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Article

Arsenic detection in animal feed of Faridpur Sadar Upazilla of Bangladesh

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Abstract: Arsenic is a common, naturally-occurring element. The metalloid substance, famous for its tasteless, odorless and occasionally lethal properties, sits at number 33 in the periodic table. Arsenic is found in both organic and inorganic. It is significantly threatening in case of Bangladesh as 61 out of 64 districts are affected by arsenic. Arsenic is spreading in various ways in the environment and creating various hazards. The serious arsenic contamination of groundwater in Bangladesh has come out recently as the biggest natural calamity in the world in terms of number of affected population. Rice polish and rice husk 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 spectophotometric method. For this purpose the FI-HG-AAS (Flow Injection Hidride 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 rice polish and rice husk were 0.805±0.111 ppm (n=10) and 0.457±0.034 ppm (n=20), respectively. In this study it was found that the level of arsenic both in rice polish and rice husk 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 arsenic contaminated area of Faridpur district.

Keywords: arsenic; animal; rice polish; rice husk

1. Introduction

Arsenic is a chemical element. Arsenic occurs in many minerals, usually in conjunction with sulfur and metals, and also as a pure elemental crystal (Cullen *et al.*, 2010). Arsenic is a metalloid. It can exist in various allotropes, although only the gray form has important use in industry (Smith *et al.*, 2004). The arsenic disaster of Bangladesh has been called the most terrible environmental catastrophe of the twentieth century. 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 component that is extremely hard to convert to water-soluble or volatile products. The fact that arsenic is naturally a fairly a mobile component, basically means that large

concentrations are not likely to appear on one specific site (Cullen *et al.*, 2010). This is a good thing, but the negative site to it is that arsenic pollution becomes a wider issue because it easily spreads. Arsenic cannot be mobilized easily when it is immobile (Rahman *et al.*, 2008).

Arsenic contamination in groundwater has been reported at different times from West Bengal, India and countries like U.S.A, Argentina, Chile, Mexico, Taiwan, Hungary, Finland, Nepal and Bangladesh (Sanyal, 1999). As regards the widespread as contamination in groundwater in parts of West Bengal, India and Bangladesh, confined within the delta bound by the rivers Bhagirathi and Ganga-Padma, two major hypotheses, both of geogenic origin, have been proposed. Arsenic can enter into food chain causing wide spread distribution throughout the plant and animal kingdoms (Kile *et al.*, 2007). The evidence of arsenic calamity in animal feed chain is scarce. Contamination of animal feed by arsenic is a newly uncovered disaster on a massive scale (Sapkota *et al*., 2007). This poses a potential dietary risk to human, although little research has focused on food as an additional source of arsenic exposure. Food may contribute up to 30-50% of the total dietary intake of arsenic when feed is generated from arsenic contaminated sources (Naidu *et al*., 2006). Arsenic is an approved animal dietary supplement and is found in specifically approved drugs added to poultry and other animal feeds. Although several research groups have begun to elucidate the effects of arsenic use in animal feed on its environmental concentrations in areas where animal waste has been land applied (Jackson *et al*., 2006).

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, 2001). It was estimated that 61 out of 64 districts and about 29% of the total tube wells in Bangladesh are contaminated with arsenic (Khan *et al*., 2006; Chakraborti *et al*., 2010) and about 85 million people are at risk of drinking arsenic contaminated water and foodstuffs (Hossain, 2008; Wahed, 2006). In a recent report Chakraborti *et al*. (2010) showed that hand tube wells of the tableland and hill tract regions of Bangladesh are primarily free from arsenic. Arsenic is one of the most toxic elements that can be found. Despite their toxic effect, inorganic arsenic bonds occur on earth naturally in small amounts (Das *et al.*, 2004). Humans may be exposed to arsenic through food, water and air. Exposure may also occur through skin contact with soil or water that contains arsenic. Levels of arsenic in food are fairly low, as it is not added due to its toxicity. But levels of arsenic in fish and seafood may be high, because fish absorb arsenic from the water they live in. It is suggested that the uptake of significant amounts of inorganic arsenic can intensify the chances of cancer development, especially the chances of development of skin cancer, lung cancer, liver cancer and lymphatic cancer (Das *et al*., 2004). However, 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 (rice polish and rice husk) in arsenic contaminated areas of Faridpur district.

2. Materials and Methods

2.1. Washing and sterilization

The study was carried out for the detection of arsenic in rice polish and rice husk. 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 (~65ºC) and stored. For sterilization, the graduated cylinders were sealed with aluminum foil. The graduated pipettes were plugged with cotton at the neck and were positioned in a canister. These glass wares were generally sterilized by dry heating at 160ºC for one and half an hour in an oven. Falcon tubes were loosely caped and wrapped using aluminum foil. Pasture pipettes were wrapped with aluminum foil. These were sterilized by autoclaving for 15 minutes at 121ºC under 15 pounds (lbs) pressure per sq. inch. After autoclaving, these were instantly dried in an oven at 60-65ºC and the caps of the falcon tubes were tightened after cooling and stored in a dust free container.

2.2. Collection of samples

Bangladesh is a nation of roughly 160.23 million people (Bangladesh Economic Review-2011) inhabiting in an area of 147570 km^2 (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 rice polish and rice husk 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 (rice polish and rice husk) 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,) of specific rice polish and rice husk. Rice polish and rice husk used as ration of respective animals were considered for possible sources of arsenic contamination. Different varieties of rice were considered. The rice polish (that generally consumed by dairy cows and poultry) and rice husk 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.

2.3. Sample preparation and digestion

Rice polish and rice husk 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 120ºC 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.

2.4. Arsenic analysis

2.4.1. Preparation of analytical solutions

2.4.1.1. 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 ($\mu g\ mL^{-1}$) trivalent arsenic (AsIII). It was kept in dark place in a refrigerator. In all cases prepared standard was used within three months.

2.4.1.2. 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.

2.4.1.3. 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.

2.4.1.4. Potassium borohydride solution

To prepare potassium borohydride solution 1.5% potassium borohydride (KBH_4) (w/v) in 0.3% sodium hydroxide (NaOH) was used. It was prepared daily and kept at room temperature during analysis.

2.5. Sample reduction

AsIII 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 80ºC for 30 minutes. Blank was prepared in the same manner. During analysis it was further dissolved to the standard curve range, if necessary.

2.6. 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) Win 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). All measurements were made in ppb. 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 resolute 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.

Items	Instrumental and chemical conditions
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

2.7. 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 coil where it reacted with a solution of 1.5% KBH_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 KBH_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 KBH_4 solution were automatically and quantitatively sucked in. The carrier liquid carrying the sample solution and KBH_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) win 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.

3. Results and Discussion

3.1. Arsenic concentration in rice polish and rice husk

Cultivation of rice mostly depends on groundwater in Bangladesh, particularly in the dry season, as the surface water sources (river, dam, pond, etc) become dry throughout the season in this region (Delowar *et al.*, 2005). Rice is widely cultivated in Bangladesh to ensure food security and it requires huge volume of groundwater (Hossain *et al.*, 2008). Groundwater of Bangladesh has been highly contaminated with arsenic (Delowar *et al.*, 2005). Thus, there is a possibility of induction of arsenic in rice, cultivated with contaminated irrigation water and soil (Islam *et al.*, 2004).

The results show that concentrations of arsenic in rice polish ranged from 0.1159 to 1.1868 ppm (Table 2) with a mean (±SEM) value of 0.805±0.111 ppm (n=10) (Table 3). Interestingly, arsenic concentration in rice polish was significantly higher than that of rice husk. Concentrations of arsenic in rice husk ranged from 0.2893 to 0.7830 ppm with a mean (±SEM) value of 0.457±0.0340 ppm (n=20).

A great fluctuation was found in the concentrations of arsenic in rice husk in comparison to that of rice polish. 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. Moreover, we know that the root of a plant (e.g Rice) absorbs and accumulates the highest levels of arsenic than other parts (Rahman *et al*., 2007) and as rice is seed grain it is assumed to have lesser and steady amount of arsenic. In China, a study to investigate the impact of irrigation with high arsenic burdened groundwater on the soil- plant system has shown that arsenic concentration in plant parts (rice polish and rice husk) decreased roots towards leaves, stems and seeds (Neidhardt *et al.,* 2012).

Table 2. Arsenic concentrations (ppm) in rice polish and rice husk collected from arsenic contaminated areas of Faridpur district.

Serial no.	Arsenic concentration in rice polish (ppm)	Arsenic concentration in rice husk (ppm)
1	**1 .1868(maximum)**	0.6461
2	0.9564	0.5892
3	1.0723	0.5415
4	0.9182	0.4754
5	1.0452	0.5527
6	1.0924	0.4867
7	0.3703	0.4900
8	0.6963	0.3035
9	**0.1159(minimum)**	0.4389
10	0.5989	0.3044
11		0.3495
12		0.3518
13		**0.7830(maximum)**
14		0.3292
15		0.3188
16		0.3828
17		**0.2893(minimum)**
18		0.4549
19		0.2908
20		0.7668

Table 3. Average concentration of arsenic (ppm) in rice polish and rice husk collected from contaminated areas of Faridpur district.

Sample name	Average(ppm)	S.D	S.M.E	N
Rice polish	0.8053	0.351423	0.11113	10
Rice husk	0.4572	0.15231	0.034058	20

3.2. A comparison of levels of arsenic in shallow tube well water with that of rice polish and rice husk collected from arsenic contaminated areas

As shallow tube well water is considered to be primary source of contamination of crops by irrigation (Rahman *et al.,* 2008). The level of arsenic of shallow tube well water is compared here with the levels arsenic found in the rice polish and rice husk 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 *et al.,* 2008). 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). In my study rice polish and rice husk were collected from Sadar upazila of Faridpur district and the average concentrations of arsenic were 0.805±0.111and 0.0.457±0.034 ppm, respectively. I found that the rice polish accumulated more arsenic than rice husk and the later, Arsenic accumulated even lesser amount in rice polish and rice husk than that of the shallow tube well water of the same area. The average concentrations of arsenic in tube well water and that found in the rice polish and rice husk are tabulated below (Table 4).

Table 4. A comparison of average arsenic concentrations in shallow tube well water with that found in rice polish and rice husk.

Arsenic concentration in rice polish (ppm)	Arsenic concentration in rice husk(ppm)	Arsenic concentration in shallow tube well water (ppm)*
0.805±0.111	0.457±0.0340	0.181*

*FAO, 2007

4. Conclusions

The present study was undertaken to detect 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 rice polish and rice husk were 0.805±0.111 ppm (n=10) and 0.457±0.034 ppm (n=20) respectively. These levels of arsenic in rice polish and rice husk is 16.1 times and 9.14 times greater than that of the permissible level of arsenic in drinking water (0.05 ppm, WHO), respectively. Interestingly rice husk contains less arsenic than rice polish. Whereas, animals are mainly fed on rice polish, which contains alarming level of arsenic in the arsenic contaminated area of Faridpur district. Therefore, to minimize or to avoid the risk of arsenic contamination in animal, irrigation should be done with deep tubewell water or with natural water such as rain water, pond and surface water. Animal should be prevented from grazing in heavily contaminated areas. Instead of arsenic sensitive cultivars, arsenate tolerant cultivars of paddy can be cultivated in the arsenic contaminated areas of our country. More research in this respect should be undertaken with the objective of mitigation of arsenic problem in Bangladesh to save the people as well as livestock.

Conflict of interest

None to declare.

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