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$_2$SO$_4$-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% HNO₃, 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°C. The suspension was boiled nearly to dryness. This pre-digestion with HNO₃ 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% HNO₃, 98% H₂SO₄ and 60% HClO₄ in the volume ratio of 10:1:4) was added. The digestion was carried out on the electric hot plate at 220°C until dense white fumes of H₂SO₄ and HClO₄ were vigorously evolved. The digestion was stopped when the residues in the flask were clear and white and only slightly moist with H₂SO₄. The HClO₄, 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 H₂SO₄-HClO₄ 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% H₂SO₄. When the organic matter had been destroyed and the soil assumed a gray color, the flask was cooled and 3 mL of 70% HClO₄ were added with a few boiling chips. The mixture was boiled on an electric hot plate at 220°C 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-D₂]

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(NO₃)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 | Kismat | Uttar  | Boaliapa | Sultanabad |
| Aquifer    |       | Motther| Motther| Kazipara, | Boalia | Boalia |
| Well depth | m     | 40     | 43     | 25     | 30     | 65     |
| Physical Parameters: |       |        |        |        |        |        |
| Temp. °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 contributors 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\(^c\) (CFU\(^d\)/100g) | | 1.2 \times 10^4     | 5.7 \times 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              | 6.489                      | 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              | 8.059                      | 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} / mg L^{-1} | \(\mu_{C_{veg}} / \text{mg kg}^{-1}, \text{dw.}\) | District                      | [As]_{water} / mg L^{-1} | \(\mu_{C_{veg}} / \text{mg kg}^{-1}, \text{dw.}\) |
|-------------------------------|--------------------------|-----------------------------------------------|-------------------------------|--------------------------|-----------------------------------------------|
| Rajshahi Division (8 Districts): |                          |                                              | Dhaka Division (13 Districts): |                          |                                              |
| Bogra                         | 0.018                    | 4.521                                        | Faridpur                      | 0.140                    | 6.588                                        |
| Jaipurhat                     | 0.001                    | 4.234                                        | Gazipur                       | 0.004                    | 4.284                                        |
| Naogaon                       | 0.006                    | 4.318                                        | Gopalganj                     | 0.187                    | 7.384                                        |
| Natore                        | 0.001                    | 4.234                                        | Kishorganj                    | 0.052                    | 5.097                                        |
| Nawabganj                     | 0.006                    | 4.318                                        | Madaripur                      | 0.191                    | 7.452                                        |
| Pabna                         | 0.032                    | 4.759                                        | Manikganj                     | 0.024                    | 4.623                                        |
| Rajshahi                      | 0.007                    | 4.335                                        | Munshiganj                    | 0.189                    | 7.418                                        |
| Sirajganj                     | 0.031                    | 4.742                                        |                                |                          |                                              |
| 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                                        |                                |                          |                                              |
| 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): |                          |                                              | Sherdpur                      | 0.022                    | 4.589                                        |
| Bagerhat                     | 0.156                    | 6.859                                        |                                |                          |                                              |
| Chuadanga                    | 0.079                    | 5.555                                        | Habiganj                      | 0.022                    | 4.589                                        |
| Jessore                      | 0.070                    | 5.402                                        | Moulavi                        | 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                                        |                                |                          |                                              |
| 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                                        |
| Bhora                       | 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: 

\[
^{As}ADD = \frac{^{As}C_{veg} \cdot CF_{veg} \cdot IR \cdot ED}{BW \cdot AT} \times (RBAF)
\]

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

Conversion Factor for Vegetables, \(CF_{veg} = 0.085\)

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

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

Exposure Duration, \(ED = (350 \times 5) \text{ day}\)

Body Weight of 6 yr Child, \(BW = 18.6 \text{ kg}\)

Averaging Time, \(AT = (365.25 \times 6) \text{ day}\)

Insertion of the values give:

\[
^{As}ADD = \frac{4.521 \times 0.085 \times 0.065 \times (350 \times 6)}{186 \times (365.25 \times 6)} \times (0.4) = 5.148 \times 10^{-4} \text{ mg kg}^{-1} \text{ day}^{-1}
\]

Hazard Quotient, \(HQ\):

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