1-2.Bioremediation of As polluted groundwater in Bangladesh

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Abstract

Biomats and groundwater were observed and analyzed to find out the microbial ability in bioremediation of As. Samples were collected from an in situ reactor in one of the highest As polluted areas 'Hazigonj' in Chandpur, Bangladesh. Epithermal Neutron Activation Analysis (NAA) of gray biomat shows that As accumulation in microbes takes place at a very large scale in the nearest flow point of As polluted groundwater. Which are several hundred times higher than that of water contains. While groundwater contains 0.03 ~ 1.71 ppm of As then biomat show 390 ~ 550 ppm in concentration. Optical and Scanning electron microscopic observation proved that biomats are consisted of autotrophic and heterotrophic bacteria mainly of coccus, bacillus and filamentous typed, associated with photosynthetic algae. Energy dispersive X-ray fluorescence spectroscopy (ED-XRF) analysis of biomats and groundwater confirmed the As attenuation taken place in an in situ reactor. That shows the most of the As in groundwater are accumulated by biomats at the nearest flow point (around 30 cm). As a result a very low concentration of As detected in the down stream, 4 m apart from the main flow point. And finally no traces of As is found in water at the down stream around 8 or 12 m apart from the main flow point. Arsenic (As) ion might be adsorbed onto the microbial cellular surface or biomethylated forming biominerals or arsenosugars respectively. And finally make As (arsenic) immobilized or volatilized. The

processes are believed to be the part of detoxification mechanism in the living organisms. The in situ experiment proved that As bioremediation might be possible with biomats that contains metabolically active microorganisms of the specific geo-aquatic environment. The method is much more economic and suitable for groundwater As pollution of Bangladesh or any other geo-aquatic eco system in the natural environment.

Key Word: Bangladesh, Arsenic pollution, Groundwater, Biomats, Neutron activation analysis, Bioremediation.

INTRODUCTION

Arsenic, the toxic metalloid is a hazardous material for its widespread carcinogenic, mutagenic and teratogenic character, responsible for many diseases such as lung, skin, liver and bladder cancer, gangrenes of toe or some of cardiovascular and neurological effects (Choudhury et al. 1998; Silver 1999; Karim 2000; Lena et al. 2001; Le 2002). Most of these kinds of disease can eventually be developed in those people, who drink water from tinted sources including As polluted ground water (Das et al. 1995; Kaiser 2000). Recently the evidence of arsenical chronic poisoning has been reported in many parts of the world and has become a serious public health problem in some of the Asian countries. The most noteworthy occurrences are in Bangladesh, India, Taiwan and Thailand including some part of Europe and America (AAN et al. 1999; Das 2000; Islam and Tazaki 2000a). Especially about 95 % or more of the total population of Bangladesh now use groundwater for drinking. Unfortunately, more than 60 % of shallow and deep-tubewell water (groundwater) contains As above the WHO guideline value of 0.01 ppm and more than 30 % of the tubewells contains As above the Bangladesh standard of 0.05 ppm. An estimated 30 ~ 35 million people in Bangladesh have been exposed to As through drinking water concentration above 0.05 ppm (BGS and DPHE 2001). Only the high levels of As in groundwater causes the widespread poisoning in Bangladesh (Harvey et al. 2002). The seriousness of the poisoning was first observed at Hazigonj in the district of Chandpur, the one of the worst affected areas in the southeast part of Bangladesh and found almost 90 ~ 94% of wells were

contaminated. (British Geological Survey and Mott. MacDonald. 1999; Smedley and Kinniburgh 2002).

In contrast, microorganisms can grow and survive in some of the most extreme and adverse environments on the earth such as high pollution, high temperature, and high pressure even in the strong acidic and heavy metals or As rich conditions (Fyfe 1997; Tazaki et al. 1998; Tazaki 2002). Meanwhile, some autotrophs and heterotrophs have also the ability to use arsenic (arsenite) as their sole or auxiliary source of energy (Ehrlich 2002). Frankenberger and Arshad (2002) suggested that bacterial or microbial methylation or some biotransformation lead to less toxic forms of As that can be used in the detoxification of the As contaminated environments. Tazaki et al. (2002) showed that microbes could play a great role in the natural bioremediation of As polluted geo-aqua ecosystem. Bacteria or microorganism in biomats selectively accumulate heavy metals and metalloids as having their own niche in the geo-aqua-ecosystem (Ariza 1998). Gadd (2002) proposed that the microorganisms are intimately involved in the biogeochemical cycling of metals or metalloids in the aquatic environment, and have potential applications in bioremediation. Krumbin (1979) also presented that the microorganisms in microbial mat have controlled bio-transfer process and the biogeochemical cycles during most of the history of life on the earth.

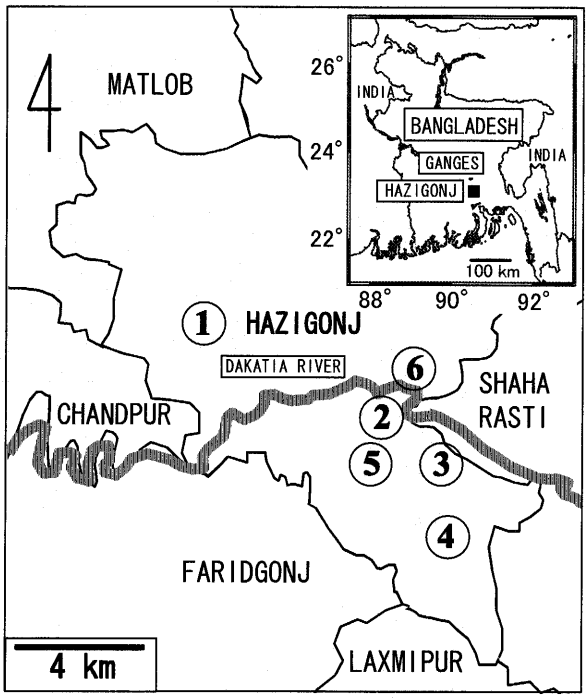
Consequently, it may be considered that including Bangladesh, geological sources of arsenic are environmental problem worldwide (Rosen 1999). Especially in Bangladesh, to mitigate the drinking water problem several kinds of usable filters are currently available for the treatment of arsenic polluted water. However, the verity of such kinds of filters is still rather limited. From the most recent research of Islam et al. (2002) suggested that arsenical attenuation might be considered on the significant role of microbial community produced around the As polluted geo-aquatic environment naturally. Besides these biogeochemical and metabolic activities of microorganisms has not also been focused for bioremedial process in As-polluted water. Microorganisms can produce arsenical minerals on their body surface and can stable As from its ionic form in the water of natural environment.

The present study is specifically designed to elucidate the biogeochemical activities of microbes in biomats, those are concerned with the arsenical bioremediation in an in

situ reactor. We also describe about the general situation of groundwater As pollution in the sampling area of Hazigonj, Chandpur, Bangladesh.

MATERIALS AND METHODS

A highly Arsenic polluted area, Hazigonj in the district of Chandpur located at (23° 15′ N and 90° 50′ E) in the southeastern part of Bangladesh (Fig. 1). The area is alluvial deposits of old Meghna flood plain (SSDI 1999). For the general investigation groundwater, biomats of different colours (gray, grayish-black, black etc.) together with pond and river water were collected in precleaned polyethylene bottles and containers from 18 spouts of 6 locations (① Dherra, ② Noadda, ③ Raycho, ④ Barkul, ⑤ Pond (Borodighi), ⑥ Dakatia River) in Hazigonj. In addition hair, nail, urine and blood were also collected in polyethylene bags and 10 ml vials from sampled people of the same locality who still drinks As polluted groundwater and few of them have already been exposed by As and suffering from various skin problems. Especially for the present study an in situ reactor was selected at the spout 1 of location 4, having a stream of groundwater flow from a hand pumped tube-well, which is used for lifting groundwater for drinking (Fig. 2). Profusely produced biomats in front of tube-well (W1, around 30 cm) and from other 3 points (W2 at 4 m, W3 at 8 m and W4 at 12 m) throughout the upstream to the down were picked up with water. Both of the water and biomat samples were collected in a natural and fixed (fixed in 1 % gluteraldehyde) condition. The samples were preserved bellow 4 ℃ and in room temperature.



① Dherra
② Noadda
③ Raycho
④ Barkul
⑤ Pond (Borodighi)
⑥ Dakatia River

Fig. 1 Locality map of the study area showing sampling locations at Hazigonj in the district of Chandpur, Bangladesh.

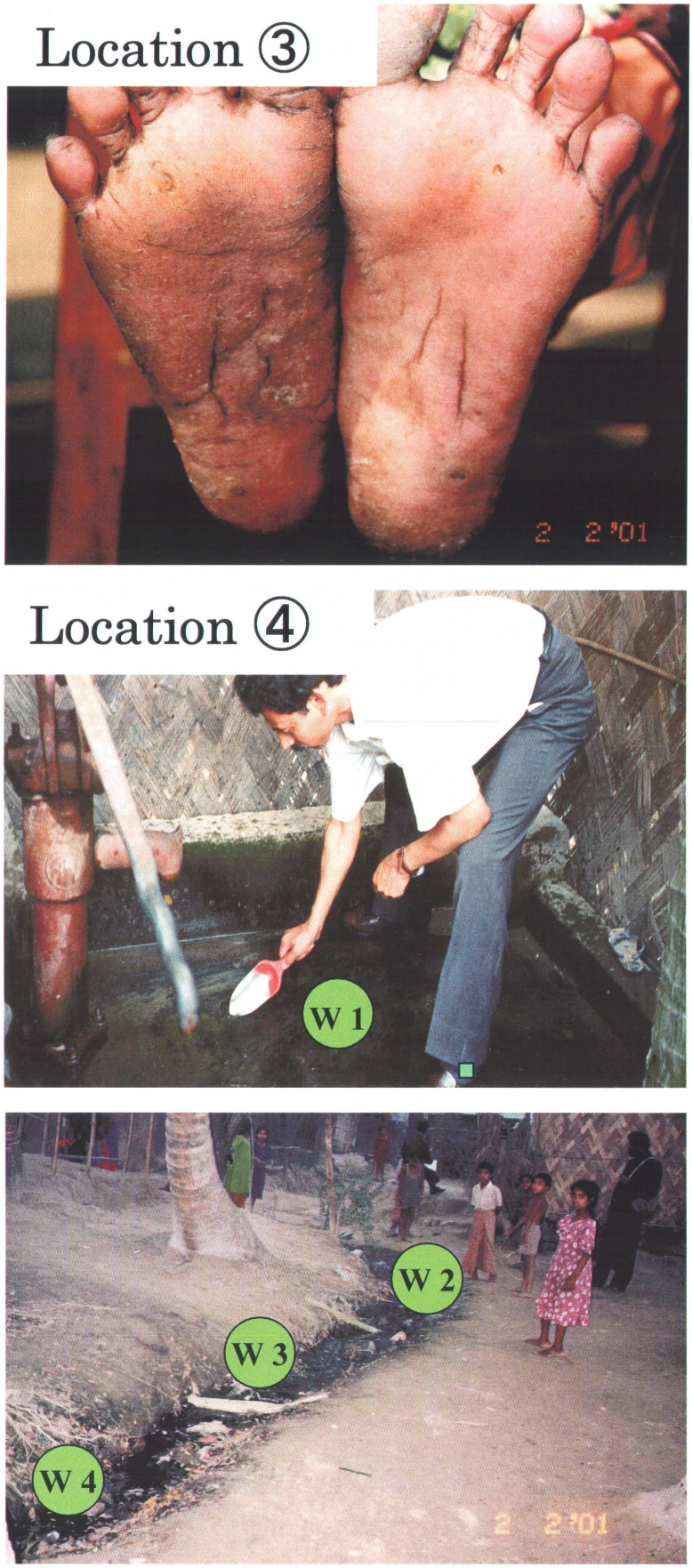


Fig. 2 Field view of sampling locations. A female patient exposed by drinking As polluted groundwater which caused keratosis on both of her legs (location 3). Grey biomats has been predominated in front of a hand pumped tube-well, which is used for ground water lifting (point W1 in location 4). Ground water flow in an in situ reactor through out the upstream to the down (point W1 ~ W4) at the spout 1 of location 4 in Hazigonj, Chandpur, Bangladesh.

An icebox was used for the collection of urine and blood samples in the field. Blood was collected by a medical practitioner, after collection those were stored in -18 ℃ till analysis in the laboratory. Specifically the water samples were tested in the field for the determination of As-concentration and its relation with Fe^{+2} or total Fe by the pack test kits. In the laboratory, all samples were analyzed by ED-XRF for their chemical composition. In addition selected biomats and hair samples were re-analyzed by Neutron Activation Analysis (NAA) to quantify the As in those samples. Besides this, optical and scanning electron microscopic observations equipped with EDX analyses also carried out for the identification and assessment of the metabolically active microorganisms and elemental concentration in biomats respectively in micro scale.

Water quality

Since the people of sampling area oftenly drink the As polluted groundwater from the hand pumped tube-well, which has caused serious health hazards. On the other hand microorganisms in biomats can survive in the same kind of polluted water and capable of absorb some elements in to their cells as in ionic form, Water quality data are thus important in identifying the reason of health hazards and the properties of microorganisms present.

To obtain data on water conditions pH, Eh, EC and DO are measured in the laboratory. pH is for indexing hydrogen ion concentration, Eh for the oxidation-reduction potential of the aqueous solution, EC for the electrical conductivity which is related to the quantity of dissolved ions and DO indicates the quantity of dissolved oxygen in the water. They were measured by a portable water quality inspection meter (pH; D-12, Eh; D-13, EC; ES-12, DO; OM-12 made by HORIBA). The measurement was carried out in the laboratory after three weeks of collection on the February $24^{th.}$ 2001. Besides this As concentration in water was tested in the field with the pack-test kits by molybdenum blue method (Kyoritsu chemical-check Lab. Corp.). In addition pack-test also carried out for Fe^{2+} and total Fe concentration. Field tests have been conducted in the field for three times (February 2^{nd} ~ 4^{th}, May 12^{th} ~ 14^{th}, in 2001 and February 11^{th} ~ 14^{th}, in 2002). Seasonal fluctuation is also been taken in the consideration.

Energy dispersive X-ray fluorescence spectroscopic (ED-XRF) analyses for the chemical composition of water, biomats, hair, nail, urine and blood samples

Water samples were filtered through acid cleaned nucleopore filter (0.45 μm) in a closed syringe (Terumo, 20 ml). After filtration, 10 ml of each water samples were pipetted using calibrated pipette in to a small porcelain crucible and then allowed to dry up at a low temperature (about 50 ℃) for 48 hours. As a result residual dried samples were obtained. The dried powdered samples were weighted out and found about 8 ~ 10 mg (average) in each sample. Then the obtained dried powder was taken onto the miler film for ED-XRF analysis. Biomats samples were dried in a decicator and then ground them well about fine particle size. These powdered samples also about 8 ~ 10 mg of each was taken on to the miler film for analysis. Hair and nail samples were made powder by using liquid nitrogen and a mortar. Blood samples were prepared on the miler film after air-drying. Urine was also prepared as the same procedure of water for about 40 hours. Analysis were carried out with an energy dispersive X-ray fluorescence spectrometer (JEOL JSX 3201 using Rh Kα) operated at an accelerating voltage of 30 kV in a vacuum condition.

Optical and scanning electron microscopic (SEM) observations

Optical microscopy

To identify the presence and variety of microbe's optical microscopic observation were carried out. Hand picked biomats, washed with distilled water were mounted on and spread over the slide simultaneously. Both of the episcopic and DAPI (4' 6-diamidino-2-phenylindole) stained samples were observed through episcopic fluorescence microscope (Nikon EFD3, Digital camera: COOLPIX 995). A filter UV-1A was used for epifluorescent microscopy. The DNA of bacterial cell in DAPI staining sample shows the fluorescence blue while red parts indicate the presence of photosynthetic pigments under the ultra violet ray (365 nm).

Scanning electron microscopy (SEM)

The scanning electron microscope equipped with an energy dispersive X-ray

spectroscopy (SEM-EDX; SEM: JEOL JSM-5200LV and EDX: PHILLIPS EDAX PV9800EX) was used in order to observe the micro morphological surface of microbes in biomats and its association with some particles which include some elements. One droop of pre fixed (gluteraldehyde, 1 %) biomat sample was mounted onto the JEOL filter, The sample was washed again with 2.5 % gluteraldehyde twice and rinsed and fixed with *t*-butyl alcohol and placed into the liquid nitrogen for freezing after Suzuki et al. (1995) methods. The sample on the filter was dehydrated in the low-vacuum chamber of SEM. After completion of freeze drying the sample was mounted on a carbon tape posted bronze stub. Dehydrated sample was coated with carbon for analyses. The analyses were carried out in 15 and 25 kV with different magnifications.

As measurement by Neutron Activation Analysis (NAA)

Epithermal neutron activation analysis had been carried out to find out the As concentration in a very trace level (ppb). Selected biomats and hair samples were analyzed for quantifying the As concentration in them. About 50 - 100 mg samples (patient hair and 2 biomats) were weighted out and placed in polyethylene bags size of 10 x 10 mm and hot sealed. As reference contained 1.0 μg arsenic were prepared from 100 ppm standard solution of atomic absorption analysis grade (100 μg/ml, Kanto Chemical Co., Inc.). 100 μl of the solution was pipetted, using calibrated pipette, onto 1 cm filter paper. The filter paper was allowed to air dry and then placed in a polyethylene bag and hot sealed for irradiation. For epithermal neutron activation analysis, cadmium foil was used. All samples and reference were covered by Cd foil with 1 mm thickness. Arsenic was determined by using the $^{75}As\ (n, \gamma)^{76}As$ at the JRR-4 reactor in Japan Atomic Energy Research Institute with $\phi = 1 \times 10^{-13}$ for 5 min. After irradiation the samples were decayed for approximately 18 hours, and then live-time counted for 1000-8000 sec using HPGe detector with energy resolution about 2.0 keV for ^{60}Co photo peak at 1332 keV. The gamma ray ($E\gamma$ =559.1 keV) from ^{76}As ($T_{1/2}$ = 26.32 hours) is used for analysis, and concentrations are determined by standard comparison.

Experimental parameters were as follows, thermal neutron flux ; 5.3×10^{13} cm^{-2} s^{-1}, epithermal neutron flux ; 1.3×10^{13} cm^{-2} s^{-1}, irradiation time ; 5 min., ^{76}As half-life ; 26.32 hours, gamma-ray energy ; 559.1 keV, data reduction ; Standard comparison.

Physical examination and questionnaire survey for the investigation of general situation at the sampling locality

A physical examination was carried out by a medical practitioner conducted between 11^{th} ~ 14^{th} February 2001. Basing on the epidermal symptoms of As exposure, mainly of arsenical spots, arsenicosis, melenosis, spoted-melanosis, karatosis and hyperkeratosis on the chest, leg, palms or on the foots. Besides this the sampled people were also asked some questions, concerning their health, self-consciousness, food and drinking habits, knowledge on arsenical pollutants in drinking water to find out the importance of motivation or remediation process. The data and necessary information were gathered throughout an open-ended questionnaire accompanied with a physical observation. Laboratory methods also applied for the analysis of collected samples those were mentioned before.

RESULTS

Water quality and the concentration of As, Fe^{2+} and total Fe

The water quality with As, Fe^{2+} and total Fe concentration of different sampling locations (①, ②, ③, ④, ⑤ and ⑥) of Hazigonj in Chandpur, Bangladesh are given in Table 1. All groundwater samples from tube-well showed a little basic in character while the water of pond and river was little acidic. A high concentration of As has been detected in the groundwater of different locations, which ranges between 2 ~ 7.5 ppm in the field and 2 ~ 3.5 ppm in the laboratory after removing PO_4. On the other hand no As was detected in the pond (Borodighi) or river water. Pack test method was used for the determination of As, Fe^{2+} and total Fe concentration both in the field and laboratory. It was noticed that the pH value (pH 7.5) of water in location 3, is a little elevated that contains high As with low Fe and high EC. Conversely, in normal pH (7 or 7.1) less amount of As with high Fe and low EC was noticed. The water sample of spout 1 from location 4 showed a little elevated pH with high concentration of Fe and less amount of As in an average EC.

Table 1 Water quality and the concentrations of As, Fe^{2+} and total Fe in different spouts of sampling locations at Hazigonj in Chandpur, Bangladesh.

Location	Points	pH	Eh (mV)	EC (mS/cm)	DO (mg/l)	As (ppm) Pack-test	Fe^{2+} (ppm) Pack-test	Total Fe (ppm) Pack-test
① Dherra	Spout 1	7.1	212	0.872	2.7	2.0	1.70	7.50
	Spout 2	7.0	219	0.679	1.6	2.5	1.80	9.00
	Spout 3	7.0	216	0.670	1.5	3.0	0.80	3.00
	Spout 4	7.2	195	2.760	1.7	5.0	1.20	1.80
② Noadda	Spout 1	7.2	280	0.466	4.9	2.0	0.05	1.00
	Spout 2	7.2	273	0.562	3.8	2.2	0.10	1.50
	Spout 3	7.1	267	0.396	5.8	3.0	0.05	0.55
	Spout 4	7.2	263	0.382	5.6	3.0	0.06	0.05
	Spout 5	7.2	264	0.415	5.6	2.5	0.08	0.06
③ Raycho	Spout 1	7.5	217	1.319	2.4	7.5	0.15	0.06
	Spout 2	7.3	208	1.748	2.3	6.5	0.06	0.50
	Spout 3	7.4	222	1.737	3.8	6.2	0.05	0.15
④ Barkul	Spout 1	7.2	250	0.549	3.7	3.0	1.90	1.50
	Spout 2	7.2	251	0.599	4.3	2.5	1.50	1.50
	Spout 3	7.1	238	0.807	3.3	5.0	0.20	0.50
	Spout 4	7.5	227	0.710	2.4	6.0	0.10	0.50
⑤Pond water	Spout 1	6.8	296	0.256	4.5	0.0	0.02	0.55
⑥River water	Spout 1	6.9	291	0.337	6.2	0.0	0.06	0.20

Eh: electrode potential *vs*. standard hydrogen electrode
EC: electric conductivity
DO: dissolved oxygen.
Pack test : was done in the field by Kyoritsu pack-test kits.

Energy dispersive X-ray fluorescence spectroscopy (ED-XRF)

The analytical results of ED-XRF are given in the Table 2 for all sorts of samples collected from location ①, ②, ③, ④, ⑤ and ⑥. Almost all groundwater from location ①, ②, ③ and ④ showed the presence of Mg, Si, K, Ca, Fe and As with traces of Na, P, Ti, Br, and Sr. Similarities also have been found in the pond and river water (locations ⑤ and ⑥) excepting the traces of As or P. A very few of the ground water sample showed not only the presence of toxic As but Cr also. Besides this almost all elements including high concentration of As were found present in biomats as well as in groundwater. Biomats contained a high concentration of Al, Si, P, S, K, Ca, Mn, Fe and As with the traces of Mg, Ti, Zn, Br, and Sr (Table 2). Arsenic (As) was detected in the hair and nail samples accomplished with Si, S, Ca, Fe, Zn and traces of Al, K, Ti, Mn and Cu (trace of Cr detected in a very few sample). In some cases Mn was found absent in hair and nail. Urine sample showed the concentration of As which was discharged from most of body of the sampled people with Si, P, S, Cl, K, Ca, and traces

of Na, Mg, Fe and Br. Where as blood showed no trace of As excepting Si, P, S, K, Ca and Fe with the traces of Zn (Table 2).

Table 2 Energy Dispersive X-ray Fluorescence spectroscopic (ED-XRF) analysis of ground, pond and river water, biomats, hair, nail, urine and blood samples collected from Hazigonj in Chandpur, Bangladesh.

Sample name	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Mn	Fe	Zn	As	Br	Sr
Ground water	●	●	○	●	●	○	○	●	●	●	○	●	○	●	●	●
Pond water	●	●	○	●	○	●	○	●	●	●	○	●	●	○	●	●
River water	●	●	○	●	○	●	○	●	●	●	○	●	●	○	●	●
Biomats	○	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●
Hair	○	○	●	●	●	●	○	●	●	●	●	●	●	●	○	○
Nail	○	○	●	●	●	●	○	●	●	●	○	●	●	●	○	○
Urine	●	●	○	●	●	●	●	●	●	○	○	●	○	●	●	○
Blood	○	○	○	●	●	●	●	●	●	○	○	●	●	○	○	○

● Detected
○ Non detected

Comparative As among the samples determined by NAA, ED-XRF and Pack-tests

A comparative concentration of As in groundwater, biomats, hair, urine and blood samples are given in Table 3. As levels in groundwater was found ranging between 0.30 ~ 1.71 ppm (ED-XRF) and 0.7 ~ 3.5 ppm (Pack-test in laboratory), while biomats contain 200 ~ 600 ppm of As (ED-XRF). Furthermore excepting blood, As levels in hair, nail and urine samples were found 0.82 ~ 1.98, 0.90 ~ 1.98 and 0.49 ~ 1.20 ppm respectively determined by ED-XRF. The similar data was obtained by Neutron

Table 3 As concentration in ground water, biomats, hair, nail, urine and blood samples analysed by ED-XRF, NAA and pack-test.

I					II				
Ground water			Biomats		Hair		Nail	Urine	Blood
*BAMWSP (ppm)	ED-XRF (ppm)	Pack-test (ppm)	ED-XRF (ppm)	NAA (ppm)	ED-XRF (ppm)	NAA (ppm)	ED-XRF (ppm)	ED-XRF (ppm)	ED-XRF (ppm)
0.1-1.5	0.30-1.71	0.7-3.5	200-600	390-550	0.82-1.98	1.60	0.90-1.98	0.49-1.20	N.D

*BAMWSP (Field test of As by Bangladesh Arsenic Metigation Water Supply Project in 1999)
ED-XRF: Energy despersieve X-ray florescence
NAA: Neutron Activation Analysis
N.D: Not detected

activation analysis (NAA) for selected biomats and hair samples. Arsenic (As) was found present 390±8 ~ 550±2 ppm in biomats and 1.6±0.1 ppm in patients hair, which is extremely higher than that of its standard or of other country people. A comparative concentration of As in hair, nail, urine and blood (patients and non-patients) is given in the table 4. The level of As in the hair or nail of Bangladesh is more than higher that of its standard or of Japanese people. In addition urine samples showed a very high concentration of As than that of its standard or of even the highly exposed population of Argentina or Mexico (Arnold et al. 1990; Le 2002; Habib et al. 2002)

Table 4 A comparative concentration of As in hair, nail, urine and blood of sampled population with that of different country people and their standard values.

Samples	Standard		Taiwan	Argentina	Mexico	Japan	Bangladesh
	Nor Env. (ppm)	Affe Env. (ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Hair	*0.08-0.25	*1.00	-	-	-	***0.20	0.82-1.98
Nail	*0.43-1.08	-	-	-	-	-	0.90-1.98
Urine	**0.01-0.02	-	**0.05-0.06	**0.27-0.37	**0.45-0.70	**0.12-0.20	0.49-1.20
Blood	**0.03-2.00 (ppb)	-	**60.00 (ppb)	**8.00 (ppb)	-	**4.60 (ppb)	N.D.

Nor Env. ; Normal Environment
Affe Env. ; Affected Eenvironment
N.D. ; Not detecetd.
* ; After Arnold et al. (1990)
** ; After Le (2002)
***; After Habib et al., (2002)
- ; No data

Optical microscopy of biomats

The grey and black biomats were collected from the surroundings of a tube-well and from the nearest stream (W1 ~ W4) of groundwater flow at the spout 1 of location 4 in the sampling area of Hazigonj for microscopic observations respectively (Fig. 2). Optical micrograph of the grey biomats showed the various colonies of microbes (W1 in Fig. 3) are metabolically active in the As polluted groundwater flow even in a highly toxic aquatic environment. DAPI (4'6-diamidino-2-phenylindole) stained epifluorescence micrographs revealed the group of bacterial cells mainly of bacillus (about 1 ~ 2 μm), coccus (about 2 μm) and filamentous typed microbes absorbed the fluorescent blue and red parts, those indicates the presence of DNA in bacterial cells,

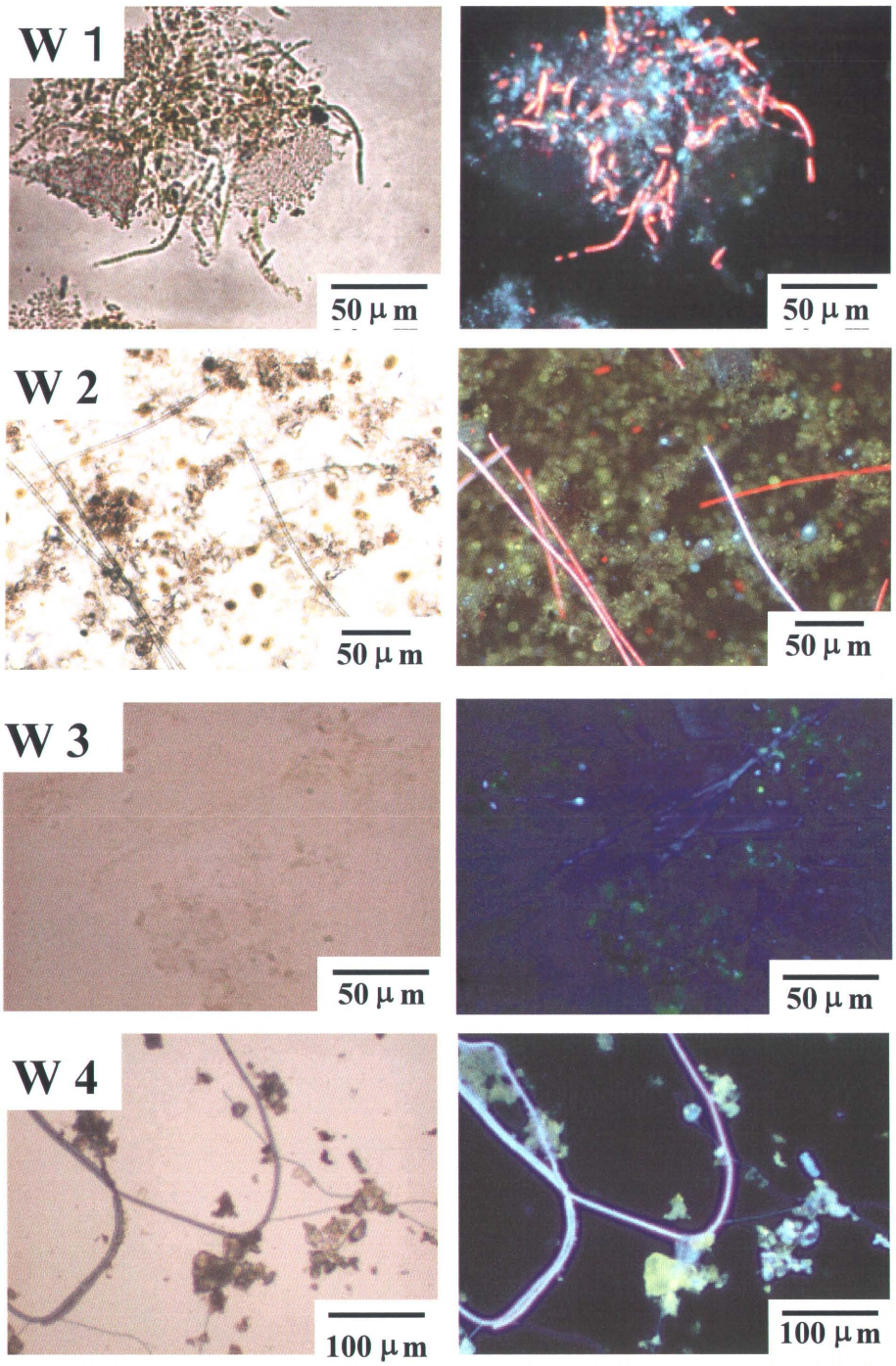


Fig. 3 Optical micrographs indicate microbial diversity in biomats mainly of bacteria and algal filaments collected throughout the upstream to the down (point W1 ~ W4) from spout 1 of location 4 in Fig. 2. DAPI (4'6-diamidino-2-phenylindole) stained epifluorescence micrographs show the florescent blue and red parts those indicate the presence of DNA in bacterial cells, and photosynthetic pigments in chromatophores of photoautphytic bacteria or in algal filaments (W1, W2) respectively. Microbial diversities in the down stream evidenced by epifluorescence micrographs, showing the fewer bacteria (W3) and filamental microbes (W4) without any photosynthetic pigment

and photosynthetic pigments in chromatophores of photo-autophytic bacteria (autotrophs) or in other algal filaments. Microbial diversity has been observed in biomat samples from the upstream to the down (W1, W2, W3, W4). In addition, it is to be noted that microbial colony in the down stream show the fewer bacteria (W3) and filamentous microbes (W4) without any photosynthetic pigments.

Scanning electron microscopy (SEM)

Scanning electron microscopic (SEM) observation recognized the microbial colonies in the grey biomats, collected from point W1 of spout 1 in location 4 (W1 in Fig. 2). A common view of entire colony indicates various kinds of microorganisms associated with different types of algal filaments and granular particles (W1-a in Fig. 4). The colonial form of bacillus typed bacterial cells (about 1.0 μm in size) adhered on to the surroundings of filament shaped substrate (W1-b in Fig. 4). The coccoidal (1.5 ~ 2 μm) and filament shaped bacteria exist in aggregation around the other filamentous microbes might be of algae (W1-c in Fig. 4).

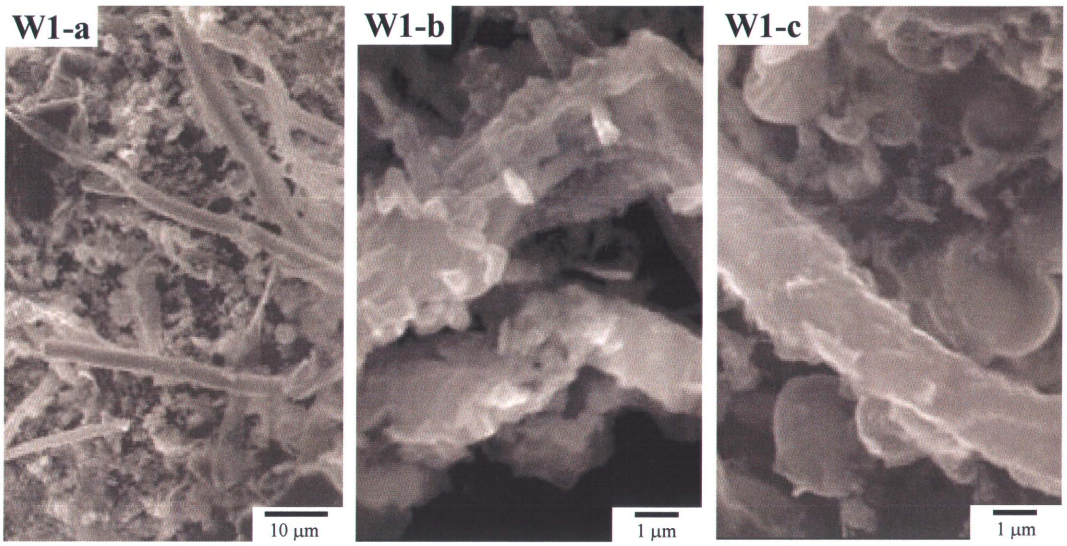


Fig. 4 Scanning electron microscopic images showing the presence of various microbes in the grey biomats, collected from point W1 of location 4 (see Fig. 2). A common view of entire colony indicates various kinds of microorganisms associated with different types of algal filaments and granular particles (W1-a). The colonial form of bacillus yped bacterial cells (about 1.0 μm in size) adhered on to the surroundings of filament shaped substrate (W1-b). The coccoidal (1.5 ~ 2 μm) and filament shaped bacteria exist in aggregation around the other filamentous microbes might be of algae (W1-c).

Spectra of ground water As attenuations by biomats.

The results of our present specific study, ED-XRF spectra plotted on graphs (Fig. 5) show the comparative arsenical attenuation in groundwater, which is corresponding to the predominated biomats produced in the different points throughout the upstream to the down (W1 ~ W4 in Fig. 5) in an in situ reactor. Noted that As, Fe, Si, Ca, and other elemental concentration were found higher in the biomats (B1 in Fig. 5) than that of water (W1 in Fig. 5) nearer (around 30 cm) to the groundwater flow point (tubewell; W1 in Fig. 2 of location 4). Comparatively low concentration of As with other heavy metals were found in water at the point W2 in the down stream, 4 m apart from the groundwater flow point. And almost no trace of As was observed excepting other elements in water at the point W3 or W4 of the same stream at a distance of 8 m and 12 m respectively (W3 and W4 in Fig. 5). Besides this biomats B2 in the down stream showed a little concentration of As, while almost no traces of As was detected in B3 or B4. However the spectra suggesting that significant amount of dissolved As in water was up taken by biomats at points W1 and W2. As a result waters at the point W3 and W4 don't show any As peak excepting the other elements (Fig. 5).

Results of physical examination and questionnaire survey

To know the general situation of groundwater As pollution in the sampling area, a total of 95 people were sampled for physical examination from 4 locations (① Dherra, ② Noadda, ③ Raycho, ④ Barkul) of Hazigonj, Chandpur in Bangladesh. The examination was carried out by a medical practitioner conducted between 11^{th} ~ 14^{th} February 2001. The patients were assessed in the field basing on the epidermal symptoms of As exposure, mainly of arsenical spots, arsenicosis, melenosis, spotted provided with previous diagnostic information's. The result showed that more than 73 % (almost 70 people out of 95) of sampled population was either patient or arsenically exposed, where as only about 27 % (almost 25 people out of 95) was found normal or un-exposed. Some of the patients have been suffering from arsenicosis, melenosis, spotted melanosis, karatosis or hyperkeratosis. Furthermore, basing on As concentration in hair, urine or nail analysed in the laboratory, arsenically exposed people were melanosis, karatosis or hyperkeratosis on the chest, leg, palms or on the

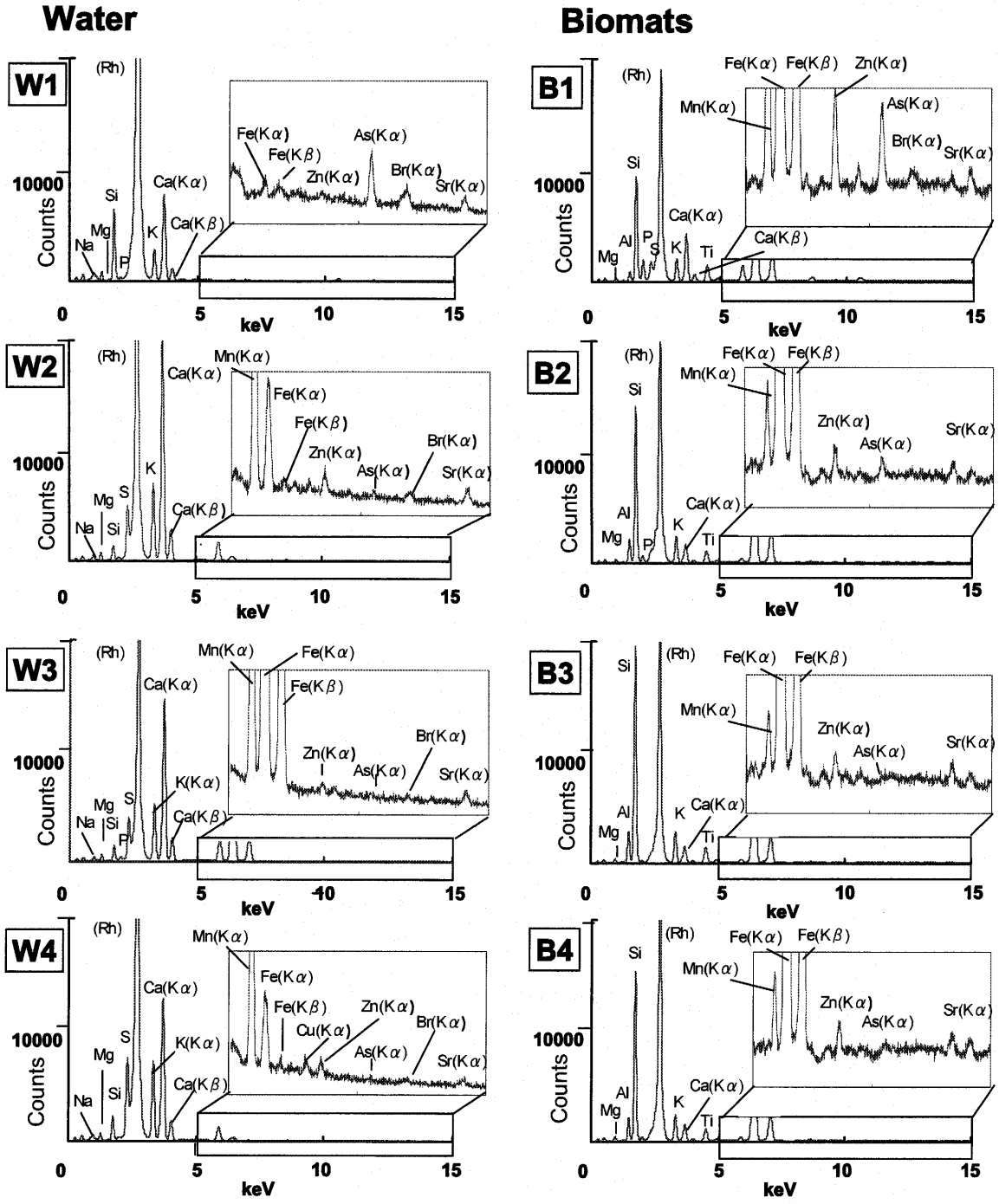


Fig. 5 ED-XRF spectra on graphs show the comparative arsenical attenuation in groundwater, which is corresponding to the predominated biomats produced in the different points throughout the upstream to the down (W1 ~ W4) in an in situ reactor. Significantly higher concentration of As is found in biomats (B1 and B2) than those of waters (W1 and W2) nearer to the groundwater flow point at location 4 (in Fig. 2) of Hazigonj, Chandpur in Bangladesh.

foots determined from the sampled population. Identified population is given in Table 5. To clarify the self-consciousness of sampled population, questions were asked about their present and past drinking habit. The result is given in Table 6. Observing the present's situation, most of the people of sampling area still drink the As polluted groundwater without any treatment due to the lack of self consciousness.

Table 5 Sampled people identified for As exposure in all locations of study area at Hazigonj, Chandpur, Bangladesh.

Location	Total personexamined	Patient	High con. As in Hair and nail	Normal
① Dherra	25	0	14	11
② Noadda	24	3	15	6
③ Raycho	22	12	9	1
④ Barkul	24	2	15	7
Total	95	17	53	25

Table 6 A comperative drinking habit of study population in the present and the past.

Location	Drinking water					
	Past			Present		
	ground	river	pond	ground	river	pond
① Dherra	23	0	2	18	0	7
② Noadda	22	0	2	15	5	4
③ Raycho	20	2	0	10	8	4
④ Barkul	24	0	0	14	1	9
Total	89	2	4	57	14	24

DISCUSSION

The general situation of As polluted groundwater and its effects on the sampling locallity at Hazigonj in Chandpur, Bangladesh

Basing on the physical examination, conducted between 11th ~ 14th February, 2001 in four villages (① Dherra, ② Noadda, ③ Raycho, ④ Barkul) of Hazigonj and on the analytical results of collected samples (pond in location ⑤, river in location ⑥ and groundwater together with blood, hair, nail and urine of patient and non patients), it is confirmed that the sampling locality 'Hazigonj' in the district of Chandpur is one of

the highest As polluted areas in southeast of Bangladesh. Because a very high concentration around 0.30 ~ 1.71 ppm of As was detected in the drinking water (groundwater) which exceeded the WHO guideline value (0.01 ppm) and even the Bangladesh standard (0.05 ppm). Excepting in blood, pond and river water a high concentration of As was also detected in the hair, nail, or in urine of 70 sampled people out of 95 (Table 5). All of these exceeded not only the standard levels of As concentration but also highly exposed Argentinian and Mexican group (Table 4). Seriousness of the problem in Hazigonj of Chandpur district was first brought home in 1998 by BRAC in Bangladesh (British Geological survey and Mott. MacDonald. 1999).

Besides this the present study revealed that a very high concentration of As (0.30 ~ 1.71 ppm) in drinking water caused serious exposures at the sampling locality. A lot of people are going to be exposed by As potential and some of them already have been potentially exposed and suffering from melanosis, keratosis, hyperkeratosis etc. Off course a very few people have no complain even though they used to drink As polluted water since long time. It might be the reason of different in physical conditions that varied from person to person, or some other reasons might be the sensitivity of the person (MIG 1998). The present data (within the limited information) evidenced that ratio of exposed population in the sampling locality is bigger than that of previous estimation for total Bangladesh (more than 25 %, around 30 ~ 35 million, reported by BGS and DPHE. 2001). More than 73 % (included 17 patient and 53 exceeding As concentration in hair, nail or in urine samples out of total 95) of sampled population have already been found exposed (Table 5). Reasonably, it might be considered that Hazigonj in Chandpur district is one of the highly As polluted and seriously affected areas in the southeast part of Bangladesh.

The results of questionnaire also confirmed that most of the people of sampling locality are not still conscious enough for safe drinking water. They could not change their drinking habit and often takes the As polluted groundwater excepting a very few (Table 6). In these cases normally there might be of two ways of mitigation for the problem. The 1st one is the house hold filtering and the other one is assuring the supply of As free drinking water by the government of the country. Bangladesh are such a developing country, that could not assure to supply the As free drinking water for a big

size population of about 125 million. And also impossible to arrange the houses hold filtration equipment for its costs. Even though several kinds of house hold filters are currently available. However the variety of such kind of filters is still rather limited. So the necessity of suitable, sustainable, economic, method of cleaning water is quite enough for the sake of millions of lives in Bangladesh.

Specific discussion on the microbial accumulation of As for bioremediation

The main objective of the present study was to elucidate the biogeochemical activities of microbes in biomats, those are concerned with the arsenical bioremediation in an in situ reactor. Biomats, consisting of various microbes, produced near by the As polluted ground water flow are found capable to accumulate As with other elements. This accumulation of elements into the microbial cellular system takes place by the biogeochemical processes. The reason is why, microorganisms carry out many uinque reactions of geochemical significance in the environment (Trudinger et al. 1979). It is ambiguously identified that a group of coexisting microbes mainly photoautophytic (autotrophic) and heterotrophic bacteria (bacillus, coccus and filamentous types) and photosynthetic algae in biomats are metabolically active in an As polluted water system. They are found enable to transport As ions into their body surface with other ions forming biominerals and arsenosugars and make them immobilize.

Normally, the cellular systems actively transport ion through their membrane in order to maintain osmotic stability (Simkiss and Wilbur 1989). More over, the cell wall of microbes contains more or less proteins, polysaccharides, amines, or polyamines (Asada and Tazaki 2001). Microbial community in biomat can produce macromolecules outside of their cell wall, commonly consisting of polysaccharides together with some protein, DNA and RNA (Geesey et al. 1988; Geesey and Jang 1990). For an instance, when As enters the human-body once by drinking or through membrane also, it can not comes out easily as it might be forming a arsenic-protein complex (Minato 1989; Islam and Tazaki 2001; De 2002) and remains in the body (a very little comes out through urine, hair or nail). It might also be considered for the case of microorganisms, as having high molecular polymeric peptide bonded protein compound in their cell surface

(Jones 1997). More over the presence of polysaccharides, amines, or polyamines on the microbial surface can interact with metals and metalloids and has been emphasized for binding them (Beveridge and Murry 1980; Beveridge 1981, 1989; Nagai et al. 2001). Besides this, Granchinho et al. (2001) reported that marine olive green algae could accumulate As and form complex arsenic-compounds of ribofuranosides derivatives, commonly termed as arsenosugar, that was found in a high concentrations (several hundred mg of As per kg wet weight). Lunde (1973) described that the capacity of As accumulation in freshwater algae is comparable and similar to that of marine algae.

The reasons resemble with this study basing on the optical microscopic and SEM observations it revealed that biomats are consisting of autotrophic and heterotrophic bacteria (bacillus, coccus, and filamentous typed) associated with photosynthetic algae those are enable to immobilize As. In addition ED-XRF and NAA analyses confirmed that the As ion could be accumulated in biomats from groundwater and could form biomenerals and arsenosugars. Arsenosugar might be produced in algae which are predominated in biomats collected from the point W1 of spout 1 in location 4. From the Neutron activation analysis (NAA) data ambiguously proved that a several hundred times of As was present in biomats (Table 3).

Tazaki (1999) explained the microorganisms in biomats could cleanup and rehabilitate the heavy metallic pollution in the geo-aquatic environment. Furthermore, Nagai et al. (2001) suggested that bacteria in reddish brown biomats could produce lollingite ($FeAs_2$), an arsenic mineral on their cell and intercellular surface. Usually microorganisms found in different kinds of biomats (e.g. green, black, and reddish brown) could accumulate heavy metals and toxic elements (e.g. Fe, Mn, Cu, Zn, Cd, Pb, and As) in the geo-aquatic environments. These kinds of accumulation take place by adsorption, precipitation, complexation, transportation or by biomethylation (Gadd 1992; Islam and Tazaki 2000b; Lloyd and Macaskie 2000; Nagai and Tazaki 2001; Yoshida et al. 2001). As for example, Ledin (2000) and Labrenz et al. (2000) reported that the bacteria *Scenedesmus pannonicus* or the bacteria of *Disulfobacteriacea* family could survive in highly toxic environment and can reduce aqueous As with other elemental concentration to well bellow the acceptable level by the geochemical and microbial process. Furthermore, Kaise et al. (1997) described that the fresh water algae

(in culture medium) could also be accumulate As by the process of biomethylation. The inorganic form of As can be biomethylated by certain microbes to the gaseous arsines or to monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMAA) those are believed to be a part of a detoxification mechanism in living organisms was reported by Frankenberger and Arshad (2002). In addition, Gonzalez et al. (2000) suggested that the algae or bacteria are considerable not only for the metallic accumulation but also for transformation of the most toxic metallic species in to others having less environmental risk.

The similarities have been found in the present study that bacteria or algae in biomats are capable to accumulate As with other elements and immobilize them by forming biominerals and arsenosugars. The data are in agreement with some work of Tazaki et al. (2002); Frankenberger and Arshad (2002); Gadd (2002) and Granchinho et al. (2001), for the significant role of microorganism in transformation of As in to the minerals or to arsenosugars. Therefore, it might be considered that biomats are capable of concentrate a high volume of As and cleanup the polluted groundwater that was revealed in the ED-XRF spectra of this study. The spectra of water and biomats show the comparative As attenuations in groundwater, which is corresponding to the biomats produced nearest to the ground water flow point (Fig. 5).

In our study, it might be suggested that biomats consisting of autotrophic and heterotrophic bacteria mainly of bacillus, coccus and filamentous type associated with photosynthetic algae could be responsible for the accumulation of As and other ions in to their cellular system by some of the complex biogeochemical processes. Which resulting the formation of biomenirals and arsenosugars onto the microbial cell surface and make them (As) immobilize. However, bioaccumulation of As in biomat can clean up the As polluted groundwater or any water system in the geo-aquatic-environment as having their (microorganism) endurance ability in the toxic or any other adverse condition.

CONCLUSIONS

Basing on the field observation and laboratory analytical data, it can be concluded on the general situation of As pollution in drinking water (groundwater) and its effects;

and specifically for the bioremediation methods of As polluted groundwater in an in situ reactor at Hazigonj in the district of Chandpur, Bangladesh.

The general situation of As pollution in groundwater is extremely high that ranged between 0.30 ~ 1.71 ppm, which exceeded the WHO guideline value (0.01 ppm) and even the Bangladesh standard (0.05 ppm). As a result most of the people of sampling locality going to be arsenically exposed and some of them already have been suffering from serious health hazards in the form of melanosis, keratosis or hyperkeratosis etc.. It is not to be mentioned that the span of the problem is unanimously wide and important one. But currently, for the sake of millions of lives the necessity of suitable, sustainable, and economic methods of cleaning water is quite enough.

The specific in situ study of Hazigonj show that the grey biomats consisting of various microbes, mainly of autotrophic and heterotrophic bacteria (bacillus, coccus and filamentous typed) and photosynthetic algae produced near by the As polluted ground water flow. ED-XRF and NAA analysis of gray biomats show that arsenic (As) accumulation in microbes takes place at a large scale (390 ~ 550 ppm) in the nearest flow point than that of others in the down stream, without any metabolic hazards.

Consequently, it might be finally concluded that microbes in biomats play an important role for cleaning the As polluted geo-aquatic environment, as having their endurance ability in any toxic or polluted environment. Reasonably it might be considered that a group of selective microorganism mainly of bacteria and algae certainly play an important role in the natural bioremediation of As polluted geo-aqua-ecosystem. This might be one of the most suitable, sustainable, and economic methods for the cleaning of As polluted groundwater in Bangladesh, which could sake millions of lives from the sufferings of drinking water.

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