Title:

Health Risk Assessment for Bangladeshis due to Arsenic Exposure from Consumption of Vegetables Grown with Natural Arsenic Contaminated Groundwater

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SUPPLEMENTARY INFORMATION (SI)

1.0 Materials

The chemicals used in this investigation were of high purity analytical grade. Distilled deionized water (DDW), obtained through the deionization plant (TYP-2500, Deng Yuan, China), was used throughout. The certified reference materials (CRM) of arsenic standard solutions for AAS (TraceCERT®) (Fluka, Switzerland) were used for calibration purposes. A number of standard solutions, reducing solutions, ternary acid mixture and other solutions were prepared according to respective standard procedures [1-3]. The other reagents were from Merck (Germany), Fluka (Switzerland) and Sigma Aldrich (USA). These were used without further purification. Two certified reference materials [Tomato Leaves (SRM 1573a) and Lake Sediment (NIES CRM 31)] and one internal reference material (prepared very cautiously by control water with CRM Standard Solution for AAS) were digested and analyzed in five or four replicates for total As concentration under the identical experimental conditions to check accuracy of the method.

2.0 Digestion of the Environmental Samples

A. Digestion of Water Samples:

Prior to analysis of water samples for total arsenic (As_T), the water samples were subjected to mild digestion with HNO_3 [1]. 100 mL of well-mixed, acid-preserved water sample was taken in an Erlenmeyer flask (125 mL). 5 mL of 68% HNO_3 was added to it and the flask was placed on a hot plate to evaporate (without boiling) to 15-20 mL. Additional conc. HNO_3 was added and heating was continued until digestion was complete as shown by a clear solution. It was transferred to a 100 mL volumetric flask with two rinsings. The pH of the solution was adjusted to 4.0 with 1.0 M NaOH solutions followed by dilution with distilled, deionized water up to the mark. It is to be noted that the water samples were not pretreated for analyzing cations of alkali and alkaline earth metals such as Na, K, Mg and Ca.

B. Digestion of Biological Samples:

Oxidation of the organic matter of plant tissue and release of the elements such as arsenic was carried out through *Wet Oxidation* by means of oxidizing acids such as the HNO_3-H_2SO_4-$HClO_4$ ternary mixture [2-4]. All biotic samples (dried and powered) were accurately weighted (1.0 ± 0.005 g) and taken in a 500-mL Erlenmeyer flask. After adding 10 mL of 69% HNO_3, the flask was swirled to moisten the entire mass of the tissue and was placed on a steam plate for 30 minutes. It was then placed on the electric hot plate at 180^0C. The suspension was boiled nearly to dryness. This pre-digestion with HNO_3 required about 45 minutes.

The digestion flask and the contents were cooled slightly. Then 10 mL of the ternary acid mixture (prepared by mixing 69% HNO_3, 98% H_2SO_4 and 60% $HClO_4$ in the volume ratio of 10 : 1: 4) was added. The digestion was carried out on the electric hot plate at 220^0 C until dense white fumes of H_2SO_4 and $HClO_4$ were vigorously evolved. The digestion was stopped when the residues in the flask were clear and white and only slightly moist with H_2SO_4. The $HClO_4$, at that point, had been largely removed. This digestion with ternary acid mixture required about 2 hours.

The digestion flask and the contents were cooled and 10 mL of 82% HCl was added. The flask was swirled and the digestate was collected in a 100-mL measuring flask. After rinsing the flask with small amounts of 6N HCl, it was washed twice with distilled, deionized water and diluted to make a final volume of 100 mL.

C. Digestion of Soil Samples:

Digestion of soil samples were carried out by modified method of *Small and McCants* with oxidizing acids such as the H_2SO_4-$HClO_4$ acid mixture [2-3, 5]. Accurately 0.5 ± 0.005 g of the soil sample was heated in a 250-mL Erlenmeyer flask until fuming with 2 mL of 98% H_2SO_4. When the organic matter had been destroyed and the soil assumed a gray color, the flask was cooled and 3 mL of 70% $HClO_4$ were added with a few boiling chips. The mixture was boiled on an electric hot plate at 220^OC for about 2 hours. Heating was discontinued when the soil was near to dryness.

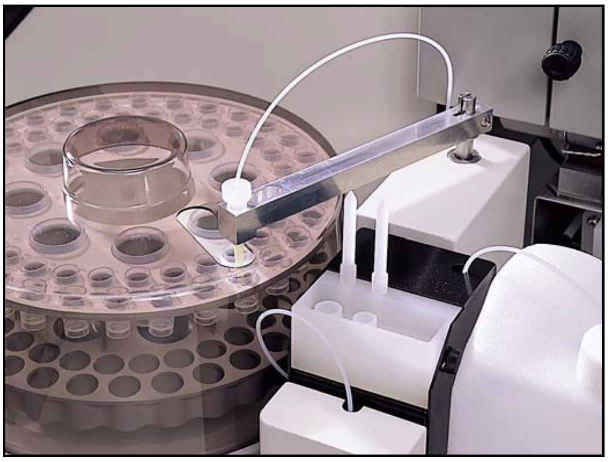
The digestion flask and the contents were cooled and 10 mL of 82% HCl was added. The flask was swirled and the digestate was collected in a 100-mL measuring flask. After rinsing the flask with small amounts of 6N HCl, it was washed twice with distilled, deionized water and diluted to make a final volume of 100 mL.

3.0 Graphite Furnace - Atomic Absorption Spectrophotometer (GF-AAS)

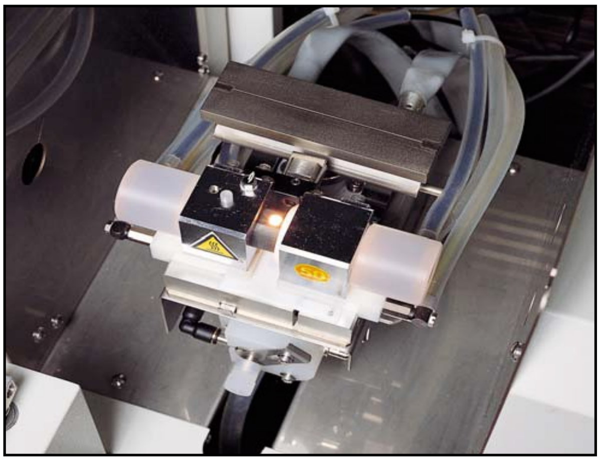
Shimadzu AA-6800

(Operates through Windows® compatible '*WizAArd*' software)

[Background Correction: BGC-SR and BGC-D_2]



Autosampler: ASC-6100 (Shimadzu)



Burner: GFA-EX7 (Shimadzu)

***Minimum Detection Limit*:** **Arsenic:** 0.3 $\mu g\ L^{-1}$

B. Operational conditions and Temperature Program of GF-AAS

The operational conditions employed for estimation of concentrations of arsenic are tabulated in the following Table (S1).

Element	As
Wavelength (nm)	193.7
Slit width (nm)	0.2
Lamp mode	BGC-D2
Lamp	HCL
Lamp current (mA)	12
Measurement mode	Peak height
Standard (µg L^{-1})	0, 4, 12, 20
Calibration curve	Linear
Correlation coefficient	0.999
Replicate	3
Mixture modifier	10 ppm $Pd(NO_3)_2$
Sample volume (µL)	20

The temperature programs of GF-AAS for analysis of concentrations of arsenic are tabulated in the following Table (S2).

Element	Step	Temperature (°C)	Time (sec)	Heating mode	Flow rate (L min^{-1})
As	1	150	20	RAMP	0.10
	2	250	10	RAMP	0.10
	3	600	10	RAMP	1.00
	4	600	10	STEP	1.00
	5	600	3	STEP	0.00
	6	2200	2	STEP	0.00
	7	2500	2	STEP	1.00

4.0 Analyses of Groundwater Samples:

The physical and chemical properties of the five natural groundwater used for irrigation purpose are mentioned in the following Table.

Table S3. Analyses of shallow and semi-deep groundwater samples used in irrigation.

Sample ID[†]	Units	RTW 01	RTW 02	RTW 03	RTW 04	RDP 05
Location		Kismat Kukhondi, Motiher	Kismat Kukhondi, Motiher	Uttar Kazipara, Paba	Boaliapara, Boalia	Sultanabad, Boalia
Aquifer		Shallow	Shallow	Shallow	Shallow	Semi-deep
Well depth	m	40	43	25	30	65
Physical Parameters:						
Temp.	^{0}C	26.3	27.1	26.8	25.9	27.6
pH		6.77	6.84	7.12	7.41	7.02
E_h	mV	15.4	13.8	25.3	-14.2	5.1
DO	mg L^{-1}	0.90	0.77	1.43	1.69	1.18
EC	μS cm^{-1}	706	672	785	800	732
Chemical Parameters:						
Fe	mg L^{-1}	6.249	3.532	0.410	1.025	0.278
As_T	mg L^{-1}	0.903	0.507	0.103	0.044	0.005
Ca	mg L^{-1}	85.360	79.915	67.001	90.472	49.838
Mg	mg L^{-1}	41.112	30.312	54.295	28.413	30.092
Mn	mg L^{-1}	0.253	0.210	1.048	0.442	0.123
Cr	mg L^{-1}	0.02	< d. l.	< d. l.	0.01	< d. l.
Na	mg L^{-1}	24.437	67.921	31.634	24.557	50.238
K	mg L^{-1}	3.218	4.852	8.090	3.913	4.921

[†] RTW stands for Tube-Well of Rajshahi and RDP for Deep-Pump of Rajshahi.

The distribution of the eight metals concentrations in five groundwater samples that were employed for irrigation purposes is presented in the following Box-and-Whisker plots.

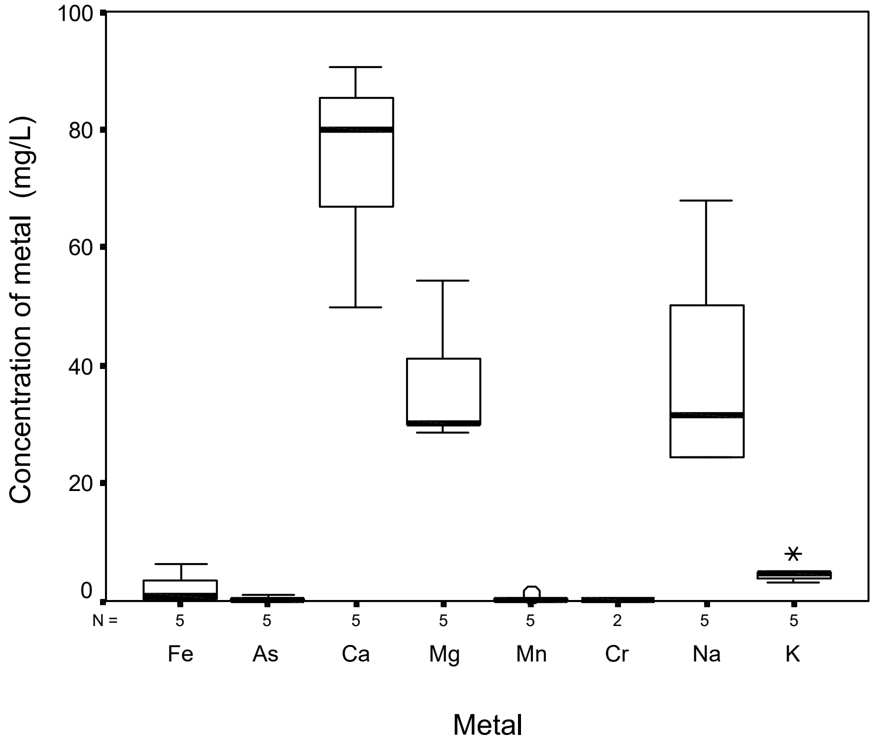


Figure. Box-and-Whisker plots for eight metals in five groundwater samples. [(▬) indicates median; lower and upper box boundaries 25th and 75th percentiles of each distribution; Whiskers as vertical lines ending in horizontal lines at the largest and smallest observed values; (*) indicates outside value and (^{O}) far outside value.]

Since Ca^{2+} and Mg^{2+} ions are the two principal contributor of water hardness, their higher values roughly indicate that the groundwater used in our study are hard. The elevated level of Fe and Mn concentrations is a consequence of predominance of reducing conditions in the aquifers, that facilitates reductive dissolution of both iron and manganese oxides. All the wells except for one have As_T concentrations exceeding the WHO limit recommended for drinking. The higher concentration of arsenic is probably due to the development of strongly-reducing conditions of aquifer, which is supported by our observed low values of redox potential.

5.0 Analyses of Cultivated Soil:

The studied soil of the field was under an agro-ecological zone of the Ganges Fluvial Floodplain. Major physical and chemical properties of control and fertilizer fortified soils are listed in the following Table.

Table S4. Selected physical, chemical and microbiological properties of tested soils.

Parameter	Unit	Original soil (Blank)	Fertilizer[d] amended soil (Control)
pH	-	7.51	7.94
Electrical conductivity	(dS m^{-1})	7.37	7.69
Organic matter (OM)	(%)	0.76	1.03
Water content	(%)	11.3	15.0
Sand	(%)	89.6	81.5
Clay + silt	(%)	10.4	18.5
Total As	(mg kg^{-1})	6.802	9.935
Total P	(mg kg^{-1})	27.36	240.8
Extractable Fe[a]	(mg kg^{-1})	8.16	12.2
Fecal Coliform[b]	(CFU[c]/100g)	1.2×10^4	5.7×10^5

[a] extracted by ammonium oxalate-oxalic acid buffer; [b] measured by membrane filtration (MF) method; [c] CFU stands for Colony Forming Unit; [d] minimum amounts were applied.

6.0 Concentrations of Arsenic in the Studied Eight Vegetables

Table S5. Concentration of arsenic in edible plant tissues of the vegetables as a function of groundwater arsenic concentration.

Vegetable	Type	n	[As]water / (mg/L)	[As]EPT / (mg/kg, dry wt.)	[As]EPT / (mg/kg, fresh wt.)
Okra	Aboveground	10	0.005	2.701	0.436
			0.005	1.854	0.253
			0.044	4.689	0.741
			0.044	4.318	0.547
			0.103	5.279	0.840
			0.103	5.179	0.716
			0.507	6.432	0.981
			0.507	6.332	0.836
			0.903	10.015	1.408
			0.903	11.129	1.539
Radish	Belowground	5	0.005	3.238	0.362
			0.044	6.139	0.760
			0.103	5.074	0.615
			0.507	10.017	1.100
			0.903	18.071	2.031
Aurum_Leaf	Aboveground	10	0.005	4.603	0.420
			0.005	4.007	0.429
			0.044	7.854	0.711
			0.044	6.364	1.168
			0.103	10.435	1.023
			0.103	9.705	1.080
			0.507	16.444	1.505
			0.507	21.942	2.256
			0.903	24.937	2.514
			0.903	31.811	3.330
Amaranth	Aboveground	11	0.005	3.691	0.411
			0.005	4.351	0.514
			0.005	2.815	0.273
			0.044	6.827	0.912
			0.044	6.104	0.657
			0.103	11.495	1.446
			0.103	7.216	0.800
			0.507	23.300	2.426
			0.507	8.372	0.914
			0.903	38.075	5.431
			0.903	14.155	1.430
Indian Spinach	Aboveground	6	0.005	2.443	0.205
			0.005	1.827	0.141
			0.044	4.485	0.346
			0.103	5.002	0.392
			0.507	7.891	0.689
			0.903	12.088	1.017
Potato	Belowground	2	0.005	3.384	0.395
			0.903	18.885	2.183
Carrot	Belowground	5	0.005	1.925	0.213
			0.044	5.022	0.550
			0.103	6.128	0.641
			0.507	8.019	0.824
			0.903	13.473	1.440
Eggplant	Aboveground	5	0.005	2.325	0.384
			0.044	3.641	0.793
			0.103	7.422	1.060
			0.507	17.730	3.565
			0.903	21.148	4.046

7.0 Average concentrations of arsenic in groundwater and vegetables for eight administrative divisions of Bangladesh

Table S6. Average concentrations of arsenic in groundwater and vegetables for eight administrative divisions of Bangladesh.

District	$[As]_{water}{}^{a}$ / (mg L^{-1})	$^{As}\overline{C}_{veg}$ / (mg kg^{-1}, dw.)	District	$[As]_{water}{}^{a}$ / (mg L^{-1})	$^{As}\overline{C}_{veg}$ / (mg kg^{-1}, dw.)
Rajshahi Division (8 Districts):			*Dhaka Division (13 Districts)*:		
Bogra	0.018	4.521	Dhaka	0.041	4.911
Jaipurhat	0.001	4.234	Faridpur	0.140	6.588
Naogaon	0.006	4.318	Gazipur	0.004	4.284
Natore	0.001	4.234	Gopalganj	0.187	7.384
Nawabganj	0.006	4.318	Kishorganj	0.052	5.097
Pabna	0.032	4.759	Madaripur	0.191	7.452
Rajshahi	0.007	4.335	Manikganj	0.024	4.623
Sirajganj	0.031	4.742	Munshiganj	0.189	7.418
Rangpur Division (8 Districts):			Narayanganj	0.048	5.030
Dinajpur	0.003	4.267	Narsingdi	0.041	4.911
Gaibandha	0.022	4.589	Rajbari	0.049	5.047
Kurigram	0.022	4.589	Shariatpur	0.151	6.774
Lalmonirhat	0.001	4.234	Tangail	0.020	4.555
Nilphamari	0.002	4.250	*Mymensingh Division (4 Districts):*		
Panchagarh	0.003	4.267	Jamalpur	0.014	4.454
Rangpur	0.008	4.352	Mymensingh	0.016	4.488
Thakurgaon	0.001	4.234	Netrokona	0.040	4.894
Khulna Division (10 Districts):			Sherpur	0.022	4.589
Bagerhat	0.156	6.859	*Sylhet Division (4 Districts)*:		
Chuadanga	0.079	5.555	Habiganj	0.022	4.589
Jessore	0.070	5.402	Moulavi Bazar	0.020	4.555
Jhenaidah	0.046	4.996	Sunamganj	0.048	5.030
Khulna	0.035	4.809	Sylhet	0.022	4.589
Kushtia	0.104	5.978	*Chittagong Division (11 Districts)*:		
Magura	0.028	4.691	Bandarban	NS[b]	-
Meherpur	0.116	6.181	Brahmanbaria	0.101	5.927
Narail	0.088	5.707	Chandpur	0.436	11.601
Satkhira	0.133	6.469	Chittagong	0.032	4.759
Barisal Division (6 Districts):			Comilla	0.142	6.622
Barguna	0.001	4.234	Cox's Bazar	0.003	4.267
Barisal	0.092	5.775	Feni	0.054	5.131
Bhola	0.010	4.386	Khagrachhari	NS[b]	-
Jhalakati	0.023	4.606	Lakshmipur	0.179	7.248
Patuakhali	0.003	4.267	Noakhali	0.162	6.960
Pirojpur	0.030	4.725	Rangamati	NS[b]	-

[a] represents mean arsenic concentration in groundwater.

[b] three districts of Chittagong Hill Tract were not surveyed.

8.0 Calculation of Average Daily Dose (ADD) and Hazard Quotient (HQ)

For Arsenic Exposure due to Ingestion of Vegetables for *Highly Exposed Child* Population Sub-group in Bogra District

Modified equation: $$ {}^{As}ADD = \frac{{}^{As}\overline{C}_{veg} \; . \; CF_{veg} \; . \; IR \cdot ED}{BW \cdot AT} \times (RBAF) $$

Here, Conc. of Arsenic in Vegetables, ${}^{As}C_{veg}$ = 4.521 mg kg^{-1} (dw.)

Conversion Factor for Vegetables, CF_{veg} = 0.085

Ingestion Rate of Vegetables by 6 yr Child, IR = 0.065 kg day^{-1} (ww.)

Relative Bio-availability Factor, RBAF = 0.4 (i.e., 40%)

Exposure Duration, ED = (350 × 5) day

Body Weight of 6 yr Child, BW = 18.6 kg

Averaging Time, AT = (365.25 × 6) day

Insertion of the values give:

$$ {}^{As}ADD = \frac{4.521 \times 0.085 \times 0.065 \times (350 \times 6)}{18.6 \times (365.25 \times 6)} \times (0.4) = 5.148 \times 10^{-4} \; mg\; kg^{-1}\; day^{-1} $$

Hazard Quotient, HQ :

$$ HQ = \frac{{}^{As}ADD}{{}^{As}RfD} = \frac{5.148 \times 10^{-4} \; mg\; kg^{-1}\; day^{-1}}{0.0003 \; mg\; kg^{-1}\; day^{-1}} = 1.716 $$

References:

1. APHA (2017) Standard Methods for the Examination of Water and Wastewater. 23rd ed. American Public Health Association. United Book Press, Inc., Baltimore, Maryland. ISBN: 9780875532875.
2. Hesse PR (2002) A textbook of soil chemical analysis. 1st ed. (reprint). CBS Publishers & Distributor, Delhi, India, pp. 371-435.
3. Shimadzu, Atomic Absorption Spectrophotometry Cook Book. In: Section 6 (Food staffs analysis). Shimadzu Corporation, Kyoto, Japan.
4. Jackson ML (2005) Soil chemical analysis. In: Plant Tissue Analysis-Mineral Constituents. Revised 2nd ed. Parallel Press, Madison, Wisconsin. ISBN: 1893311473.
5. Sakamoto H, Susa Y, Ishiyama H, Tomiyasu T, Anazawa K (2001) Determination of trace amounts of total arsenic in environmental samples by hydride generation flow injection-AAS using a mixed acid as a pretreatment agent. Analytical Sciences 17: 1067-1071. http://dx.doi.org/10.2116/analsci.17.1067