; Ministry of Agriculture, Fisheries and Food 2000, 56–67 µg; MAFF 1998 and 63 µg; MAFF 1997), Canada (59.2 µg; Dabeka et al. 1993), Sweden (60 µg; Jorhem et al. 1998), Denmark (118 µg; NFAD 1990) and Basque country (291 µg; Urieta et al. 1996). In comparison, the estimated dietary As intakes are lower in Belgium (12 µg; Buchet et al. 1983), Croatia (11.7 µg; Sapunar-Postruznik et al. 1996), and the Netherlands (15 µg; De Vos et al. 1984). The contribution of drinking water, rice, other crops, meats, fishes and spices to dietary exposures was not included in these estimates of Bangladesh that may be several folds higher than that from vegetables.

The vegetables like brinjal, carrot and onion in Bangladesh had the higher concentrations of As in contrast to bean, khirai, potato, pulse and tomato; and brinjal had the highest As concentration. The mean and range of the total As concentrations in all the vegetables from the markets of Dhaka were 114 and 1.0–293 µg/kg, respectively. The highest mean values of As were found for the carrot, 174 µg/kg, followed by brinjal, 160 µg/kg and onion, 136 µg/kg. The mean accumulation ability of the vegetables decreased in the order of carrot > brinjal > onion > potato > khirai > bean > pulse > Tomato in Bangladesh. The mean accumulation ability of the vegetables decreased in the order of brinjal > lettuce > carrot > kalabachin > onion > bean > potato > pimiento in Almeria, and lettuce > brinjal > onion > kalabachin > carrot > pimiento > tomato > potato in Salamanca. The mean and range of As concentrations for vegetables grown in Spain were 65 and bdl–130 µg/kg, respectively, for Salamanca, 102 and bdl–423 µg/kg, respectively, for Almeria. Highest As concentrations were 130 µg/kg for lettuce in Salamanca and 423 µg/kg for brinjal in Almeria. The brinjal, carrot, kalabachin, lettuce and onion from Salamanca, and brinjal, carrot, kalabachin and lettuce from Almeria of Spain showed moderate to elevated levels of As. In both regions of Spain, brinjal and lettuce showed the highest As concentrations. The average intake of total As from the leafy and non-leafy vegetables sampled from the market of Dhaka was estimated to be 14.82 µg/day. Daily consumption of As-rich vegetables can result in an excessive intake of As in the group of populations of Bangladesh and some regions of Spain, based on the provisional tolerable intake of As for adults recommended by WHO. The method's recovery of As using a certified reference material exhibited that the percentage recoveries of total As were 102.6 %. The precision of the technique, expressed as relative standard deviation, was between 5 % and 10 %.

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