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Microalgal diversity and arsenic concentration in irrigation water, soil and rice plant samples collected from West Bengal, India

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Abstract
Arsenic is one of the toxic and carcinogenic metal presents in the soil, water and some biological samples from the Eastern parts of India. This causes many health problems with human and animals including cancer, arsenicosis. Arsenic water pollution is one of the human problems in the recent century. Rice (*Oryza sativa* L.) is one of the major food crops in many countries. The objective of the present study was to determine the concentration of arsenic in irrigation water, paddy field soil and in the rice plants cultivated with the arsenic contaminated irrigation water. Irrigation water samples, paddy field soil and microalgal samples, and rice and turmeric plants were collected from 10 different places of Medinipur, Howrah, Bardhaman and Hooghly districts of West Bengal, India.
The samples were analyzed by atomic absorption spectroscopy (AAS) to determine arsenic concentrations. Physiochemical parameters of the water samples were also analyzed and microalgal samples were microscopically identified and recorded. The concentration of arsenic in the water samples of all the studied samples was found to be below detectable level (<0.005) to 0.006 mg/kg dry weight of arsenic. The concentration of arsenic in the soil samples of all the studied samples was found to be between 5.87 and 13.78 mg/kg dry weight of arsenic. The concentration of arsenic in the root of all the studied rice samples was ranged from below detectable level (<0.25) to 15.06 mg/kg dry weight of arsenic. The concentration of arsenic in the shoot of all the studied rice samples was found to be below detectable level (<0.25) to 4.69 mg/kg dry weight of arsenic. The concentration of arsenic in the grain of all the studied rice samples was found to be below detectable level <0.25 mg/kg dry weight of arsenic, which did not exceed the permissible limit in rice (1.0 mg/kg) according to WHO recommendation.

Keywords: Microalgal diversity, arsenic concentration, irrigation water

1. Introduction

Natural arsenic contamination of groundwater resources in parts of Asia is posing a serious threat to the health of millions of people. The identification of the ground water contamination by arsenic (*As*) in the deltaic region, particularly in the Gangetic alluvium of Bengal including Bangladesh and West Bengal of India has been termed the world's biggest natural calamity in known human history. The issue of contaminated drinking-water has been taken up by governments and development partners and many efforts are under way to mitigate the problem. However, the same water resources are used extensively for irrigation purposes throughout the region too. Since an initial investigation on *As* accumulation in rice undertaken by Food and Agriculture Organization of the United Nations (FAO) with support from the United Nations Development Programme (UNDP) by Duxbury and Panaullah (2007) [7], further scientific studies in the last couple of years have reported potential risks from *As* in irrigation water because of land degradation affecting agro-ecosystem services. The most well-known concern is *As* entering the food chain, affecting food safety. This poses a potential dietary risk to human health in addition to the risk from drinking contaminated groundwater. Continuous buildup of *As* in the soil from *As* contaminated irrigation water may reduce crop yields, thus affecting the nutritional status and incomes of rural farming communities. Plants may accumulate arsenic via root uptake from soil solution and certain species may accumulate substantial concentrations. Uptake, accumulation and toxicity vary within and between plant species. In general, more arsenic in the soil leads to higher concentrations in plants. Crops receiving irrigation through arsenic contaminated water accumulate the toxic element in them.

Rice (*Oryza sativa* L.) is one of the major food crops in many countries. Arsenic accumulation in rice is viewed as a newly recognized disaster for South-East Asia, where rice is a staple food (Meharg, 2004) [1]. As the cultivation of rice requires huge volume of water, long term use of arsenic contaminated groundwater for irrigation may result in the increase of arsenic concentration in the agricultural soil and eventually accumulation in rice plants. The paddy soil gets contaminated by the irrigation water and thus enhances more probable condition for bioaccumulation of arsenic in rice plants. The maximum acceptable limit for agricultural soil is 20.0 mg/kg recommended by the European Community. The permissible limit of arsenic in the rice grain part according to WHO recommendation is 1.0 mg/kg dry weight. Regardless of the sampling locations the arsenic accumulation follows the order of root > straw > husk > grain. Consumption of rice straw containing considerable amount of arsenic by cattle could potentially lead to increased arsenic levels in meat or milk. The arsenic content in rice plant is correlated to the degree of arsenic contamination of irrigation water and soil (Bhattacharya *et al.,* 2009) [2]. Imamul Huq *et al.,* (2006) [5]. And Chakravarty *et al.,* (2003) [8] reported that vegetables grown in the garden and receiving irrigation with arsenic contaminated water had significantly higher arsenic than those grown in the unaffected areas.

The objective of the present study was to determine the concentration of arsenic in irrigation water, paddy field soil and in the root, shoot and grains of rice plants and turmeric plant cultivated with arsenic contaminated irrigation water. This study would help to evaluate the transfer of arsenic from irrigation water and paddy soil to the rice plant.

2. Materials and Methods

2.1 Study Area: The administrative structure of West Bengal consists of 18 Districts. Water, soil, rice plants, turmeric plants and microalgal samples were collected from 10 different places of Medinipur, Howrah, Bardhaman and Hoogly districts of West Bengal, India.

2.2 Collection of Samples: All the samples were collected during the month of November, 2014. Water, soil and microalgal samples were collected from the rice and turmeric cultivation areas. Water samples were collected in sterile two (2) liter plastic canes and brought it into the lab within two days for further analysis. Rice and turmeric plants were collected with soil samples in sterile poly bags and brought it into the lab and kept for shade drying for arsenic analysis for three (3) days. The microalgal samples were also collected from nearby places of the root of the rice and turmeric plants in sterile eppendorf tubes.

2.3 Instrumentation: The microalgal samples were microscopically observed by using Olympus CH20i microscope and photographed by Sony digital still camera model DSC-W320 and identified by comparison with monographs. The flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) method was used for arsenic analysis. A Perkin Elmer system of Flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS), Model AAnalyst 700 and FIAS 400, was used for total *As* in water, under the following conditions: sample loop 0.5 mL; reducing agent, 0.5% NaBH4 in 0.125% NaOH at 5 mL/min; 10% HCl, at 10 mL/min; and argon at 100 mL/min as carrier gas for the FI system. An *As* electrode less discharge lamp and electric oven temperature for the quartz cell at 900 "C was used in AAS. Physio-Chemical parameters of the water samples were analyzed by YSI multi-parameter water analyzing kit.

3. Results

Study Area and Collection of Samples

West Bengal is located in strategic position of Eastern India, lies between 21° 31´ and 27° 14´ N latitude and between 85° 91´ and 89° 53´ E longitude. Water, soil, rice plants, turmeric plants and microalgal samples were collected from 10 different places of Medinipur, Howrah, Bardhaman and Hoogly districts of West Bengal, India.

Fig 1: Sampling sites of rice, microalgae, soil and water samples collected from West Bengal, India. 1N-Hajigarh, 2N-Mirzapur Bankipur, 3N-Belmuri, 4N- Madhusudanpur, 5N- Balichak, 6N-Bhogpur, 7N- Mecheda, 8N- Ghoraghata, 9N- Kulghachia, 10N- Jhapandanga.

Microalgal Diversity

The microalgal species were microscopically observed, photographed and identified are listed in Figure 2 and 3. *Scenedesmus sp. and Chlorella vulgaris* were found to be dominant microalgal species in the collection sites.

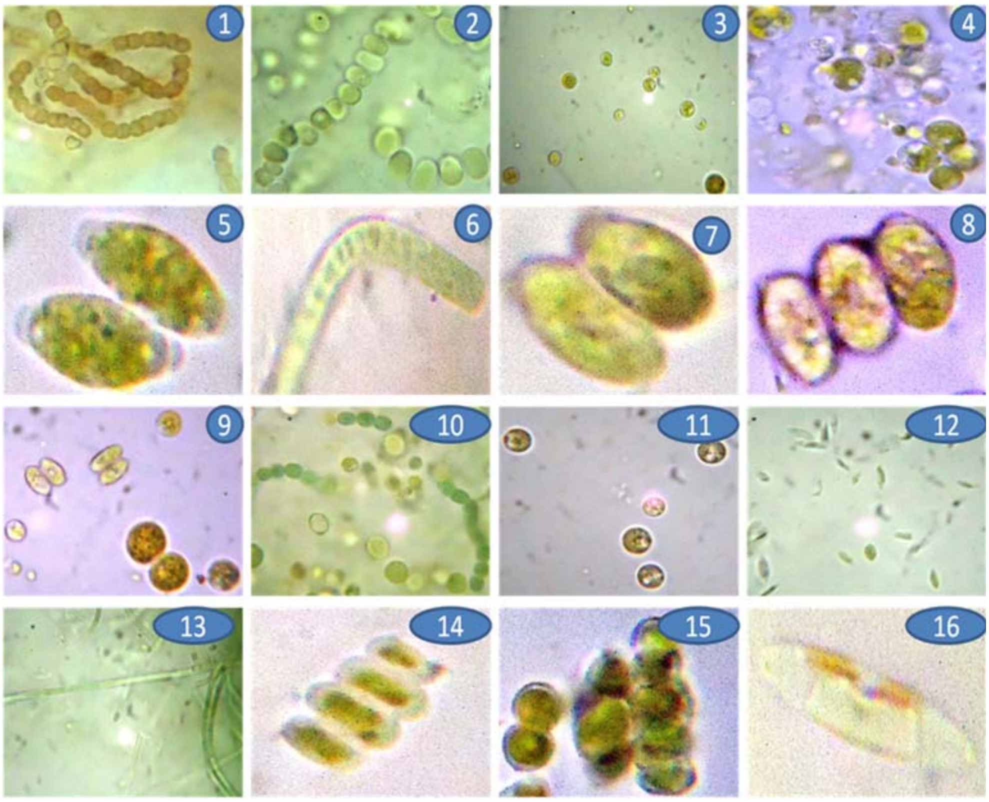

Fig 2: Microalgal Species which are identified from arsenic polluted areas. Note: 1N to 10N is representing the sampling sites. 1. *Anabaena circinalis* Var. crassa Ghose (1N), 2. *Anabaena fertillissima* Rao (1N), C. B, 3. *Chlorella vulgaris* Beyerinck (1N), 4. *Crucigeniella rectangularis* (A. Braun) (1N), 5. *Euglena* sp. (1N), 6. *Oscillatoria* sp. (1N), 7. *Scenedesmus bijugatus* (Turpin) Kutz (1N), 8. *Scenedesmus* sp. (1N), 9. *Scenedesmus* sp. (1N), 10. *Anabaena oryzae* Fritch (2N), 11. *Chlorella vulgaris* Beyerinck (2N), 12. *Scenedesmus acutus* var. obliquus Rabenh (3N), 13. *Phormidium* sp. (4N), 14. *Scenedesmus acunae* Com (5N), 15. *Scenedesmus* sp. (5N), 16. *Achnanthidium saprophilum* (Kobayasi & Mayama) (6N).

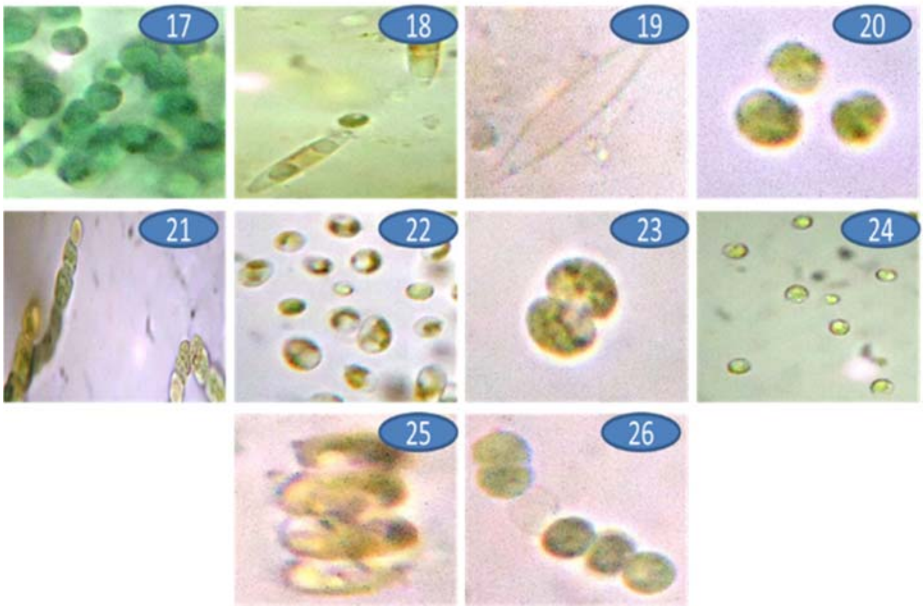

Fig 3: Microalgal Species which are identified from arsenic polluted areas. Note: 1N to 10N is representing the sampling sites. 17. *Anabaena* sp. (6N), 18. *Closterium libiulla* Focke (6N), 19. *Craticula cuspidta* (Kutz.) D. G. Mann (6N), 20. *Chlorella vulgaris* Beyerinck (7N), 21. *Calothrix brevissima* G. S. West (8N), 22. *Chlorella vulgaris* Beyerinck (8N), 23. *Cosmarium exiguum* W. Archer (8N), 24. *Chlorella vulgaris* Beyerinck (9N), 25. *Scenedesmus bijugatus* var. blcellularis (Chodat) Philipose (9N), 26. *Nostoc linkia* var. arvensis Rao, C.B (10N).

Shade Drying of the Samples

The soil samples, rice plants and turmeric plants were dried Under shade condition for arsenic analysis. The plant samples were cut into small pieces before the analysis of arsenic.

Fig 4: Samples which were kept for shade drying at room temperature for analysis of arsenic concentration. 1N to 10N= Rice Plants (1N-Hajigarh, 2N- Mirzapur Bankipur, 3N-Belmuri, 4N- Madhusudanpur, 5N- Balichak, 6N-Bhogpur, 7N- Mecheda, 8N- Ghoraghata, 9N-Kulghachia, 10N- Jhapandanga), 11N-Rhizome, 12N-Turmeric Leaves, 13N-Soil, 14N-Root of Rice Plants, 15N-Shoot of Rice Plants, 16N-Grain of Rice Plants.

Arsenic Analysis

Table 1: Arsenic concentration found in water, soil, root, shoot and grains of rice and turmeric samples collected from various places of West Bengal, India. (Note: BDL-Below Detectable Level, DL-Detectable Level).

Place Of Collection	Arsenic Concentration (mg/l or mg/kg)				
	Water	Soil	Root	Shoot	Grains
Hajigarh	BDL (DL: 0.005)	**5.87**	5.12	BDL (DL: 0.25)	BDL (DL: 0.25)
Mirzapur Bankipur	BDL (DL: 0.005)	8.51	4.39	BDL (DL: 0.25)	BDL (DL: 0.25)
Belmuri	BDL (DL: 0.005)	7.33	9.74	4.69	BDL (DL: 0.25)
Madhusudanpur	BDL (DL: 0.005)	11.52	15.06	0.71	BDL (DL: 0.25)
Balichak	BDL (DL: 0.005)	8.88	9.58	BDL (DL: 0.25)	BDL (DL: 0.25)
Bhogpur	0.006	13.78	10.55	0.29	BDL (DL: 0.25)
Mecheda	BDL (DL: 0.005)	8.69	1.48	0.30	-
Ghoraghata	BDL (DL: 0.005)	12.57	13.14	BDL (DL: 0.25)	BDL (DL: 0.25)
Kulgachia	BDL (DL: 0.005)	12.49	10.28	0.39	BDL (DL: 0.25)
Jhapandanga	0.005	10.94	BDL (DL: 0.25)	0.69	BDL (DL: 0.25)

The concentration of arsenic in the water samples of all the studied samples was found to be below detectable level (<0.005) to 0.006 mg/kg dry weight of arsenic. The maximum concentration (0.006) of arsenic in the water samples was found in Bhogpur. The concentration of arsenic in the soil samples of all the studied samples was found to be between 5.87 and 13.78 mg/kg dry weight of arsenic. The maximum concentration (13.78) of arsenic in the soil samples was found in Bhogpur and minimum concentration (5.87) of arsenic in the soil samples was found in Hajigarh. The concentration of arsenic in the root of all the studied rice samples was ranged from below detectable level (<0.25) to 15.06 mg/kg dry weight of arsenic. The maximum concentration (15.06) of arsenic in the soil samples was found in Madhusudanpur and minimum concentration (BDL) of arsenic in the soil samples was found in Jhapandanga. The concentration of arsenic in the shoot of all the studied rice samples was found to be below detectable level (<0.25) to 4.69 mg/kg dry weight of arsenic. The maximum concentration (4.69) of arsenic in the shoot samples was found in Belmuri and minimum concentration (BDL) of arsenic in the soil samples was found in Hajigarh, Mirzapur Bankipur, Balichak and Ghoraghata. The concentration of arsenic in the grain of all the studied rice samples was found to be below detectable level (<0.25 mg/kg) dry weight of arsenic.

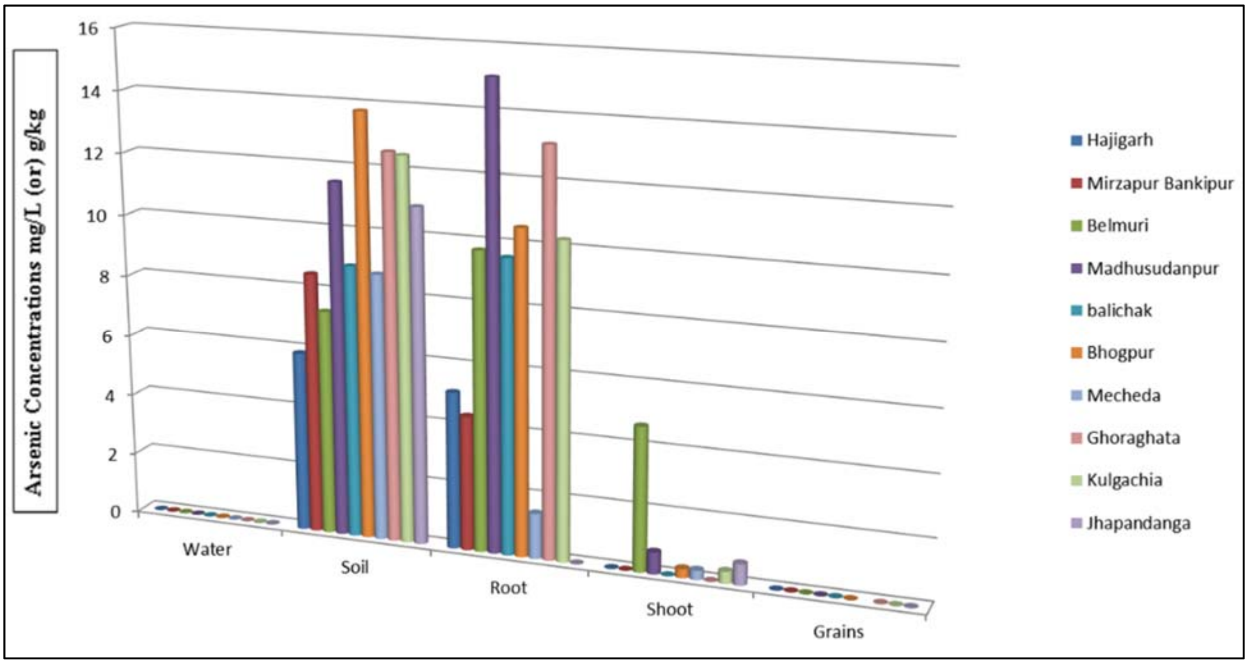

Fig 5: Arsenic concentration found in water, soil, root, shoot and grains of rice and turmeric samples collected from various places of West Bengal.

Physio-Chemical analysis of water samples

Physio-Chemical parameters of the water samples were analyzed and the results were presented in Table 2. The maximum temperature 37° C was found at Mecheda and Ghoraghata, the minimum temperature 36.75° C was found at Balichak. The maximum conductivity (1.711 milli-Siemens/cm^c) was found at Ghoraghata and minimum (0.185 mS/cm^c) was found at Balichak. The maximum specific conductivity (1.775 mS/cm) was found at Ghoraghata and minimum (0.192 mS/cm) was found at Balichak. The maximum resistivity (5212.7 Ω. cm) was found at Balichak and minimum (562.067 Ω. cm) was found at Ghoraghata. The maximum TDS (1.115 g/l) was found at Ghoraghata and minimum (0.121 g/l) was found at Balichak. The maximum salinity (0.86 ppt) was found at Ghoraghata and minimum (0.09 ppt) was found at Balichak. The maximum dissolved oxygen percentage (122.11 DO %) was found at Madhusudanpur and minimum (8.3 DO %) was found at Hajigarh. The maximum dissolved oxygen in milli grams (12.74 mg/l) was found at Madhusudanpur and minimum (1.34 mg/l) was found at Ghoraghata. The maximum dissolved oxygen in charge (50 DO ch) was found at Madhusudanpur and minimum (20.6 DO ch) was found at Balichak. The maximum pH (6.64 pH) was found at Madhusudanpur and minimum (6.42 pH) was found at Ghoraghata. The maximum pH associated with milli Volts (64 pH mV) was found at Ghoraghata and minimum (50.7 pH mV) was found at Madhusudanpur. The maximum oxidation reduction potential in milli Volts (-66.9 ORP mV) was found at Hajigarh and minimum (-135 ORP mV) was found at Madhusudanpur. The maximum Alkalinity (750 mg/l) was found at Kulgachia and minimum (175 mg/l) was found at Balichak. The maximum Chloride (362.38 mg/l) was found at Ghoraghata and minimum (0 mg/l) was found at Madhusudanpur and Balichak. The maximum Potassium (1259.0 mg/l) was found at Hajigarh and minimum (1.5 mg/l) was found at Madhusudanpur. The maximum Calcium (400 mg/l) was found at Mecheda and the minimum (10 mg/l) was found at Balichak. The maximum Sodium (1465.0 mg/l) was found at Belmuri and minimum (3.2 mg/l) was found at Ghoraghata.

Table 2: Comparison of water parameters analyzed from the water samples collected from rice fields of various places (lowest and highest values are highlighted with colors red and green respectively).

place	Temp.	Cond. (mS/ cm^c)	Cond. (mS/ cm)	Resis. (Ω. cm)	TDS (g/l)	Salinity (ppt)	DO (%)	DO (mg/L)	DO (ch)	pH	pH (mV)	ORP (mV)	Alkalinity (mg/l)	Chloride (mg/l)	Potassium (mg/l)	Calcium (mg/l)	Sodium (mg/l)
Hajigarh	36.9	1.042	1.081	925.46	0.679	0.51	8.3	1.51	26.7	6.51	58.2	-66.9	375	99.97	1259.0	200	17
Mizapur Bankipur	36.85	0.411	0.426	2348.61	0.267	0.2	86.1	7.68	35.9	6.59	53.4	-83	500	12.47	302	125	958.2
Belmuri	36.92	0.453	0.47	2123.45	0.296	0.22	48.4	4.05	33.9	6.57	54.9	-116.3	500	4.1	58.8	75	1465.0
Madhusudanpur	36.82	0.231	0.237	4243.44	0.148	0.11	122.11	12.74	50	6.64	50.7	-135	250	0	1.5	25	986
Balichak	36.75	0.185	0.192	5212.7	0.121	0.09	27.7	1.44	20.6	6.53	57.1	-108.8	175	0	7.5	10	15.7
Bhogpur	36.88	0.436	0.45	2220.25	0.283	0.21	67.2	3.37	43.1	6.53	57.1	-108	250	62.48	63.9	40	80.8
Mecheda	37	1.666	1.73	578.873	1.083	0.84	39.3	3.04	48.2	6.43	63.1	-100.6	625	224	498.1	400	33.8
Ghoraghata	37	1.711	1.775	562.057	1.115	0.86	16.9	1.34	28.8	6.42	64	-130.2	625	362.38	62.1	200	3.2
Kulgachia	36.74	1.365	1.411	708.448	0.888	0.68	87.4	6.03	48.2	6.43	63.4	-134.9	750	187.44	7.8	275	11.2
Jhapandanga	36.76	0.514	0.531	1884.61	0.333	0.25	109.1	8.73	49.2	6.52	57.9	-103.8	375	54.98	233.1	105	216.2

4. Discussion
In the parlance of the World Health Organization, arsenic has been responsible for the largest mass poisoning of a population in history (Smith *et al.,* 2000) [9]. West Bengal is the richest reservoir of rice bio-diversity and the rice bowl of the country. Pollution by arsenic occurs naturally through the dissolution of minerals and ores, and concentrations in groundwater in some areas are elevated as a result of erosion from local rocks (McArthur *et al.,* 2001) [10]. West Bengal has extremely rich natural water resources. Most of the people in West Bengal are drinking arsenic contaminated water above the permissible limit (0.01 mg/L). Millions of people in Western Bengal and Bangladesh have been drinking groundwater from wells that contain 100-2,000 µg/L As, and many of these people have succumbed to diseases that are caused by the arsenic contaminated ground water (Mandal *et al*. 1996) [14]. Continuous drinking from arsenic-laced wells can cause various health disorders including birth complications and cancer (Waalkes *et al,* 2007; Li *et al.,* 2008; Tokar *et al.,* 2011) [13, 12, 11]. To understand the severity of the arsenic crisis in West Bengal, India, a detailed, 3-year study was undertaken by Mohammad Mahmudur Rahman *et al.,* 2005 [6]. in Murshidabad, one of the nine arsenic-affected districts in West Bengal. Of the 26 blocks in Murshidabad, 24 were found to have arsenic above 50 mg/L.

In this present study the concentrations of arsenic was studied in the irrigation water, paddy field soil and in the root, shoot and grains of rice plants and turmeric plant cultivated with arsenic contaminated irrigation water. The results revealed that arsenic supplied with irrigation water and the agricultural field soils are thankfully accumulates much less in rice highly responsible for the transfer and uptake of grains (Table 1). The concentration of arsenic in the grain of all the studied rice samples was found to be between 0.25±0.014 to 0.73±0.009 mg/kg dry weight of arsenic, which did not exceed the permissible limit in rice (1.0 mg/kg) according to WHO recommendation. The microalgal species were microscopically observed, photographed and identified. *Scenedesmus sp. and Chlorella vulgaris* were found to be dominant microalgal species in the collection sites. Physio-Chemical parameters of the water samples such as Temperature, Conductivity, Specific Conductivity, Resistivity, Total Dissolved Solids, Salinity, Dissolved Oxygen (%),Dissolved Oxygen (mg/L), Dissolved Oxygen (ch), pH, pH (mV), Oxidation Reduction Potential, Alkalinity, Chloride, Potassium, Calcium and Sodium are analyzed and results were compared (Table 2).

5. Conclusion
This study would help to evaluate the transfer of arsenic from irrigation water and paddy soil to the rice plant.

6. Acknowledgement
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