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CASE REPORT

CLINICAL MANIFESTATIONS OF ARSENIC TOXICITY AND EXTENT OF GROUNDWATER CONTAMINATION IN GOPALNAGAR VILLAGE OF MURSHIDABAD DISTRICT

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ABSTRACT

The problem of arsenic toxicity in Murshidabad district of West Bengal is geogenic in origin. Hariharpara is one of the severely affected blocks of Murshidabad district, where one-third of the groundwater samples contain arsenic above 50 µg/L, which is the maximum recommended level of consumption in absence of alternate source of drinking water, proposed by World Health Organization (WHO). Potential health hazards faced by the residents of Hariharpara block include skin problems like warts, cones and hyperkeratosis. Thickening of arteries, myocardial infarction and cyanosis of toes and fingers are severe manifestations of arsenic toxicity. Consumption of arsenic contaminated drinking water over sustainable period of time could lead to neurological disorders, chronic hepatitis and cirrhosis of liver and cancers of lung, liver, kidney, bladder and intestine. Gopalnagar village of Hariharpara block was selected as the site of analysis of biochemical and clinical effects of arsenic toxicity. The soil and groundwater samples collected from this village were alkaline in nature and showed huge amount of bacterial load and total dissolved solids (TDS). These uncharacterized bacteria could be fecal coliforms and pathogenic in nature. Presence of these bacteria could lead to diseases like typhoid, cholera and bacterial gastroenteritis. Presence of iron and phosphate above the Bureau of Indian Standard (BIS) recommended level in soil and groundwater samples of Gopalnagar resulted in various health hazards in the residents of the village. High level of iron could lead to formation of complexes between iron and arsenic and result in diseases like cyanosis, liver or bowel cancer, joint pain etc., whereas, high level of phosphate could lead to diarrhea or constipation, osteoporosis and deposition of calcium phosphate in muscle and soft tissues. The clinical manifestations of arsenic toxicity were documented by surveying 50 families of Gopalnagar village. The manifestations among the residents range from mild symptoms like dark coloured rashes and black spot in extremities to moderate symptoms like hardened tissue of hand and feet, diarrhea etc. In extreme cases some people show bent fingers, respiratory problems, as cities and even death. Both male and female members are exposed to arsenic contaminated soil and water. Due to the economic and social backwardness of the villagers alternate sources of drinking water and proper medical facilities are unavailable to them.

INTRODUCTION

Heavy metal contamination in soil and water is a serious problem, both nationally and globally. Arsenic contamination in soil and groundwater is among one of the global menaces. Arsenic is released in soil by natural biogeochemical processes. However, anthropogenic activities increase the concentration of arsenic in soil. Application of arsenic containing insecticides, pesticides and weedicides in soil raises the concentration of this naturally occurring element [1]. From soil leaching adds arsenic to groundwater. Arsenic exists in both inorganic and organic forms. Inorganic forms of arsenic like arsenite As (III) (+3), arsenate As (V) (+5) and arsenide (-3) are more toxic than its organic forms like arsenobetaine and arsenocholine. It is very difficult to remove minute quantities of arsenic from drinking water because of the excess solubility, mobility and bioavailability of inorganic forms of arsenic (compared to its organic forms). Being very reactive in nature arsenic forms large number of complexes with metals like iron and non metals like sulphur, phosphorous and others. It forms oxides upon exposure to oxygen. It also forms arsenides with various metals. Therefore, more than 200 complexes have arsenic as one of the major constituents in them. Various methods have been applied in removal of arsenic from drinking water. Though methods like precipitation, coagulation, oxidation and adsorption can be applied there is no efficient and cost effective method for removal of arsenic from drinking water, till date. Also, dissolution and desorption of minerals containing arsenic and improper disposal of arsenic containing sludge again add the arsenic in groundwater [2, 3, 4, 5]. Biomagnification is the accumulation of toxic substance in cereals, fruits, vegetable and in other food items and subsequent increase in toxicity as it moves to the next level in the food chain. Biomagnification of arsenic in rice, mustard, and other leafy vegetables further increases the problems associated with arsenic contamination. Rice, the staple crop of many parts of India, accumulates maximum amount of arsenic amongst the cereals [6, 7]. Arsenic is highly toxic to human body if consumed for a sustainable period of time. It shows various health effects like respiratory, circulatory, pulmonary and gastrointestinal disorders. Along with this, chronic exposure to arsenic would give rise to skin lesions, dark spots on hand and legs, neurological disorders involving headache and mental confusion, hematological disorders like anaemia, leucopenia, etc. Cancers of liver, lung, intestine, kidney and skin might also result from chronic exposure to arsenic due to chromosomal aberration and genetic mutation. Arsenic enters into the body through contaminated food and water. Inside the body arsenic is converted into monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) by the liver. This is followed by excretion of arsenic through urine. Therefore, arsenic will also damage liver and renal systems in long run [4, 8, 9, 10].

Murshidabad is one of the severely arsenic affected districts of West Bengal. The 26 blocks of the district are separated from Bangladesh by the river Bhagirathi. Differential arsenic concentration has been observed in groundwater, of the blocks situated in the western and eastern sides of the river. Blocks on the eastern side of Bhagirathi river are more arsenic contaminated than those on the western side of the river [11]. Table 1 depicts the status of various blocks of Murshidabad district with respect to Arsenic contamination. The problem of arsenic contamination in Murshidabad district is geogenic in origin. In this district the concentration of arsenic in groundwater varies from 1 μg/L to 2040 μg/L [12, 13]. The problem has affected 19 blocks and a population of approximately 40,08,480. Some blocks of Murshidabad district like Raghunathganj, Suti, Farakka faces the problem of water logging which further aggravates the problem of arsenic contamination of soil and groundwater. As a remedial measure West Bengal state government has set up arsenic removal plants and provided domestic filters to produce arsenic free water. Also, Central Ground Water Board dug deep tube wells in some of the blocks to have an access towards arsenic free water. However, in certain regions in southern part of the district, aquifers are beyond 350 m bgl, which requires high capacity drilling rigs for exploration [14].

Table 1 Status of various blocks of Murshidabad district with respect to Arsenic contamination

Arsenic Affected Blocks of Murshidabad District	Bhagobangola I	Bhagobangola II	Jalangi	Lalgola	Domkal	Berhampur	Beldanga I	Beldanga II
Groundwater samples with >10 μg/L of arsenic	61.2%	71.6%	77.8%	73.0%	69.6%	46.3%	61.1%	36.5%
Groundwater samples with >50 μg/L of arsenic	30.6%	43.0%	50.9%	34.5%	35.0%	15.6%	34.7%	12.5%
Maximum Concentration of arsenic found in the block (μg/L)	1285 μg/L	1852 μg/L	2040 μg/L	1028 μg/L	1300 μg/L	635 μg/L	1700 μg/L	345 μg/L

METHOD

Analysis of Surveys and Reports

Surveys, data and reports of Public Health Engineering Department (PHED), School of Environmental Science (SOES), under Jadavpur University, and State Water Investigator Directorate (SWID) on arsenic contaminated regions of Murshidabad district, was analyzed.

Collection of soil and groundwater samples from Gopalnagar village of Hariharpara block

Soil sample was taken and added to distilled water to get a concentration of 1mg of soil per 1ml of distilled water. The soil sample and water was mixed well to bring the ions and electrolytes in the polar phase and the sediments were allowed to settle down. This mixture was used for the measurement of biochemical parameters of the soil sample. Water samples were used undiluted, if not mentioned otherwise, for the same measurements. All the measurements were done at room temperature.

Measurement of pH and TDS

pH of soil and water samples was measured at 25^{o}C. Total Dissolved Solid (TDS) in 100 ml water sample of Gopalnagar, was estimated from the difference of weight of evaporation dish before (dry and empty evaporation dish) and after the evaporation of entire 100 ml of water in evaporation dish by application of heat. The TDS per liter of water was calculated subsequently by unitary method.

Estimation of total phosphate

Equal volume of soil and water samples were mixed with equal volume of Ammonium Molybdate solution and with 30% concentrated HCl. The colour of the complex formed was intensified by adding 200 μl of Stannous Chloride solution. The intensity of the colour was measured by measuring absorbance of the complex formed, at 690 nm wavelength using a UV-Vis Spectrophotometer. Using solutions of known concentration of Potassium Dihydrogen Phosphate, a standard curve was prepared and the concentration of phosphate in soil and water samples was calculated from there.

Estimation of total iron

The method used for the estimation of iron measures only the presence of Fe^{3+} in the sample. Therefore, to measure total iron present in the sample, 50 μl of 0.15 M $KMnO_4$ solution was added to 0.9 ml of sample. $KMnO_4$, a good oxidizing agent, converted all Fe^{2+} in the sample into Fe^{3+} ions. To 0.9 ml of sample, 5 ml of 0.10 M Ammonium Thiocyanate was added and mixed well. To stop hydrolysis of complex formed 1 M H_2SO_4 was added. The absorbance of the coloured complex was measured at 480 nm using a UV-Vis Spectrophotometer. In this case also, a standard curve was prepared using known concentration of $FeCl_3$ solutions and concentration of iron for soil and water samples was calculated from the standard curve.

Estimation of total sulphate

Total sulphate in the samples was estimated by Barium Chloride method, where 500 µl of conditioning reagent was added to 8 ml of sample and mixed well. The conditioning reagent contained Glycerol, concentrated HCl, Isopropanol and Sodium Chloride. The volume of the solution containing 8 ml of sample and 500 µl of conditioning reagent was made up to 10 ml with distilled water. A pinch of Barium Chloride was added to the solution and optical density was measured at 420 nm using UV-Vis Spectrophotometer. Standard curve was prepared using known concentrations of Sodium Sulphate. The concentration of sulphate in the sample was calculated from the standard curve.

Estimation of bacterial load and isolation of potentially arsenic resistant bacteria

Water and soil samples were plated on LB agar plates without any antibiotic. This gave an estimate of bacterial load in soil and water sample. Then, water and soil samples were serially diluted and plated on LB agar plates without antibiotic. Dilution was done to obtain single colonies of potentially arsenic resistant bacteria.

Survey of Gopalnagar village

Gopalnagar is a highly arsenic affected village of Hariharpara block of Murshidabad district. The village was visited by our team and the team members personally interacted with the villagers to document the health hazards faced by the villagers due to their prolonged exposure of arsenic toxicity.

RESULT AND DISCUSSION

Level of Arsenic contamination in Hariharpara and the potential health hazards

Surveys, reports and data on arsenic contaminated regions from Public Health Engineering Department (PHED) and School of Environmental Science (SOES) were analyzed. Hariharpara is one of the severely arsenic affected blocks of Murshidabad district. In Hariharpara block, the percentage of water samples having arsenic concentration greater than 10 µg/L is 63.2%. About 33.4% of water samples of Hariharpara block are contaminated with 50 µg/L of arsenic, the recommended limit of arsenic by World Health Organization in absence of any alternative source of drinking water. Residents of this block depend on ground water for drinking [15, 16, 17]. Therefore, people of this block consuming drinking water with more than 10 µg/L of arsenic might show skin problems like warts and cones on soles and palms. Consumption of drinking water with 10 µg/L to 100 µg/L of arsenic for prolong period of time might result in hyperkeratosis followed by hyperpigmentation and hypopigmentation. Thickening of arteries and myocardial infarction might occur in villagers of this block, drinking water with greater than 0.6 mg/L of arsenic. Cyanosis of toes and fingers could be observed upon consumption of arsenic contaminated water at a level of 0.6 to 0.8 mg/L. Drinking of arsenic contaminated water for sustainable period of time would give rise to neurological disorders involving mental confusion, headache and lethargy. Arsenic also causes chromosomal aberrations and mutations leading to cancers of lung, liver, kidney, bladder and intestine. Therefore, regular drinking of water with 0.29 mg/L and 0.30 to 0.59 mg/L of arsenic might show prevalence of developing skin cancer at a rate of 27.1 and 106.2, respectively, by the age of 60 (Table 2). A mortality rate of 405.12 could be observed in male who are dependent on water contaminated with arsenic at a concentration of 0.03 to 0.06 mg/L. Consumption of arsenic contaminated water for a long period of time would also result in chronic hepatitis and cirrhosis in the residents of this block. This could be due to extra burden on liver for detoxification of arsenic. Renal system also plays a vital role in elimination of arsenic through urine. Therefore, renal system might also get severely affected due to prolong intake of arsenic contaminated water. Diet is the main source of arsenic intake. Consumption of arsenic contaminated water, cereals, vegetables and fruits grown in arsenic contaminated soil and intake of fresh water fishes cultured in arsenic contaminated water would introduce approximately 50 µg of arsenic per day in residents of this block. Introduction of 0.05 to 1 mg of arsenic/kg/day to a person's body would give rise to neurological, haematological and cardiovascular disorders (Table 2) [4, 13]. Gopalnagar village of Hariharpara block was chosen for the downstream work. Soil and water samples were collected from this village and various biochemical parameters like pH, TDS, phosphate, iron, sulphate and bacterial load was measured. Also the clinical manifestation of arsenic toxicity was documented by conducting a survey, where 50 families participated.

Table 2 Potential health hazards encountered by residents of blocks of Murshidabad district

Arsenic concentration or exposure level	Associated health effects	Blocks where it could be observed
> 0.6 mg/L	Thickening of arteries and myocardial infarction	All the aforesaid blocks
>1 mg/m³	Disorders of respiratory system	All the aforesaid blocks
0.6-0.8 mg/L	Cyanosis of fingers and toes	All the aforesaid blocks except Beldanga II and Berhampur
> 0.6 µg/L	Gastrointestinal disorders like several abdominal colic, vomiting, diarrhoea, burning lips and painful swallowing	All the aforesaid blocks except Beldanga II and Berhampur
> 3 mg/day	Haematological disorders like anaemia and leucopenia	Jalangi and Bhagobangola II
0.05-1mg/kg/day	Neurological effects like Headache, lethargy, mental confusion	All the aforesaid blocks
0.29-0.59 mg/L	Cancers of skin, lung, bladder, kidney, liver and intestine	All the aforesaid blocks
> 10 µg/L	Warts and cones on soles and palms	All the aforesaid blocks
> 10-1000 µg/L	Hyperkeratosis followed by heperpigmentation and hypopigmentation	All the aforesaid blocks
>0.02 mg/kg/day	Ascites, jaundice, enlarged tender liver	All the aforesaid blocks

Alkaline pH and high TDS in samples from Gopalnagar village of Hariharpara block

Water and soil samples were collected from Gopalnagar village of Hariharpara block. pH of soil and water samples was measured at 25^{o}C and found to be alkaline in nature. pH of soil and water was 8.37 and 7.30, respectively (Table 3). Arsenic is more commonly found in alkaline soil and water sample. Total Dissolved Solids (TDS) was found to be 446 mg/L in the water sample from Gopalnagar (Table 3) [18]. Such a huge amount of TDS in drinking water would damage liver due to excessive strain for detoxification. Therefore, liver diseases could be observed in the residents of Goplanagar, along with some bone and dental problems. Bureau of Indian Standards (BIS) has set the maximum recommended limit of 500 mg/L of TDS in drinking water [19]. Though the residents of Gopalnagar are consuming water with acceptable level of TDS, but they might suffer from several diseases due to presence of various toxic metals and compounds in the water. There is no serious ill effect associated with drinking of water containing high TDS. But presence of heavy metals like arsenic, cadmium and harmful compounds like perchlorate and PCBs might give rise to various health problems.

High iron content in soil and water samples of Gopalnagar

The iron content in soil and groundwater sample of Gopalnagar was measured by ammonium thiocyanate method using UV-Vis Spectrophotometer. Various studies have indicated the easy formation and frequent occurrence of complexes between arsenic and iron in soil and water. Arsenic form ferric arsenate like $As_2Fe_3O_8$, $AsFeO_4$, $FeH_6As_3O_{12}$, etc. Arsenic is easily adsorbed by metal oxides, especially by iron oxides. Also, the rate of oxidation of As (III) is increased by breakdown of iron citrate complexes by photons. Therefore, iron compounds are used for deposition and filtration of arsenic as arsenic form complexes with iron very easily and get precipitated. Therefore, we measured iron content in soil and water sample from Gopalnagar. There was 954.3 μg/L of iron in soil sample of Gopalnagar, whereas 1129.8 μg/L of iron was present in the groundwater sample (Table 3). High iron content in soil of Gopalnagar might result mainly from various anthropogenic activities like over exploitation of soil leading to removal of upper humus layer and exposure of the lower layers of the soil, rich in minerals. Similarly, huge amount of iron could be observed in water samples due to leaching from soil. The groundwater sample of Gopalnagar showed iron content greater than its recommended limit in drinking water. BIS has set the permissible limit to 0.3 mg/L of arsenic in drinking water [19]. Therefore, intake of groundwater with 1.13 mg/L of iron might give rise to cirrhosis, liver cancer, bowel cancer, joint pain and other health problems in residents of Gopalnagar. Excessive iron might get deposited in various organs like pancreas, heart and liver. Also, being a potent oxidizer, it can damage tissues by various other ways.

Phosphate content in soil and water samples of Gopalnagar

The total phosphate was measured in soil and groundwater samples of Gopalnagar to have an indirect idea of arsenic uptake by plants, as arsenic and phosphate are taken up by common uptake mechanism. Both phosphate and arsenic are taken up by phosphate transporters present in root of plants. These transporters show less affinity toward arsenic compared to phosphate. The groundwater sample of Gopalnagar showed 60.5 μg/L of phosphate, whereas 90 μg/L of phosphate was found in its soil sample (Table 3) [18]. Phosphorous is the second most abundant element in the body after calcium. It is important for development of the bones and production of steroid hormones. But excess phosphate in the body can be detrimental to health. U.S. Environmental Protection Agency has set the recommended limit of 25 μg/L of phosphate in drinking water [20]. Therefore, consumption of drinking water with huge amount of phosphate by the people of Gopalnagar village would give rise to digestive problems like diarrhea or constipation, increased risk of osteoporosis and deposition of calcium phosphate in muscles and soft tissues leading to their hardening.

Sulphate content in soil and water samples of Gopalnagar

Arsenic also forms large number of complexes with sulphur such as arsenic trisulphide, tetra arsenic tetra sulphide, arsenic sulphate ($As_2(SO_4)_5$), etc. Therefore, we estimated sulphate content in soil and water samples of Gopalnagar by Barium chloride method using UV-Vis Spectrophotometer. The total sulphate content in soil sample was 1.3 mg/L (Table 3). The level of sulphate was undetectable in groundwater sample of Gopalnagar by the above mentioned method. The presence of high sulphate in soil could be result of decomposition and mineralization leading to deposition of organic sulphate in soil. Leaching might introduce the sulphate from soil to water. The sulphate content in water sample of Gopalnagar was less than the recommended limit of 250 mg/L as set by U.S. EPA [20]. Intake of water with sulphate concentration above this limit might show some laxative effects.

Table 3 Biochemical Parameters of Soil and Groundwater sample of Gopalnagar Village

Sl. No.	Biochemical parameter	Soil	Groundwater
1.	pH	8.37	7.30
2.	TDS	-	446 mg/L
3.	Phosphate	90 μg/L	60.5 μg/L
4.	Iron	954.3 μg/L	1129.8 μg/L
5.	Sulphate	1.3 mg/L	-

High bacterial load in water sample of Gopalnagar

Both soil and water sample of Gopalnagar block contained millions of bacteria. The undiluted samples when plated on LB agar plate showed lawn of bacteria. Dilution of water sample up to 10^{-8} dilution allowed the isolation of single bacterial colonies on LB agar plate. These bacteria could not be characterized at this moment and therefore, might include potentially arsenic resistant bacteria [18]. Along this, it might also include other kinds of pathogenic bacteria. Therefore, residents of this village consuming groundwater might show various diseases due to these pathogenic bacteria. Fecal coliforms might also be present in this water sample that would result in diseases like typhoid, cholera, dysentery, bacterial gastroenteritis etc.

Severe condition of villagers of Goplanagar

Gopalnagar is a village of Hariharpara block and is situated at 24.028° latitude and 88.434° longitude. This village is on Swaruppur road adjacent to Swaruppur More (Figure 1). Next aim was to conduct a survey amongst the villagers of Gopalnagar of Hariharpara block for a closer view at the socio-economic problems and health effects of arsenic contamination amongst the villagers. All the 50 families of the village are well aware of the arsenic contamination in soil and groundwater of the village. Most of the families are Below Poverty Line (BPL) and the residents of the village belonged to Schedule Caste (SC) and Other Backward Castes (OBC). This exemplifies the social and economic backwardness of the village. Agriculture and animal husbandry is the main source of income for 90% of villagers. Jute, rice, wheat, mustard and arum are predominantly cultivated crops. As the soil of the village is contaminated with arsenic therefore, accumulation of arsenic in food crops, its biomagnification and introduction in food chain enhances this serious problem. Animal husbandry with arsenic contaminated water might introduce arsenic in milk and milk products. Hariharpara block is also famous for fisheries. Fresh water fishes when cultured in arsenic contaminated water would accumulate arsenic in them. Cereals, milk and milk products and fishes are transported from this village to other blocks and districts of West Bengal. In this way, arsenic gets spread to the uncontaminated regions of West Bengal. This makes it a serious problem, nationally. Therefore, residents of arsenic unaffected blocks might show some of the arsenic related symptoms in long run. The villagers of Gopalnagar village, though well aware of arsenic contamination and its ill effects, could not take any kind of measures to combat arsenic toxicity. The only measure taken by the Panchayat is implantation of only 2 water taps for giving arsenic free water. So, people residing far away from those water taps consume arsenic contaminated groundwater. The residents of the village showed dark coloured rashes and black spots in feet, hand, chest, neck and back, darkened teeth and nails, hardened tissue of hand and feet, diarrhea etc. In extreme cases some of the people also showed arsenicosis, bent fingers, respiratory problems, ascitis, etc. Two of the villagers died in last 5 years due to arsenicosis (Figure 2). Also, there were families where only some of the members showed disorders and symptoms related to arsenic. Though males would be more susceptible to ill effects of arsenic, females working in the fields submerged with water were more affected by arsenic. Also, not all the villagers of the village were arsenic affected. Children except one, of this village did not show any kind of health effects due to arsenic contamination. These indicated the importance of various factors like dose of arsenic, exposure period, nutritional status, occupation and genetic predisposition in appearance of symptoms and severity of complications. Some of the symptoms and complications might have resulted from high phosphate and iron content in the soil and in the groundwater of the village. High TDS, phosphate, iron and bacterial load all together resulted in this serious condition of villagers of Gopalnagar. These villagers due to their economic backwardness could not afford for any kind of treatment and thus dependent only on medicines from small Homeopathy chamber of Panchayat Samiti. Neither could they purchase purified and packaged drinking water for consumption purpose and household chores. Under the "Swajal Dhara" scheme implemented by the Government of West Bengal, the water of river Ganges is purified and supplied to the villagers. However, there is just one tap in the entire village supplying water under the "Swajal Dhara" scheme, which is highly insufficient according to the daily requirement of the villagers.

CONCLUSION

Potential health hazards posed by arsenic toxicity in Hariharpara could be assessed from the reports of PHED and SOES. Real time studies in Gopalnagar village depicted the presence of various chemical as well as biological contaminants in soil and groundwater, which makes the groundwater completely unsuitable for consumption. Clinical manifestations of arsenic toxicity exhibited mild to fatal symptoms amongst the villagers. The irony of situation is in spite of being aware of the ill effects of arsenic contamination, the villagers are still consuming arsenic contaminated water and using it for household chores. This is due to the absence of alternate sources of arsenic-free water and arsenic-free arable land. Along with the conventional strategies of the government, some innovative technologies related to water shed management should be implemented. Biotechnological tools like bioremediation and inhibition of biomagnification could be used to mitigate the arsenic contamination of soil and groundwater.

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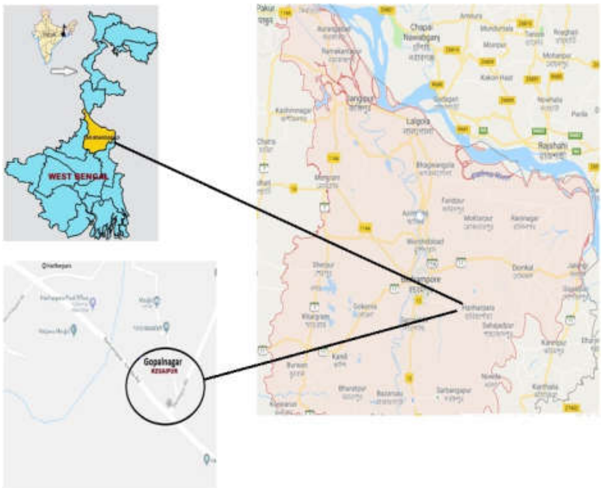

Figure 1 Map of the area of Study

Figure 2 Clinical manifestations of prolonged exposure to Arsenic toxicity

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