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Arsenic Content in Inactive Tissue: Human Hair and Nail

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAR designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MEH, SMH, KN and RH managed the literature searches, analysis of the study performed the Neutron Activation Analysis and managed the experimental process. Authors MSS and MSA performed the bio-accumulation analysis and took part in the statistical calculation. Authors MAHB and MAP took part in the interpretation of the results and MAP prepared the final version of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Nuclear reactor based Neutron Activation Analysis (NAA) method was applied for the determination of arsenic content in human scalp hair, nail and water samples at the south western part of Bangladesh. Average Arsenic (As) content in male and female hair samples were 0.93 μg/g and 3.71 μg/g respectively and also As content in nail samples

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for male and female were 1.40 µg/g and 2.03 µg/g respectively. A significant correlation in hair and nail for male (r = 0.615) and female (r = 0.728) were observed. This correlation indicated higher affinity to accumulate in external tissue of human body with exposure time. On the other hand, a significant correlation (r = 0.79 and r = 0.72; p = 0.01) was found in female hair and nail As c content in relation to water content (average 0.89 µg/g). Similar association (r = 0.49 and r = 0.53; p = 0.01) was observed in both matrices for male with water content at the significance level of correlation coefficient. More than 20% people of study area were arsenic affected due to highly contamination of groundwater. Arsenic in both matrices were relatively higher, compare to World Health Organization (WHO) recommended value (>1 µg/g). Results indicated that arsenic content in human hair and nail were greater inconsistency perspective to gender variation.

Keywords: Arsenic; hair; nail; tube-well water; neutron activation analysis technique.

1. INTRODUCTION

Arsenic contamination in the human body is one of the most severe problems in Bangladesh. Its harmful consequences have negatively impacted environmental and socio-economic condition in the country. The people in the study area used Arsenic (As) contaminated groundwater for different purposes. Most available inorganic form of arsenic frequently present in tube-well water which is much more toxic than organic form of arsenic [1,2,3]. Drinking water act as a primary source of inorganic arsenic exposure [4,5]. These uses of contaminated groundwater expedited As accumulation in human body through the food chain. For the last few decades, drinking water is the main factor for public health concern in different regions of the world [6]. Metabolic activities in human body get available As in soluble form through their enzymatic processes. However, the trivalent forms (methylated metabolites of arsenic) are more toxic than the inorganic form of arsenicals [2,3,7-9]. Arsenic concentrates in different organs (such as bladder, liver, kidney, skin and lung) of human body and also attribute to carcinogenic diseases [10-4]. A few non-cancerous health hazards associated with arsenic poisoning such as skin lesions, cardiovascular disease, neurological problems, hypertension and diabetes mellitus [15].

Hair and nail acts as a biomarker to assess environmental contamination of human by poisonous trace metal [16-21]. As a diagnostic tool, hair and nail analysis is the important way to evaluate environmental exposure [22] due to the content of keratin [5,23]. In Bangladesh, groundwater along with the deltaic and alluvial sediments is directly contaminated by As. High level arsenic contamination in groundwater (geochemical reaction such as oxidation and reductive dissolution) was found in west-Bengal India [24-26]. More than 80 million people of Bangladesh are arsenic affected due to ground water contamination within 64 districts [27,28].

Arsenic content and gender variability for inactive tissue (hair and nail) depends on critical parameters including contamination pathway, contamination rate and body mechanism in relation to metabolic activity. It is indicated that elemental composition of human hair may be changed in the presence of some factors, such as cosmetic treatments, age, sex (frequent washing, bleaching, coloring, perming), geographical region and individual physiological differences, ethnicity, living habits [15,29,30]. Metabolic activity is absent in inactive tissue. That is why it acts as a reliable diagnostic tool for environmental contaminant assessment. But hair analysis is more difficult than nail due to external contamination. Some problems have been encountered recognized with hair analysis due to internal and external contamination of different substances [31,32].

In the present study, the neutron activation analysis (NAA) method was applied to determine arsenic. The NAA method is an accurate technique for the total arsenic (TA) quantification in nails with regards to minimal sample and relatively low instrument detection limits [33-35]. The neutron activation analysis (NAA) method was shown high accuracy and precision for the arsenic analyzing compare to the AFS method [33]. The quality control (QC) measure is significant to make sure sensitivity and accuracy of the methods [36].Chemical methods like AAS or ICP-MS are also not very much suitable as the sample need to be digested for the analysis [33]. Here, no chance to external contamination in sample analysis with chemicals. The NAA method is very sensitive for the analysis of hair and nail, because these can be analyzed in solid form which means that as received from the donor. The object of this research work is to identify arsenic content in both matrices (hair and nail) perspective to ground water contamination and try to determine the possible consequences of As content in relation to sex at Jhenaidah district in Bangladesh.

2. MATERIALS AND METHODS

2.1 Study Area

This study area is situated in the south-western ('*Achintanagar'* village) part of Bangladesh in the Jhenaidah district (Fig.1). In the study area, groundwater is highly arsenic contaminated. A few people have been identified as arsenicosis patients who carried different wart like spots in whole body. Non-government organizations (NGOs) and other development organizations attempt to identify contamination level in respect to patients and water quality assessment.

2.2 Sample Collection

Samples (Scalp hair and toenails in both sexes) were collected from 51 subjects (25 male and 26 female; as coded as FH=female hair, FN=female nail, MH=male hair and MN=male nail), who were exposed to arsenic contagion due to ground water contamination by using 'random technique' as described by Sharma et. al. [22]. Shallow tubewell (from community) water samples (n =9) were collected from those who were drinking arsenic contaminated water, contained arsenic above 0.05mg/l ('*Achintanagar*' Village). The mean age of the participant (donor) was 46 year. The sex ratio for male and female was 1:1.04. By the historical observations and interviews, arsenicosis diseases have been widely distinguished in human body during past 25 years. A small bundle of hair was cut from the scalp of the patient using clean scissors. A few authors are recommended to use this technique for hair sampling [22,37].The length of the bundle of hair is < 3 cm. The average weight of each sample was 50 mg. Consequently, nails (1-3mm) were collected from the same subject using a clean nail clipping (washed with ethanol). Most of the studies using nail clipping for the elements analysis of finger nail [38,39] and toenail [40,41]. As a bio-marker toenails is better than fingernails with respect to external contamination and to maximize the exposure time window [42-44]. Collected hair and nail samples were preserved with intensive attention to avoid the external contamination. Water samples were dried at 100^0C until they reached close contact to bottom of the flux, and then it were absorbed onto filter paper as similarly prepared for gamma ray counting.

Fig. 1. Location map of the study area ('*Achintanagar*' village, Jhenaidah district)

2.3 Sample and Standard Preparation

In order to remove external contamination, each of the hair and nail samples were washed 3 times in a petri dish followed by the cycle: acetone + deionized water + acetone and then allowed to dry (at room temperature) naturally. A number of studies are recommended hair [18,38,43,45-48] and nail [2,11,17,39,44,49] washing procedure with water and acetone to remove visible external contamination. The dried clean hairs and nails were cut into small pieces and were placed into the ultra-clean polyethylene (PE) irradiation vials. For standardization the relative method is used. Thus three types of standard reference materials (SRMs) IAEA-SL-1, IAEA-Soil-7 and NIST coal fly ash (1633b) were placed into the ultra-clean PE irradiation vials. All the samples and SRMs were bundled together and put into the PE irradiation tube.

2.4 Irradiation and Counting

The total arsenic (TA) content in human hair, nail and water was determined by Neutron Activation Analysis (NAA) technique as described until that time [33,50]. Irradiation was performed at the dry crystal thimble (DCT) facility of the TRIGA Mark –II research reactor with power set at 1 MW for 20 minutes with thermal neutron flux of 1.5×10^{13} cm^2/sec for the determination of As. Irradiate samples were placed into HPGe (high purity germanium detector; CANBERRA, 25% relative efficiency, 1.8 keV resolution at 1332.5 keV of ^{60}Co) in conjunction with a digital gamma spectrometer (ORTEC, DSPEC Jr^{TM}). After two days decay, first counting was performed 3600 sec for the estimation of arsenic. Indicated target elemental toxicity [$^{75}As(n,\gamma)^{76}As \rightarrow ^{76}Se$; $t_{½}$=26.3 h; E_{γ} = 559.1 keV, 657 keV] with the execution program (software) Maestro- 32 (ORTEC).Relative standardization method was applied to concentration calculation. Gamma peak analysis and concentration determination, resulted from (n,γ) reaction and it may vary for different elements. Arsenic contents were calculated using software Genie-2000 (Canberra) and MAESTRO-32(ORTEC).In addition, Hypermet PC version 5.12 was used for manually peak checking. Al- 0.1%Au foils were used for the determination of the neutron flux gradient within irradiation tube bundle with foils at three positions. Quality control was performed using three international reference materials (SRM) IAEA-Soil7, IAEA-SL-1 and NIST 1633b Coal Fly Ash within 10% deviation.

3. RESULTS AND DISCUSSION

In this study, groundwater contamination and its extent through food chain and negative consequences on living organism were evaluated. Out of these 51 subjects, 25 were male and 26 were female. The age variation was 5 to 72 years of the target subject. Most of the people at the study area was affected by arsenicosis disease due to use of highly As contaminated ground water. Nail and hair samples were analyzed to identify the degree of As content and their variation in respect of gender. Results indicated that the average As content in male and female hair samples are 0.93 µg/g and 3.71 µg/g respectively. As content in nail samples for male and female are 1.40 µg/g and 2.03 µg/g respectively. Many studies were documented for As content in hair and nail samples to correlate the present situation of Bangladesh [27,51]. For example, A few authors reported different levels of As content in human hair ranging from 0.47 to 6.64 µg/g, 0.02 to 8.17 mg/kg and 0.906 to 0.795 mg/kg [51,52,53]. Arsenic content was reported from patients at the arsenic affected area of west Bengal in India range from 0.17 to 14.39 mg/kg [48]. World health organization (WHO) recommended value for human hair 1 *ppm*. Details descriptive statistics of As content of different objects are shown in Table 1.

Table 1. Descriptive statistics of As content in different samples

Sample ID	*n*	Mean	Variance	SD	RSD (%)
MH	25	0.926±0.08	0.308	0.555	59.98
FH	26	3.707±0.36	16.28	4.035	108.8
MN	25	1.397±0.09	0.471	0.686	49.14
FN	26	2.034±0.19	3.505	1.872	92.06
W	9	0.89±0.13	0.719	0.848	95.69

Note: n = Sample Number, *SD*= Standard Deviation, *RSD*= Relative Standard Deviation

It is reported that a few factors were responsible for affecting element accumulation in hair and nail matrices such as geochemical environment, dietary habits, lifestyle etc [54,55]. Average As concentrations (0.89 µg/g) in tube-well water at the study area were higher than permissible limit (0.05 ppm) for drinking water in fact of Bangladesh. The other particular source could be attributing to As contamination in this study area. Groundwater is a primary source for irrigation activities in Bangladesh. Due to its long term practice, soil may build up facility to accumulate As from irrigation water. A significant amount of As content is increased in agricultural soil at the end of the season [56]. A few studies have been reported to arsenic contamination in rice grain and vegetables in Bangladesh [57,58,59]. Food contamination and its daily dietary may reflect to degree of toxicity.

Metal content in hair of human body rely on hair colour, age, sex and smoking habitat in spite of insufficient information on this subject [17,60-64]. According to the data from descriptive statistics (Table 1), As content in both matrices (hair and nail) indicated a partial source of human exposure with particular place and conditional movement of toxic element at the study area. Arsenic tends to bind with sulfhydrayl groups (Keratin) in hair and nails composition [65] which may reflect to current exposure. Table 2 illustrated the association of finding from several studies of As content of inactive tissue from human body.

Table 2. Comparative study of found As content with world reference

As content	**Taksagi et. al. [66]**	**Samanta et. al. [48]**	**Carreiro et al. [67]**	**Lin et al. [45]**	**Saad and Hassanien et al. [68]**	**Karagas et.al. [65]**	**Oluwol et al. [69]**	**Mand et al. [46]**	**Our study**
Mean content of As (Nails)	N/A	4.73	N/A	.71	N/A	0.61	N/A	5.53 (ave)	MN=1.397, FN= 2.034
Mean content of As (Hair)	0.61	N/A	0.43	N/A	0.54	N/A	0.28	N/A	MH=0.926, FH= 3.707

N/A: not available; ave: average

Elemental content of As in both matrices exhibit a significant correlation for better understanding with degree of exposure and similarities. A significant (p<0.01) correlation between toxic elements are quite similar on described as Rodushkin et al. [32]. In Fig. 2, it was observed that significant correlation in hair and nail for male r=0.615 and female r=0.728. Lin et. al. [45] reported a significant (r=0.53, p<.01) correlation between hair and nails. This correlation indicated higher affinity to As accumulate in inactive tissue of human body. In the study area that had the highest amount of arsenic in ground water also showed the highest amount of arsenic in human hair and nail especially for females.

A significant correlation (r=0.79 and r=0.72; p=0.01) was found in female hair and nail As content in relation to water content (average 0.89 µg/g). Similar association (r=0.49 and r=0.53; p=0.01) was observed in both matrices for male with water content at the significance level of correlation coefficient are shown in Fig. 3. Karagas et. al. [65] found a significant correlation (r=.46, p<.001) of arsenic contamination between water and nails. Arsenic content in fingernails and water exhibit a positive significant (r=0.68, p<0.03) correlation at the arsenic affected area of west Bengal, India [46].

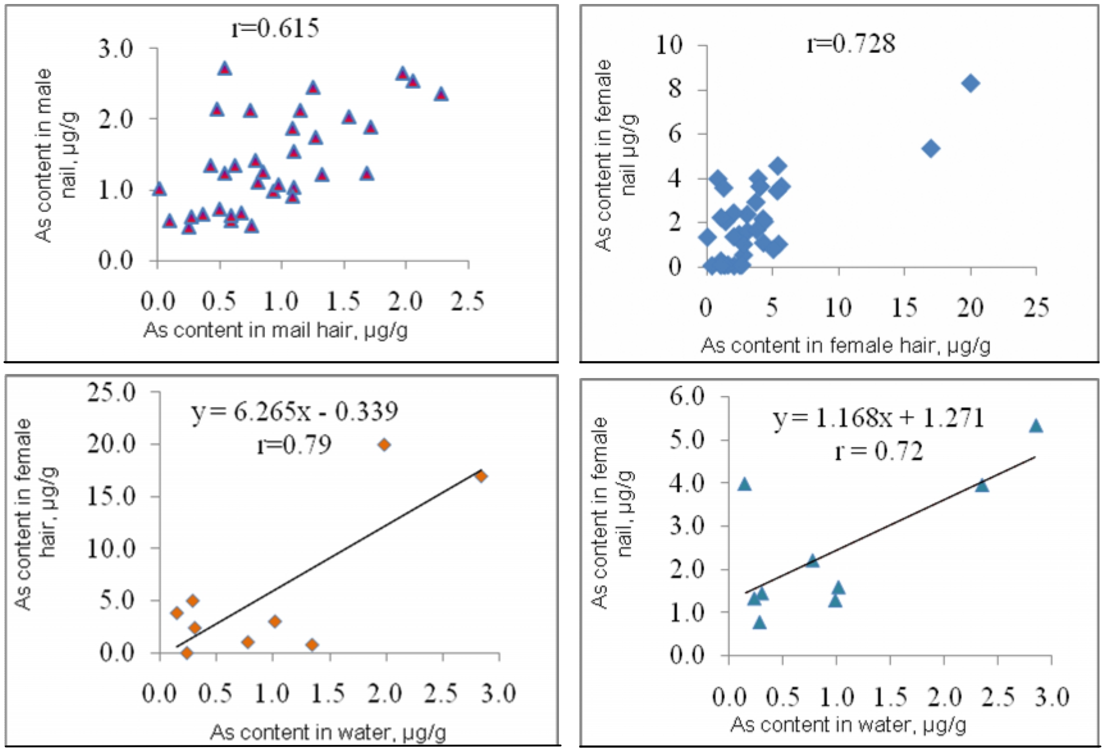

Fig. 2. Shows the relation of As content in gender variation for hair and nail

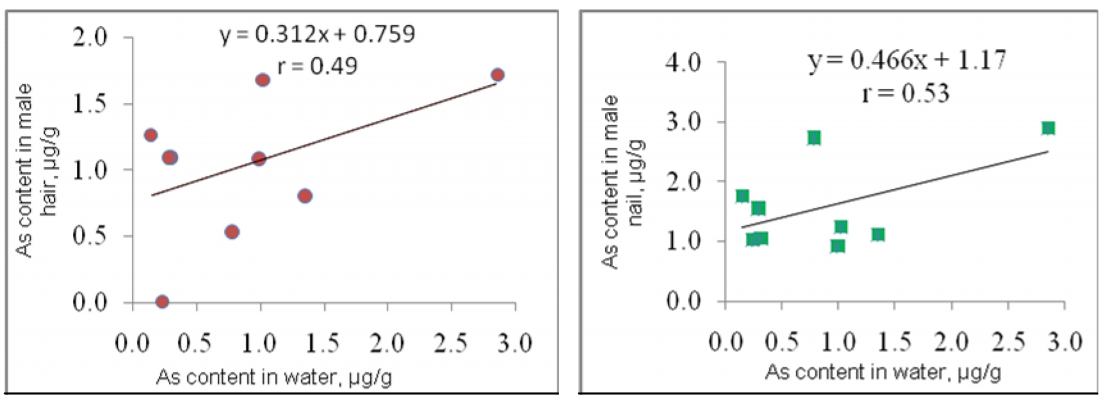

Fig. 3. Shows correlation between average arsenic content in both matrices (hair and nail) and water samples

Note: Indeed, a community tube-well s contributes to water supply for drinking purpose in two or three family members. For statistical correlation analysis, arsenic content in both matrices for male and female were taken average from the community tube-well water

Shows Fig. 4, arsenic content in human hair and nail showed greater variability perspective to gender variation. Results showed As content in female hair were greater than male hair. Arsenic contents in female nails were two times higher than male nail. A significant difference of As arsenic content in both male and female hair was observed due to using of

As-enriched groundwater [70]. Karagas et al. [65] reported the Arsenic content variation in relation to sex in toenails. Arsenic content in the male subjects was higher than in the female subject [22].

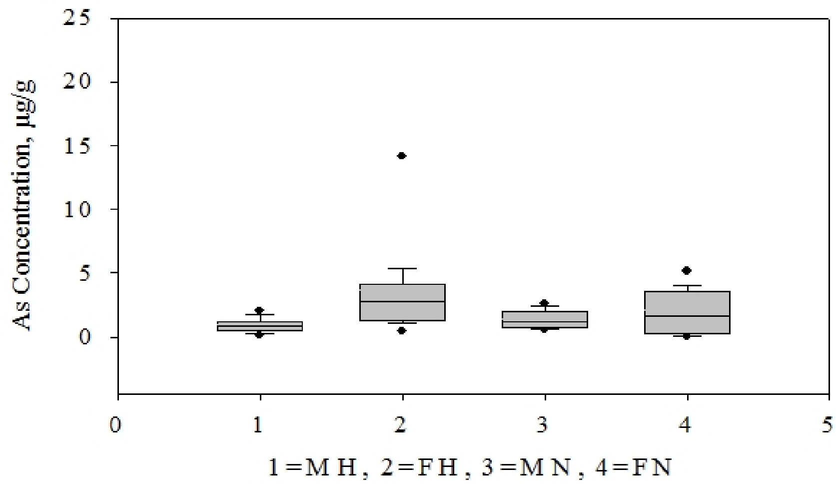

Fig. 4. Shows As content in both matrices in relation to gender (male and female)

According to results, As uptake may be attributed to live duration at residence, exposure time, immune system, metabolism activity. The methylation processes have confirmed a reactivation the toxic effect of arsenic through biochemical reaction [71]. A number of studies have been confirmed to more efficient methylation power in women [72-80]. It may be the important point to explain the gender variability of As content in hair and nails. Others such as hair conditioner, shampoo, smoking habit, water intake (per day) and sampling season may have significant effect on arsenic toxicity in relation to exposure condition. A person (male), spends at least 12-14 hour within 24 hours spend outside of the house for working purpose at different places. At that time, they did not continue to take contaminated food in fact it may be reduced As content in both matrices. Others such as, age and occupation may play as a confounding factor for As toxicity.

From observation, 20% of the people at the study area were arsenic affected due to highly contamination of groundwater. Sampson et al. [81] reported that arsenicosis symptoms (hyperpigmentation, Blackfoot disease, raindrop hypopigmentation) have been develop after 8-10 years of consuming As contaminated (elevated As levels) water. Spontaneously the subject group used this contaminated water in spite of their arsenicosis symptoms because lack of logistic support to get available arsenic free water for household purpose. The socio-economic condition [70] and nutritional status [81] has several direct effects on As toxicity. Most of the people of this area live in house and mainly take rice. The high level of arsenic content was found in the studied population at the residence location. Based on observation, it is difficult to change (lack of financial and energy resources) this situation due to far from other sources to easily carry home water. This research will be extended to investigate the As content in food consumed by the population and soil.

There are a number of techniques to treat arsenic. However, most of them rely on converting all of the arsenic to a valence of +5 (some will be +3) because of easier to remove. The technologies to remove As include adsorption on alumina, reverse osmosis membrane separation, ion exchange, or lime softening. A Significant effort has been made in recent decades to identify arsenic contamination by the government and non government organizations (NGOs) in Bangladesh. The Government of Bangladesh has taken a number of initiatives to supply arsenic free drinking water through Bangladesh Arsenic Mitigation Water Supply Project (BAMWSP). The NGOs and international development partners involved (Asia Arsenic Network, IDE Bangladesh, the NGO Forum, World Vision, Hospital, BRAC, Dhaka Community Care Bangladesh and Water Aid Bangladesh) in different arsenic mitigation activities in Bangladesh. Particularly, UNICEF Bangladesh works with the Department of Public Health Engineering (DPHE) and NGOs in some of the highly arsenic-contaminated areas in the country. The 'SHEWA-B programme', funded by the U.K. Department for International Development (DFID), uses communication materials to raise awareness about sanitation, hygiene, and safe water, primarily through a network of 10,000 Community Hygiene Promoters (CHPs). 'The DART project', funded by the Canadian International Development Agency (CIDA) works in the area where arsenic contamination is serious due to lack of easy alternative water supplies.

Initial survey in the present study indicates that a large number of subject populations may chronically poisoned from arsenic in the study area. The affected people need to be seriously investigated to examine their health hazards. In addition, they need to be supported to mitigate the risks. Attempt should be made to supply them arsenic free drinking water and seek out the problem for the sustainability measure and proper health care.

4. CONCLUSION

Arsenic accumulation and elimination activity in body mechanism demonstrated its toxicity depends on environmental exposure in the present study. Accordingly, it may be concluded that higher values of As content in human hair and nails tissue were accumulated may be due to the longtime use of contaminated groundwater or foods. Results indicated that the ground water was contaminated with arsenic and acts as the primary source of arsenic poisoning among the peoples of the study area.

There are limited studies of the assessment on possible health risks linked with consumption of the As contaminated ground water through NAA particularly in Bangladesh. Consequently this study was conducted at '*Achintanagar*' village to assess the concentration of Arsenic in water, human hair and nail. The result As content in groundwater, it may attribute elevated transfer to human food chain.

This is not the conclusive evidence that the groundwater is the cause of As concentration increasing in the test samples. But there may be some association of groundwater contamination to increase As concentration in all the subject samples examined in the study area. There is insufficient evidence in the literature for a possible As concentration in human hair and nail due to groundwater. This study is so far the first evidence that groundwater can cause As contamination in inactive tissue. A detail biochemical research will be needed to establish these critical findings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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