Exploration of biochemical properties of soil and groundwater in arsenic affected blocks of Murshidabad district and isolation of potential arsenic resistant bacteria

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ABSTRACT: Arsenic is one of the major contaminants of soil and groundwater, responsible for a number of health hazards. Various blocks of Murshidabad district show arsenic concentration above the maximum permissible limit in soil and water samples. In the present study, we have focused on some highly arsenic contaminated regions of Murshidabad district. We have analyzed various biochemical parameters of soil and water samples of this district. The soils of these regions show high alkalinity and the groundwater samples also exhibits a basic pH. The total dissolved solids of these water samples varied from 200 mg L^{-1} to 300 mg L^{-1} . Also, the bacterial load in the water samples was extremely high. Since, groundwater is a source of drinking water in the blocks of Murshidabad district, consumption of such high amount of total dissolved solids would take a toll on the detoxifying and excretory system of the body i.e., on the hepatic and renal systems. The uncharacterized microorganisms in the soil could be fecal coliforms or other pathogenic bacteria, and their consumption would have serious health consequences. After serial dilutions (10^6 to 10^9 folds) of the groundwater and the soil samples, we could still isolate some bacteria thriving in these samples. Since, the soil and groundwater of these regions are highly arsenic contaminated, bacterial colonies isolated after serial dilutions could be potentially arsenic resistant bacteria.

1 INTRODUCTION

Arsenic toxicity is one of the serious problems both from national and global perspective. Arsenic is a toxic metal, which contaminates soil and groundwater (Hendryx, 2009; Huq *et al.*, 2006; Pais & Benton, 1997). In India arsenic contamination of soil and groundwater is a severe problem in lower Gangetic plain and Ganga-Brahmaputra deltaic region (Rahman *et al.*, 2005). U.S. Environmental Protection Agency prescribed the maximum permissible limit of arsenic in drinking water to be $10 \mu\text{g L}^{-1}$ (EPA, 2006). However, World Health Organization notified that in absence of any alternate source of drinking water, the maximum permissible limit of arsenic would be $50 \mu\text{g L}^{-1}$. In countries like Canada and Australia, this permissible limit is $5 \mu\text{g L}^{-1}$ and $7 \mu\text{g L}^{-1}$, respectively. Arsenic is released in the soil by natural biogeochemical cycles, from there it leaches into the groundwater by natural processes. Arsenic enters the body through water and food (Kapaj *et al.*, 2006). There are evidences that staple food crops like rice have accumulated arsenic. This leads to biomagnification of arsenic within the food chain. West Bengal is one of the severely arsenic affected states of India. Within West Bengal nine districts are affected by arsenic toxicity. Murshidabad is one of the severely arsenic affected districts of West Bengal. The river Ganga separates it from Bangladesh. Eastern bank of Bhagirathi river constitutes more arsenic contaminated blocks of Murshidabad district (64.7% above $10 \mu\text{g L}^{-1}$ and 32.5%

above $50 \mu\text{g L}^{-1}$) compared to the western bank of the river (30.1% above $10 \mu\text{g L}^{-1}$ and 11.7% above $50 \mu\text{g L}^{-1}$). In this study, our main aim is to analyze the conditions of soil and groundwater in highly arsenic contaminated regions of Murshidabad district (Fig. 1). For this purpose, we have qualitatively and quantitatively checked various parameters of soil & groundwater samples from these affected regions. Also, we have attempted the isolation of potential arsenic resistant bacteria from these regions.

2 METHODS/EXPERIMENTAL

2.1 Measurement of pH of soil and groundwater samples

Soil samples were diluted to obtain a concentration of 1 mg soil/1 mL distilled water. Soil samples were mixed well with water by constant stirring so, that the soluble solutes i.e. ions and electrolytes come in the polar phase. The particulate matter in the soil (soil sediments) was allowed to settle down. The pH of the solution was measured at 25°C . Similarly, the pH of the water samples was measured at the same temperature.

2.2 Estimation of bacterial load in the soil and groundwater samples and isolation of potential arsenic resistant bacteria

Water samples, and soil samples dissolved in water was plated in a LB-agar plate to estimate the bacterial load in the soil and groundwater samples.

Figure 1. Soil samples collected from a) Mahisasthali village.

Further, serial dilutions of water and soil samples were made and these diluted stocks were plated in LB-agar plates for isolation of single colonies of potential arsenic resistant bacteria.

3 RESULTS AND DISCUSSION

3.1 *Estimation of pH and total dissolved solids in the soil and groundwater samples from severely arsenic affected regions of Murshidabad district*

Water samples were collected from Bhagobangola I, Hariharpara, Chunakhali and Asrampara, which are the regions amongst the severely arsenic affected blocks of Murshidabad district. Soil samples were also collected from Bhagobangola I, Hariharpara and Chunakhali. pH of the soil and water samples was measured at 25°C. Both soil and water samples from the four arsenic contaminated blocks were found to be alkaline in nature. Soil sample of Hariharpara showed the maximum pH (8.37) amongst all the other soil samples. Among the water samples, water sample from Bhagobangola I showed the maximum pH (7.60). All the soil samples showed pH above 8. Total dissolved solids (TDS) in the water samples were found to be very high. TDS in water samples from Hariharpara was 200 mg L⁻¹. The other three sources showed TDS concentration of 300 mg L⁻¹.

3.2 *Isolation of potential arsenic resistant bacteria from the soil and groundwater samples*

Next, with an aim to isolate arsenic resistant bacteria from these samples, soil and water samples from aforementioned arsenic affected blocks were plated with and without dilutions on LB agar plate. Huge number of bacterial colonies was observed when undiluted soil and water samples were plated. As PHED had marked these blocks as severely arsenic contaminated, we wanted to identify arsenic resistant microorganisms from soil and water samples of these blocks. Therefore, we isolated bacteria from these soil and water samples by serially diluting the samples and spreading them on LB agar plate. Single bacterial colonies could be

observed in the plates. Considering the huge arsenic toxicity of the aforementioned regions, these colonies could be categorized as colonies of potential arsenic resistant bacteria.

4 CONCLUSIONS

We have concentrated our study on four blocks of Murshidabad district. These were Bhagobangola I, Hariharpara, Chunakhali and Asrampara. All of these blocks had been marked by PHED (Public Health and Engineering Department) as severely arsenic affected blocks. We collected soil and water samples from these areas and measured the pH and total dissolved solids (TDS). Both soil and water samples of these areas were found to be very alkaline. Soil & water samples from the above-mentioned arsenic affected blocks were plated with and without dilutions on LB-agar plate. With serial dilutions single bacterial colonies could be obtained on the LB-agar plate. Considering the huge arsenic toxicity of these regions, the soil and water microbiota obtained from these regions could be potentially arsenic resistant. Huge number of bacterial colonies was observed when undiluted soil and water samples were plated. Therefore, people of these blocks were drinking water contaminated with millions of bacteria. As characterization of these bacteria was not done therefore, it could only be assumed that these water samples might be contaminated with toxic bacteria including fecal coliforms and other pathogenic bacteria.

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