

ARSENIC ACCUMULATION OF ANIMAL FEED (GRASS AND WATER HYACINTH) IN FARIDPUR SADAR UPAZILLA

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ABSTRACT

Elevated level of groundwater arsenic (As) in Bangladesh has resulted as a massive calamity of exposing a large population to health risk and affecting livelihood and sustainable development of the country. The extensive use of arsenic contaminated groundwater in irrigation poses a potentially long term detrimental effect to human health as well as it is an environmental hazard. Arsenic is spreading in various ways in the environment and creating various hazards. This study was performed to detect the level of arsenic concentration in animal feed chain. Grass and Water hyacinth samples of two commonly used animal feeds were collected from arsenic contaminated areas of Faridpur district. After collection, the samples were prepared by a series of steps such as, washing, drying and digestion; finally arsenic was determined by atomic absorption spectrophotometric method. For this purpose the FI-HG-AAS (Flow Injection Hydride Generator Atomic Absorption Spectrophotometer) method was used. The arsenic absorbed by the animal feed (grass and water hyacinth) samples was determined. The mean arsenic concentration in Grass and Water hyacinth were 0.397 ± 0.075 ppm (n=20) and 0.365 ± 0.035 ppm (n=20), respectively. In this study it was found that the level of arsenic both in Grass and Water hyacinth is greater than that of the maximum permissible level in drinking water (0.05 ppm, WHO). This study was performed to detect the level of arsenic in animal feed of As contaminated area of Faridpur district.

Key words: arsenic, animal, grass, water hyacinth

INTRODUCTION

The arsenic disaster of Bangladesh has been called the most terrible environmental catastrophe of the twentieth century. WHO described the condition as “The largest mass poisoning of a population in history” (WHO, 2004). More than 75 million people of Bangladesh are being poisoned by groundwater arsenic contamination. 61 out of 64 districts across the country face the hazard of As poisoning (Arthur, 2006). Peoples of Bangladesh poses major health risks due to the presence of significant concentrations of As in groundwater and its affect on the crop production (Meharg *et al.*, 2003). Arsenic is a crystal-shape metalloid element which is brittle in nature and grayish white in color. As is a naturally-occurring poisonous chemical element and always occurs as compounds with others (Ali *et al.*, 2003). It is widely distributed in the soil profile as component of minerals and found in nominal amounts in all organisms. Chemically, arsenic compounds are of two types-inorganic and organic. Inorganic arsenic is more toxic (≈ 10 times) than organic. Inorganic arsenic has again two natural ionic forms-trivalent and pentavalent. As (III) has higher ability to form complex with coenzymes in human and animals. Inorganic Arsenite and Arsenate are commonly found in Bangladesh groundwater (Alex 2006). Intermittent incidents of arsenic contamination in groundwater can arise both naturally and industrially. The natural occurrence of arsenic in groundwater is directly related to the arsenic complexes present in soils. Arsenic can liberate from these complexes under some circumstances. The alluvial and deltaic sediments containing pyrite has favored the arsenic contamination of groundwater in Bangladesh (Ahmed *et al.*, 1997). Most regions of Bangladesh are composed of a vast thickness of alluvial and deltaic sediments, which can be divided into two major parts-the recent floodplain and the terrace areas. The groundwater in Bangladesh has declined progressively due to the excessive extraction of water for irrigation and domestic water supply, lack of water management and inadequate

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recharge of the aquifer. The ground water declined beyond 8 meters in 12% areas of Bangladesh in 1986. This extent rose to 20% areas in 1992 and 25% areas in 1994. The study on forecasting ground water level fluctuation in Bangladesh indicated that 54% areas of Bangladesh are likely to be affected up to 20 meters in some areas particularly in northern part of the country. Many scientists are currently studying this compound since it may add significantly to the overall carcinogenicity of inorganic arsenic. Once inorganic forms have been converted to MMA and DMA, they are excreted from the body. Thus, the presence of DMA and MMA in the urine, skin, hair, and nails are often evidence of arsenic poisoning. The inhalation and ingestion of As is perhaps the responsible for human carcinogen affecting skin and lungs (Bates *et al.*, 1992).

A national survey indicated accumulation of high amounts of As in rice grains (0.4 to 1.0 mg/kg) present in areas where the concentrations of As in irrigation water and soils are high. This is creating a real food-related health hazard for the local communities (Chandiramani *et al.*, 2007). There have also undesirable levels of As observed in some other crops, like vegetables, tubers, fruits, and even wheat in Bangladesh (Chandiramani *et al.*, 2007). The presence of As in food through the water-soil crop routes has raised a worldwide concern in terms of food safety. The World Health Organization (WHO) recommendation the acceptable level of As in drinking water is 50 µg/L in Bangladesh, on the other hand the global standard is 10 µg/L. The accumulation of groundwater As in the soil fields and then its entering into the food chain through the crops (Huq *et al.*, 2001). With the above discussion the key research field of this studies was located the pathway of As being incorporated in the food chain through the water-soil-crop pathway. For the above reasons the present study was conducted to detect arsenic level, to estimate the concentration of arsenic and to compare the level of arsenic in animal feeds (Grass and Water hyacinth) in arsenic contaminated areas of Faridpur district.

MATERIALS AND METHODS

Washing and Sterilization

The study was carried out for the detection of arsenic in Grass and Water hyacinth. The experiment was conducted at the Arsenic Detection and Mitigation (ADM) Laboratory, Department of Pharmacology, BAU, Mymensingh, Bangladesh. All glass and plastic wares and sample containers were cleaned by brushing in the laboratory using detergent and soaked overnight in 10% HCl (v/v). After overnight dipping they were washed thoroughly in running tap water and rinsed two times by double distilled water. They were then oven dried (~65°C) and stored. These glass wares were generally sterilized by dry heating at 160°C for one and half an hour in an oven. Falcon tubes and Pasture pipettes were sterilized by autoclaving for 15 minutes at 121°C under 15 pounds (lbs) pressure per sq. inch. After autoclaving, these were instantly dried in an oven at 60-65°C and the caps of the falcon tubes were tightened after cooling and stored in a dust free container.

Collection of Samples

Bangladesh is a nation of roughly 160.23 million people inhabiting in an area of 147570 km² (Bangladesh Economic Review-2011). In this study, one worse arsenic affected district Faridpur was selected. In Faridpur sadar upazilla 5 unions (Ambikapur, Aliabad, Kanaipur, Kaijuri, Majchar) were selected and animal feed samples such as grass and water hyacinth were collected. All required samples were collected during the month of May (summer season) in the years 2012. In all cases, two types of samples (Grass and Water hyacinth) were collected in the five days of sample collection period. A standardized personal interview of each owner was carried out based on a prearranged questionnaire. Owners were briefly questioned by visiting door-to-door during sample collection and information obtained from the interview was recorded. Questionnaire was structured including general information (area, cultivation season, harvesting season, varieties, type of irrigation) of specific grass and water hyacinth. Information about history of water irrigation by the study subjects including water source were obtained on the basis of the questionnaire. Water hyacinth and grass used as ration of

respective animals were considered for possible sources of arsenic contamination. The water hyacinth (that generally consumed by cattle) and grass were collected in zip-type bag, labeled and kept in a polyethylene bag and finally transferred to the laboratory and stored in desiccators until analysis.



Fig. 1. Water hyacinth



Fig. 2. Grass

Sample Preparation and Digestion

Grass and Water hyacinth samples were sun dried to reduce water percent. About 0.95-1gm sample was taken separately into digestion tube and 10 ml of 69% concentrated HNO_3 and 70% of perchloric acid mixture at the ratio of 5:3 was added. The samples left to react overnight in a chemical "hood", then heated in a block digester (M-24 plazas/samples, JP Selecta, Spain) at 120°C until colorless clear watery fluid appears. Tubes were gently shaken several times to facilitate destroying all the carbonaceous material. This digestion converts all arsenicals to inorganic arsenic for FI-HG-AAS determination. Digestion was considered complete when production of reddish-orange fumes and foam within the tube had subsided, the solution had become clear and did not bubble or react upon agitation. Tubes were removed from the digestion block, cooled, diluted to 50 mL adding Millipore water and filtered through filter paper (Whatman No. 41) and stored in 50 ml polythene bottles. The sample solution at that stage was ready for determination of its total arsenic. In each set, blank reference material were prepared following same digestion procedures.

Arsenic Analysis

A. Preparation of Analytical Solutions

1. Standard

One ml of arsenic standard (1000 ppm, As_2O_5) was added into a 100 mL volumetric flask. To it 0.8 g potassium iodide and 10% HCl (v/v) was added up to 100 mL mark. It was poured into a heat resistant beaker and was boiled for a while on an electric heater. After cooling down, 0.5 g ascorbic acid was added and it was transferred into a brown bottle. The standard concentration became 1 ppm (g mL^{-1}) trivalent arsenic (AsIII). It was kept in dark place in a refrigerator. In all cases prepared standard was used within three months.

2. Carrier liquid

One percent solution of HCl (v/v) was used as carrier liquid. To prepare this solution, 5 mL of HCl was taken into a 500 mL volumetric flask and it was diluted to 500 mL with Millipore water.

3. Blank

Ten percent solution of HCl (v/v) was used as blank. In a 500 mL volumetric flask 50 mL HCl was taken and it was dissolved to 500 mL with Millipore water.

4. Sodium borohydride solution

To prepare potassium borohydride solution 1.5% Sodium borohydride (NaBH_4) (w/v) in 0.3% sodium hydroxide (NaOH) was used. It was prepared daily and kept at room temperature during analysis.

B. Sample Reduction

As III shows maximum affinity to form their hydrides; therefore, it was essential to reduce AsV to AsIII before performing analysis by FI-HG-AAS. Pentavalent form of arsenic in water/digestate was reduced to trivalent form as described by Wahed *et al.* (2006). Prior to arsenic determinations, 1 mL (5 mol) HCl and 1 mL of 20% KI (w/v) were added to a 10 mL water/digestate sample in pyrex test-tube and heated on a water bath at 80°C for 30 minutes. Blank was prepared in the same manner. During analysis it was further dissolved to the standard curve range, if necessary.

C. Arsenic Detection

Concentrations of arsenic in digested samples were determined using atomic absorption spectrophotometer (AAS), model PG -990 equipped with a computer with atomic absorption (AA) software (PG Instruments Ltd., UK) following pre-reduction with KI and KBH_4 to generate AsH_3 (Samanta *et al.*, 1999). The instrument was coupled with a Flow Injection Hydride Generator (FI-HG); model WHG-103A, (PG Instruments Ltd., UK). The detection limit of the instrument for arsenic was two ppb. Quantification of arsenic was performed by spiking samples with standards at different concentrations. For constructing standard curve working standard of 0, 2.5, 5, 10, 15 and 20 ppb were prepared immediately before use by serial dilution of the stock in 10% HCl. Samples exceeding the standard curve range were diluted again and analysed further. The concentration of arsenic in those samples was resolved by multiplying by the dilution factor, as appropriate. In every occasion the linear correlation factor was bigger than 0.99. Determinations were performed in duplicate having the relative error <1% for all the samples. The salient details of the methods adopted for the present work are summarized in Table 1.

Table 1. Instrumental and chemical conditions employed for the determination of arsenic by FI-HG-AAS.

Carrier gas pressure (Argon cylinder)	0.24 MPa
Flow rate of carrier gas (Hg regulated)	180 ml/min
Carrier liquid	1% HCl(v/v)
Reductant	1.5% NaBH_4 + 0.3% NaOH
Burner height	12 mm
Burner position	(-) 1.5 mm
Calibration graph (linear)	10-100 ppb
Sample taken per injection	2 to 25
Measurement mode	Peak height
Wavelength	193.7 nm
Integration time	15 seconds
Lamp current	10 mA
Blank solution	10% HCl
Light source	Cathode lamp

D. Analytical Procedure

The carrier gas pressure was used as energy source. Diluted (1%, v/v) HCl was used in the carrier stream to sweep the sample at the mixing chamber where it reacted with a solution of 1.5% NaBH_4 (w/v) stabilized in 0.3% NaOH. Before the formal determination, water and air in the fluid measurement system was cleaned off by placing the sample suction tube into the carrier liquid and carry out the operation process at least two times. Blank solution was calibrated before measuring the sample solution. In every analysis set first two data of blank was ignored. The sample, carrier liquid and NaBH_4 solution suction port was placed in respective solution. Then the start key of the HG was pressed. The sample solution, the carrier liquid and NaBH_4 solution were automatically and quantitatively sucked in. The carrier liquid carrying the sample solution and NaBH_4 began their permanent flow and the reaction takes place after their convergence. The carrier gas into the gas-liquid separation tube brings along the resultant and the mixed gas enters the electric quartz absorption-tube

atomizer. The resulting absorption of the lamp radiation was proportional to the arsenic concentration. Sample solution concentrations were determined by direct comparison with the calibration curve and the reading was automatically transferred to atomic absorption (AA) software. The washed liquid was driven out automatically. The sample was replaced after getting the result. The data were analyzed statistically using student's t-test as described by Bailey (1981). The As concentration in the supplied sample was multiplied by the dilution factor to obtain As level in the sample.

RESULTS AND DISCUSSION

Arsenic concentration in Grass and Water hyacinth

Grass grows as a weed in the agricultural land and road sides. On the other hand water hyacinth grows on the canal, ponds, and low land areas surround the agricultural lands. Irrigation of agricultural land mainly depends upon the ground water in Bangladesh. But groundwater of Bangladesh has been highly contaminated with arsenic. Thus, there is a possibility of induction of arsenic in grass and water hyacinth with contaminated irrigation water and soil. The results show that concentrations of arsenic in Grass ranged from 0.129 to 1.546 ppm (Table 2) with a mean (\pm SEM) value of 0.397 ± 0.075 ppm

Table 2. Arsenic concentrations (ppm) in Grass and Water hyacinth collected from arsenic contaminated areas of Faridpur district.

Serial Number	Arsenic Concentration in Grass (ppm)	Arsenic Concentration in Water hyacinth (ppm)
1	0.190	0.263
2	0.441	0.555
3	0.568	0.538
4	0.243	0.438
5	0.181	0.162
6	0.154	0.357
7	0.246	0.470
8	0.481	0.355
9	0.161	0.339
10	0.564	0.523
11	0.129	0.479
12	0.371	0.383
13	1.546	0.188
14	0.758	0.468
15	0.775	0.388
16	0.208	0.360
17	0.233	0.542
18	0.141	0.435
19	0.201	0.024
20	0.350	0.033

($n=20$) (Table 3). Interestingly, arsenic concentration in Grass was significantly higher than that of Water hyacinth (Fig 2). Concentrations of arsenic in Water hyacinth ranged from 0.024 to 0.555 ppm (Table 2) with a mean (\pm SEM) value of 0.365 ± 0.035 ppm ($n=20$) (Table 3). A great fluctuation was found in the concentrations of arsenic in Grass in comparison to that of Water hyacinth (Fig 2). The fluctuations could be due to differences in the absorption and distribution of arsenic in the plant. Another reason could be due to variation in the soil arsenic concentration from plot to plot.

Table3. Average concentration of arsenic (ppm) in Grass and Water hyacinth collected from contaminated areas of Faridpur district

Sample Name	Average	SD	SEM	N
Grass	0.397	0.3364	0.0752	20
Water hyacinth	0.365	0.1586	0.0354	20

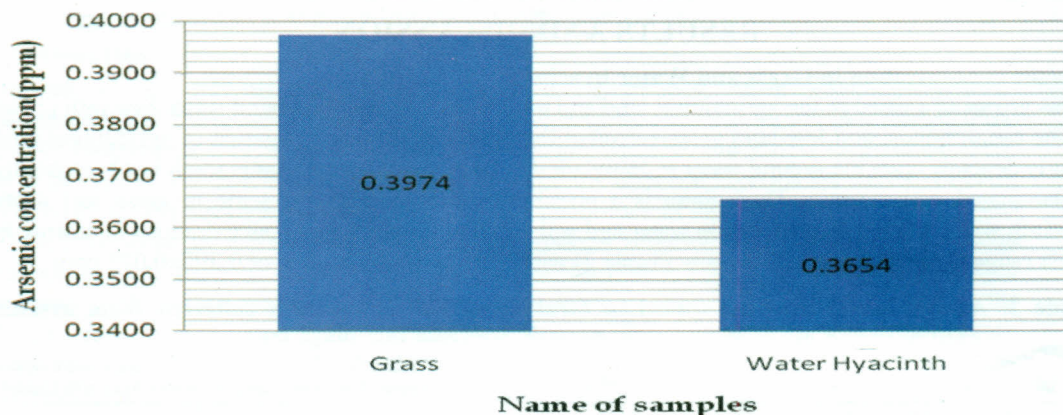


Fig. 3. Average concentration of arsenic (ppm) in Grass and Water hyacinth collected from Sadar Upazila of Faridpur district.

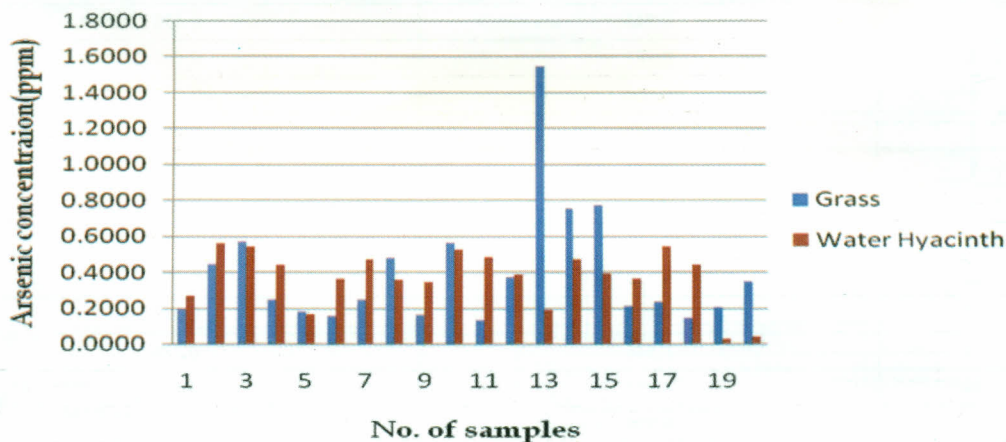


Fig: 4. A graphical presentations of arsenic concentrations in Grass and Water hyacinth collected from Sadar upazila of Faridpur district.

A comparison of levels of arsenic in shallow tube well water with that of Grass and Water hyacinth collected from arsenic contaminated areas

As shallow tube well water is considered to be primary source of contamination of crops by irrigation, the level of arsenic of shallow tube well water is compared here with the levels arsenic found in the Grass and Water hyacinth of Sadar upazila of Faridpur district. In Sadar Upazila the mean arsenic concentration in shallow tube well water is 0.136 ppm which is near about 3 times higher than the Bangladesh maximum permissible limit of 0.05 ppm (Hossain 2006)). It is reported that shallow aquifer layer is contaminated with arsenic in almost all of the districts in Bangladesh and Faridpur is the worst contaminated district (DPHE-BGS, 2000). Grass and Water hyacinth samples were collected

from Sadar upazila of Faridpur district and the average concentrations of arsenic were 0.397 ± 0.075 and 0.365 ± 0.035 ppm, respectively. This result indicates that the Grass accumulated more arsenic than Water hyacinth samples. The soils and water are contaminated by As contaminated shallow tube well water. Generally soils contain more As in the contaminated areas and higher amounts of As may be accumulated in the grass. The average concentrations of arsenic in tube well water and that found in the Grass and Water hyacinth are tabulated below (Table 4) and graphically presented in the figure 5.

Table 4. A comparisons of average arsenic concentrations in shallow tube well water with that found in Grass and Water hyacinth.

Arsenic concentration in shallow tube well water (ppm)*	Arsenic concentration in Grass (ppm)	Arsenic concentration Water hyacinth (ppm)
0.181*	0.397 ± 0.075	0.365 ± 0.035

*FAO, 2007

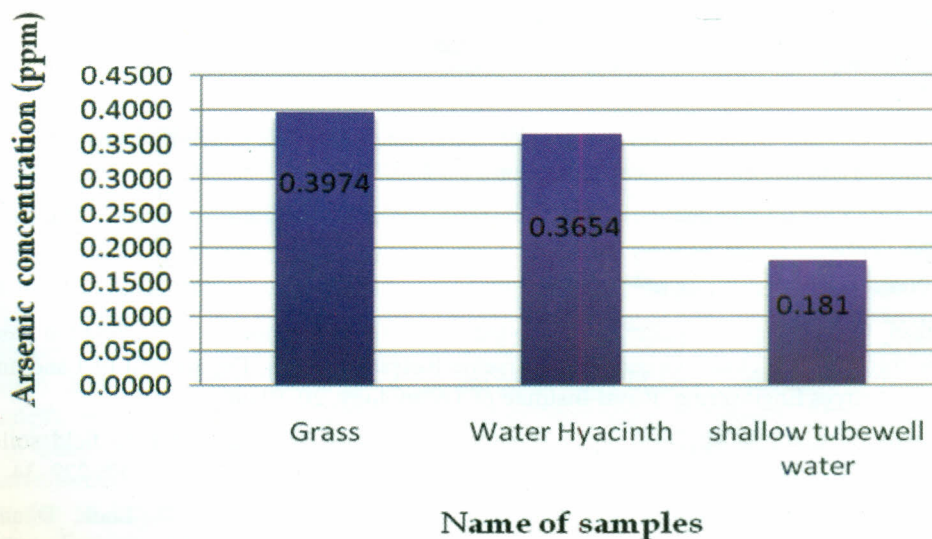


Fig. 5. A graphical comparison of average arsenic concentrations in shallow tube well water with that found in Grass and Water hyacinth collected from Sadar upazila of Faridpur district.

SUMMARY AND CONCLUSION

The present experiment was done to measure arsenic level in animal feed chain. Level of arsenic concentration was determined by FI-HG-AAS method and found that the average arsenic concentration in Grass and Water hyacinth were 0.397 ± 0.075 ppm ($n=20$) and 0.365 ± 0.035 ppm ($n=20$) respectively. Interestingly Water hyacinth contains less arsenic than Grass. Animals are mainly fed on Grass, which contains alarming level of arsenic in the arsenic contaminated area of Faridpur district. As shallow tubewell water is most frequently used for irrigation which is more contaminated with arsenic than deep tubewell water leading to arsenic contamination in animal feed chain. To minimize or to avoid the risk of arsenic contamination, animal should be prevented from grazing in heavily contaminated areas. More research in this respect should be undertaken with the objective of mitigation of arsenic problem in Bangladesh.

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