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Arsenic accumulation in microbial mats from underground water in Bangladesh

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Abstract : Underground water has been extensively used as drinking water since last 20－30 years for the reduction of infant mortality from diarrheal diseases in Bangladesh. Accordingly so many shallow and deep tube-wells were sank at that period. Recently it is discovered that, tube-well water is polluted with high concentration of arsenic and poisoning has become a serious public health problem in Bangladesh.

In the geo and aqua-ecosystem microbial mats of bacterial colony plays an important role in the remidiation of toxic heavy metals in polluted soil and water systems. Various kinds of microbial mats are forming near to those arsenic-contaminated underground water flows. Green and black biomats together with polluted water, soil and rice samples were collected from Bangladesh. The investigations were carried out to qualify the arsenic accumulation by microbial mats for bioremediation. In this study arsenic in the above samples were analyzed by ED-XRF, XRD, and optical microscopic view.

The results suggest that microorganisms in microbial mats accumulate arsenic with other elements from contaminated water.

Key words: Arsenic, microbial mats, microorganism, accumulation, underground water, contamination, bioremidiations.

1. Introduction

Arsenic is being found in underground water of many parts of world such as Bangladesh, India, Mongolia, Taiwan, Thailand, Argentina, Chile, including some parts of England and United States (Le et al., 2000 ; Feeney et al., 2000). Arsenic is listed as a hazardous material for its toxicity and causes lung and skin cancer. It also can cross the placental membrane and enter into the metabolic system of unborn children (Karim, 1999). There were also reports of arsenic in Taiwan and China that causes a condition called " blackfoot" where tissues die and gangrene sets in leading to the loss of toes and feet (Banfield and Nealson, 1999). Therefore, it may be considered that the geological source of arsenic is environmental problem worldwide (Rosen, 1999).

Recently the evidence of chronic poisoning has been reported from many districts of Bangladesh and has become a serious public health problem (AAN, 1999). Specially arsenic (As) contamination in water and soil has already affected the health of millions (Bangladesh has an area of 147570 km^2 with a population size of 124 Million) of people (Fuji and Karim, 1998). Arsenic contamination of drinking water and soil is by far the worst calamity in history from exposure to a chemical substance. Arsenic contamination of ground waters has been officially detected in Bangladesh in 1993 by the DPHE (Department of public health and engineering) for the first time in Chapai Nawabganj district. It is adjacent to an area of West Bengal (India) which had been found to be extensively contaminated in 1988. Extensive contamination in Bangladesh was confirmed in 1995 when additional surveys showed contamination of shallow and deep tube-wells across much of southern and eastern Bangladesh with some regions of Western Bangladesh also.

The contamination occurs in ground water from the alluvial sediments that make up much of the area. Ground water has been extensively used in Bangladesh only since last 20-30 years for the reduction of infant mortality from diarrheal diseases. According to an estimation 95 %or more of Bangladeshis now use ground water for drinking. Arsenic has probably been present in the ground water for thousands of years, but recently it was discovered (British Geological Survey, 1998).

In Japan, spring water of Masutomi in Yamanashi prefecture and Tsuwano in Shimane prefecture also containes arsenic. The concentrations are more than 5 and 1 ppm, respectively (Suzuki, 1971, 1972 ; Ishiga et al., 1999).

Arsenic is found available not only in the drinking or underground water but also in the soils and clayey sediments (Tazaki et al., 1998), as it is one of the most abundant elements on the earth and occurs naturally in many chemical forms (Jack et al., 1998). The element is rarely encountered as the free element, but is distributed among a great variety of mineral species (Zingaro, 1994). It is stable in the presence of water and aqueous solutions of all pH's free from oxidizing agents (Muylder and Pourbaix, 1957). It was also reported that heavy metals like lead, cadmium, zinc including arsenic is accumulated in the roots of plants from the soil and tarnslocated to the fruits (Cobb et al., 2000). Besides this, Tazaki (1999) explained that microorganisms in biomats can clean up and rehabilitate the heavy metallic pollution in the environmental system. In addition to a recent study (Nagai and Tazaki, 2000), it is found that brown biomats can accumulate As with Fe while no trace of arsenic in water detected.

As described above, ubiquitous arsenic is found at trace levels in the earth's atmosphere, water, soils, and organisms. In the earth's crust arsenic contents varies from 0.1 to several hundred ppm (Newman et al., 1998). The World Health Organization (WHO) standard for arsenic in drinking water is allowed up to 0.01 mg/l and less than 15 mg / kg of soil in the farming area. The permissible limit is highly exceeded both in the drinking water (usually ranging between 0.05 - 2.4 ppm) and in the soils (up to 220 mg/kg) of the worstly affected districts such as Sylhet, Comilla, Chandpur, Noakhali, Lakxmipur, Madaripur and others (Islam and Tazaki, 2000).

Many researches have been carried out on arsenical hazardness for its toxicity in home and abroad. In some cases spot questionnaire and indoor research have also been carried out. Basing on the total science, nobody has focused on the microbiological activities of bacteria that can accumulate arsenic together with other heavy metals in the natural environment.

In this study we reveal that biomats (bacterial colony) can play an important role in bioremidiation by accumulating arsenic in the geo-aqua-environmental ecosystem.

2. Materials and Methods

Underground water, soils, biomats (green and black) and rice samples were collected from Noadda (1) and Dherra (2) villages of Hazigonj, Chandpur, Bangladesh in 9 th May, 2000(Fig. 1). These samples were analyzed with ED-XRF for chemical composition, XRD for mineralogy and optical microscopy for the confirmation of the presence of bacteria in microbial mats.

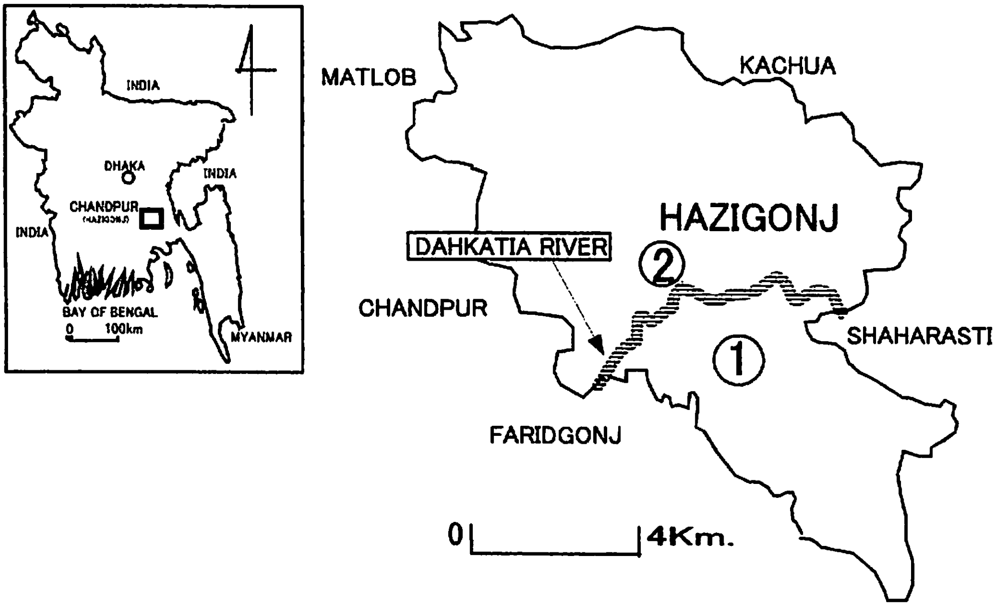


Fig.1. Locality map of Noadda (1) and Dherra (2) villages at Hazigonj in the district of Chandpur, Bangladesh.

2.1. Sample preparation for energy dispersive X-ray fluorescence spectrometer (ED-XRF)

500 μl of water samples were taken on the mailer film (normal drying within 60 hours at the rate of 50 μl/ 6 hours of interval), soil, biomats and paddy samples were also air-dried and ground to fine powder to use on the miller film for ED-XRF analyses.
Analyses 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.

2.2. Sample preparation for X-ray diffractometer (XRD)

One gram of each powdered sample was suspended in distilled water and mounted on the slide simultaneously and kept them for air drying. After being dried up they were studied by XRD using a Rigaku diffractometer having Cu Kα radiation operated at 40 kV and 30 mA.

2.3. Sample preparation for Microscopic study

Hand picked biomats were mounted and spread over on two different slides simultaneously using distilled water. Natural and DAPI (4, 6-diamidino- 2 -phenylindole) stained samples were observed by an optical microscope (Nikon OPTIPHOT- 2).

3. Results

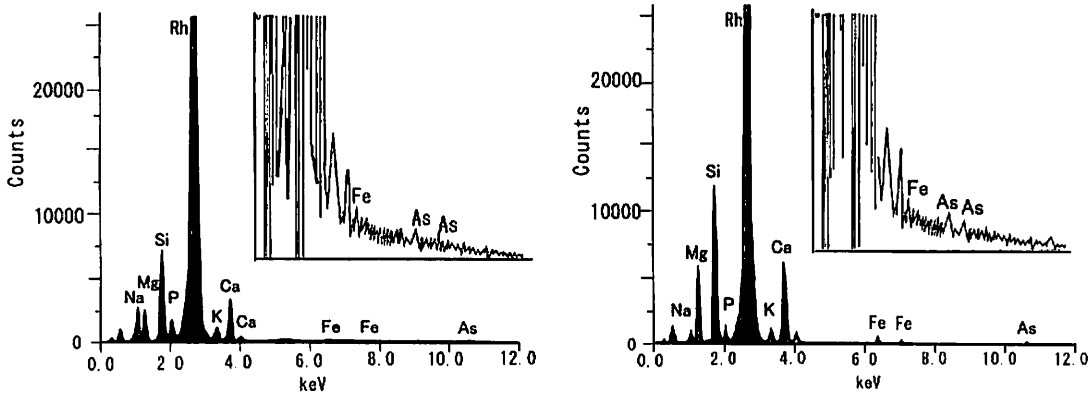
3.1. ED-XRF

The analyses showed the presence of Al, Si, K and Fe with the traces of Mg, Ca, and Ti in soils of both sampling points at Noadda (1) and Dherra (2) Villages. P, S, and K contents were rich in rice with the traces of Mg and Si (sometimes Ca and Fe). Trace of arsenic was found neither in the soil nor in rice samples at both of the sampling points. Besides this, water from Noadda (1) and Dherra (2) village richly contains Na, Mg, Si, Ca with the traces of P, K, Fe and As (arsenic was found in the spectrum). The analyses showed the presence of Al, Si, K, Ca and Fe, all rich in both green and black biomats with the traces of Mg, P, S, Ti, Mn, and As. The peaks for arsenic are found in the spectra of the underground water and both kinds of (green and black) biomats of Noadda (1) and Dherra (2) villages of Hazigonj, Chandpur in Bangladesh (Fig. 2), but their contents remain nearly 0.002 and 0.004 wt% in water, and 0.006 and 0.026 wt% in biomats of Noadda (1) and Dherra (2) villages respectively (Table 1).

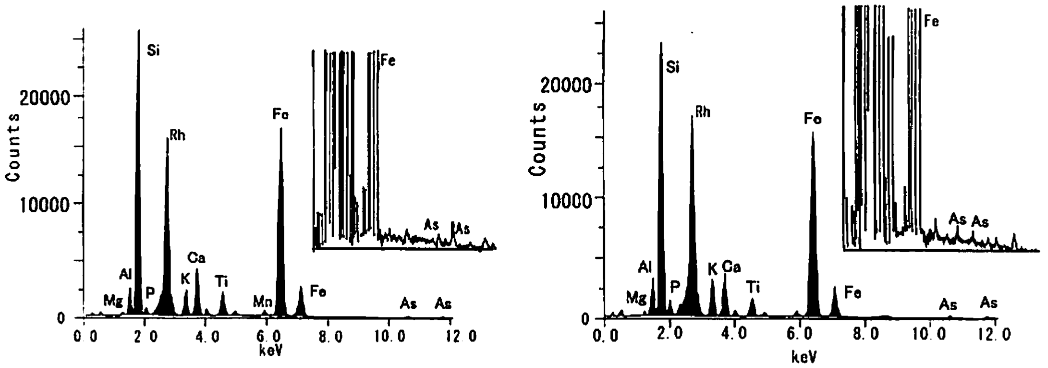
Table 1 ED-XRF analyses of underground water, soil, rice together with green and black biomats formed in arsenic-contaminated areas of Noadda (1) and Dherra (2) villages at Hazigonj in Chandpur, Bangladesh(Fig.1).

Sampling point 1 (Noadda)								
	Water		Soil		Rice		Green Biomats	
	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)
Na_2O	35.95	32.03	0.03	0.04	nd	-	0.54	0.60
MgO	27.25	37.34	1.38	2.40	3.14	6.97	1.96	3.35
Al_2O_3	0.05	0.03	16.16	11.08	1.03	0.90	10.07	6.79
SiO_2	25.41	23.35	64.30	74.80	9.04	13.47	63.27	72.37
P_2O_5	4.75	1.85	0.76	0.38	30.66	19.34	3.15	1.53
SO_3	1.66	1.14	0.33	0.29	19.51	21.19	1.30	1.11
K_2O	1.45	0.85	4.36	3.23	30.95	29.40	4.45	3.24
CaO	3.45	3.39	1.82	2.27	4.49	7.33	4.55	5.58
TiO_2	nd	-	1.50	1.31	-	-	1.72	1.48
MnO	nd	-	0.22	0.21	0.24	0.31	0.16	0.16
Fe_2O_3	0.03	0.01	9.13	4.00	0.84	0.47	8.82	3.80
As	0.00	0.00	nd	-	nd	-	0.01	0.01
Total	100.00	100.00	99.99	100.00	99.89	99.37	99.99	100.00

Sampling point 2 (Dherra)								
	Water		Soil		Rice		Black Biomats	
	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)	Wt.(%)	at/mole(%)
Na_2O	5.30	5.04	nd	-	nd	-	0.42	0.46
MgO	23.61	34.54	1.58	2.65	5.20	11.38	1.99	3.35
Al_2O_3	nd	-	12.36	8.18	0.93	0.81	8.36	5.57
SiO_2	33.36	32.74	68.81	77.29	10.11	14.83	64.33	72.79
P_2O_5	12.04	5.00	1.95	0.93	34.88	21.68	1.81	0.87
SO_3	8.68	6.39	0.53	0.45	17.04	18.77	0.77	0.66
K_2O	1.48	0.93	4.62	3.31	24.79	23.22	3.40	2.45
CaO	14.04	14.76	2.97	3.57	5.01	7.90	5.83	7.07
TiO_2	nd	-	1.19	1.00	0.14	0.16	2.50	2.12
MnO	0.09	0.08	0.18	0.17	0.27	0.34	0.23	0.22
Fe_2O_3	1.40	0.56	5.81	2.46	1.62	0.90	10.35	4.40
As	0.00	0.00	nd	-	nd	-	0.03	0.02
Total	100.00	100.04	100.00	100.00	99.99	99.98	100.00	99.99


(A) Water of sampling point (1)

(C) Water of sampling point (2)


(B) Biomat from Sampling point (1)

(D) Biomat from Sampling point (2)

Fig.2. A comparative spectrum of ED-XRF analyses for (A) underground water of sampling point (1) showing contents of Na, Mg, Si and Ca with traces of K, Fe and As. (B) Green biomats of sampling point (1) showing contents of Al, Si, K, Ca, Fe with traces of P, K, Ti, Mn and As. (C) underground water of sampling point (2) showing the presence of Na, Mg, Si, Ca with traces of P, K, Fe and As. (D) Black biomats of sampling point (2) showing contents of Al, Si, K, Ca, Ti, Fe with traces of P and As elements.

(Rh Kα ray used for ED-XRF analyses)

3.2. XRD

The XRD patterns of green biomats from Noadda (1) and black biomats from Dherra (2) villages reproduced in Figure 3 showed the strong quartz peaks at 3.4Å, 4.3Å and 1.8Å, and small peak of illite (10.1Å) in green biomats. The black biomats also showed the same peaks.

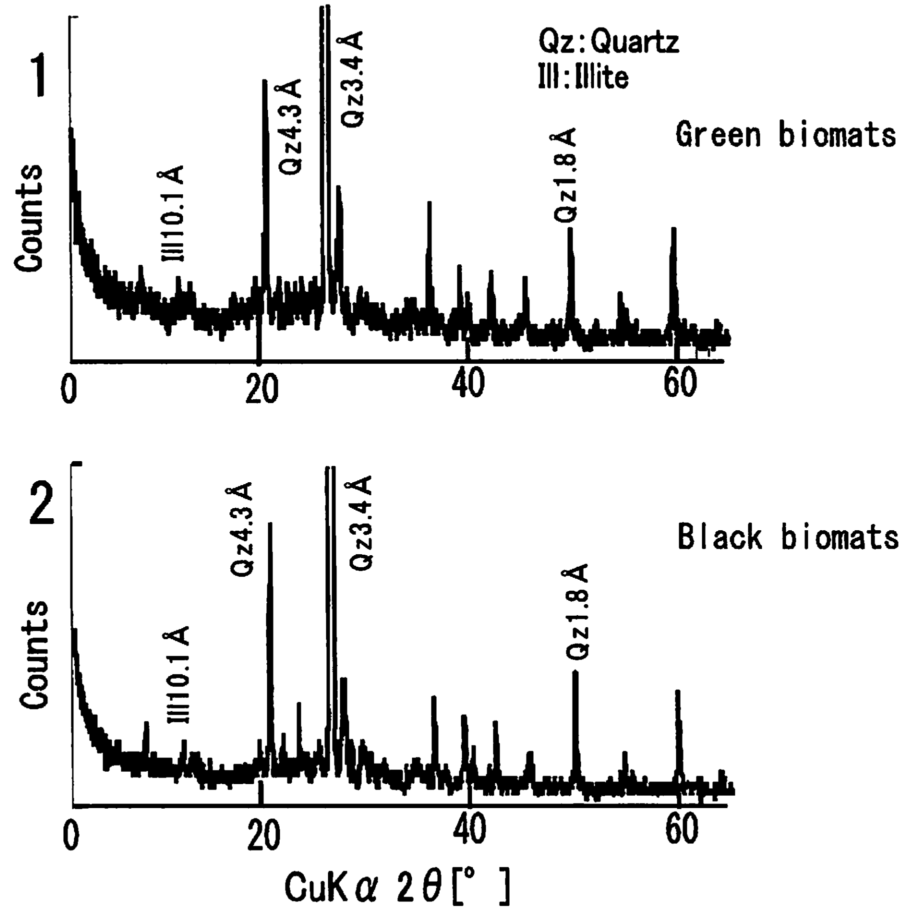


Fig.3. X-ray powder-diffraction patterns of green biomats from Noadda village (1) and black biomats from Dherra village (2) formed near to the underground water flow of Hazigonj, Bangladesh.

3.3. Optical microscopy

Optical micrograph of green biomats from Noadda （1）, showed filamentous photosynthetic bacterial community both in natural and DAPI stained view （Fig. 4 B and C）. On the other hand the dominance of the coccus type of bacterial community was observed in the black biomats of Dherra （2） village （Fig. 5 B and C）.

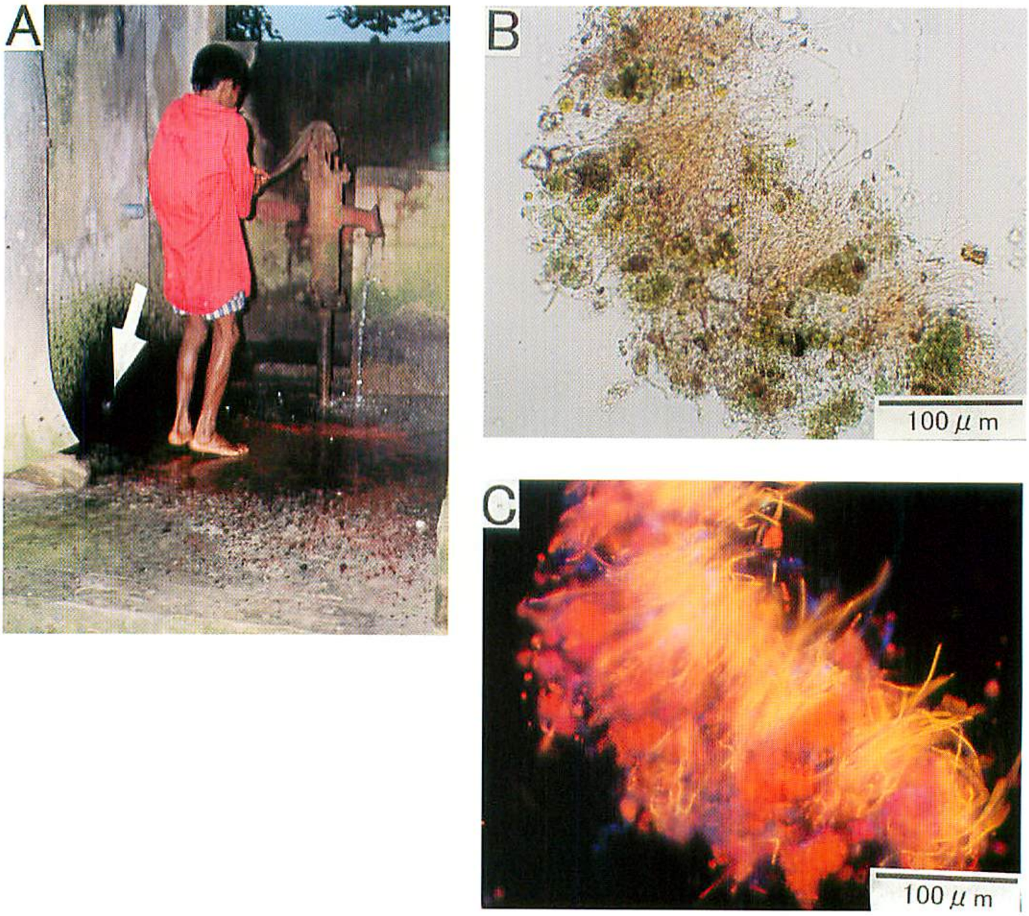


Fig.4. Field view of biomats (→) in front of an arsenic-contaminated tube-well water flow at Noadda village (Fig.1-1) of Hazigonj, Bangladesh (A). Optical micrograph of green biomats showing the colony of photosynthetic bacteria (B), and florescence micrograph showing DAPI-stained photosynthetic bacteria (C).

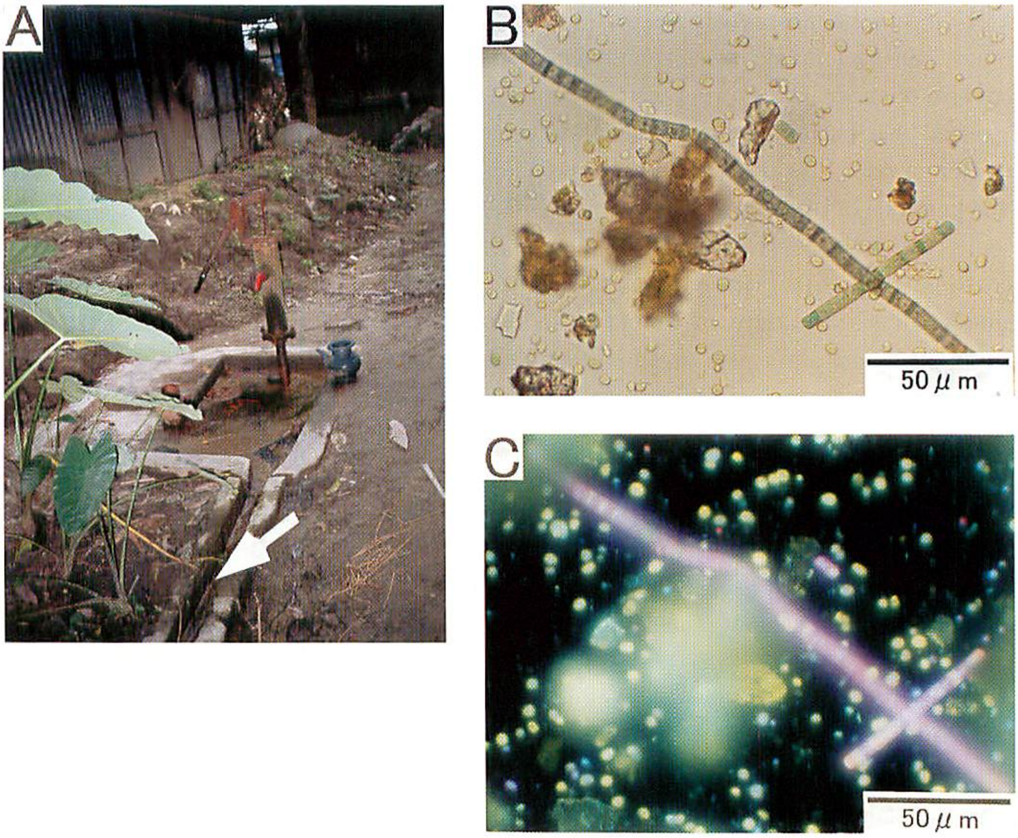


Fig.5. Field view of biomats (→) in front of an arsenic-contaminated tube-well water flow at Dherra village (Fig.1-2) of Hazigonj, Bangladesh (A). Optical micrograph of black biomats showing the colony of coccoidal-bacteria (B), and florescence micrograph showing DAPI-stained coccoidal-bacteria (C).

4. Discussion

The results revealed that soil and rice grown near to the contaminated water were not polluted by arsenic (according to ED-XRF). However arsenical accumulation is being found in the microbial mats, indicating that they have the ability to accumulate heavy metals and metalloids including arsenic from the contaminated water flow. According to Gonzalez et al. (2000), the interaction between arsenic and microorganisms (algae, bacteria) is considerable not only for metallic accumulation, but also for the transformation of the most toxic metallic species into others having less environmental risk. Environmental microbe-metal interactions are the need to remediate extensive metal contamination of water and soils, although microorganisms are not alchemists and can not change a toxic metal to a less toxic element. However, microbially catalyzed precipitation or volatilization of metals can clean up the environmental geo-aquatic ecosystem (Lovley, 2000). From the most recent research (Nagai and Tazaki, 2000), it is found that brown biomats can accumulate arsenic with Fe. Furthermore Mn contained arsenical minerals were also obtained from spring water (Nagai and Tazaki, 2000). Tazaki et al. (1997) suggested that bacterial biomineralization might be helpful for cleaning soil and water pollution. Ledin (2000) reported that metal ions are also accumulated by microorganisms in the presence of other ions and showed that *Scenedesmus pannonicus* can accumulate the arsenic, which is consistent to Demon et al. (1989). Furthermore, bacteria are being increasingly important in bioremidiation because they are capable of filtering and degrading a large variety of polutants in the soil and underground water. Therefore, microbialmats certainly play an important role in the accumulation of heavy metals and toxic substances and can remidiate a polluted geo-aqua system.

5. Conclusion

ED-XRF, XRD and optical microscopic analyses suggest that microorganisms in biomats might have the ability to accumulate arsenic with other elements. Green and black biomats are often found near to the underground tube-well water flow. Biomats might play an important role of cleaning the environmental pollutant.

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