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# Effect of Soil Arsenic on Soil Microbial Populations in West Bengal, India

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**Abstract** **Original Research Article**

Arsenic, predominantly of geogenic source, has contaminated ground water and soil of the West Bengal, covering 111 blocks under 12 districts. Arsenic ingestion causes terrible health hazards to human being. Reported groundwater as content of 50 – 1600 µg/L, is several times more than permissible limit. Arsenite (III) binds to proteins and Arsenate (V) affects energy production in biological cells which results in skin melanosis to cancer. Microbes populations are indicators of soil health, influences soil fertility and may also offer possibilities of alleviation. Influence of as toxicity and tolerance in different soil microbes needs to be studied. Microorganisms are capable to use arsenate (V) or arsenite (III), in their metabolism through their *ars* genetic system. The present investigation involves enumeration of bacteria, actinomycetes and fungi in the affected areas of Nadia District. Soil samples were collected from 21 zones. The present study was showed how the microbial population varies in different arsenic contaminated area. The samples were analysed in a Parkin Elmer Atomic Absorption Spectrometer with Flow Injection Analysis system (FIAS 400). The data was analysed in using standard statistical tools. The total arsenic loading in the sample soils ranged between 0 to 20.00 mg $kg^{-1}$; bacterial population varied from $44x10^{-8}$ to $203x10^{-8}$, and actinomycetes from 10 $x10^{5}$ to 61 $x10^{5}$ and fungi from $4x10^{5}$ to$46x10^{5}$ CFU per gram soil. Total soil As content and microbe populations were highly significant with bacteria -0.802** actinomycetes --0.763** and fungi -0.732**.
**Keywords**: Arsenic, microbial population.


## INTRODUCTION

In last few years, the groundwater in the Gangetic alluvial zones of West Bengal (covering 38,865 sq. km area) is affected by arsenic contamination resulting in arsenic – related health hazards for millions [1, 7]. The widespread arsenic contamination in groundwater in different parts of West Bengal, distributed over 3200 villages of 111 blocks, located primarily in twelve districts (namely Howrah, Hoogly, Burdwan, Malda, Murshidabad, North and South 24 parganas, Kolkata, Nadia and Coochbehar, North & South Dinajpur) adjoining the river Bhagirathi, is of great concern.

Arsenic is widely distributed in all geological materials varying concentration. Arsenic enters into terrestrial and aquatic ecosystem through a combination of natural process such as weathering of rocks, biological activity etc. In present days, arsenic pollution in soil, water environment is a global problem. Actually arsenic is a semi metal or metalloid, toxic for most of the organisms [2, 7]. Arsenic (As) is known to be one of the most hazardous substances in the environment and the attention of which has increased in the past two decades due to the mounting number of affected people exposed to it [3]. Arsenate (V) and arsenite (III) are the two dominant form of in inorganic arsenic. Arsenite (III) is most toxic than arsenate (V) [12]. Arsenite is generally found in reducing environment but arsenate is generally predominant in well oxidized condition [4]. Arsenite has an ability to bind to sulphydryl groups of proteins and dithiols such as glutaredoxin. But arsenate is a chemical analog of phosphate and can inhibit oxidative phosphorilation of a cell. Arsenic is ubiquitus in soil and its concentration range from 1-40 mg as kg-1 in uncontaminated soil but can reach much higher levels in contaminated soil [5].

Soil is the home of various types of microorganisms. A group of soil microorganisms capable of transforming arsenic into different forms [6, 7]. Microbial metabolism generally influences the biogeochemical cycle of arsenic effecting both its speciation and toxicity. In anaerobic environment arsenate can be used as an electron acceptor by reduction of arsenite. On the other hand in aerobic situation arsenite can be oxidized to arsenate by aerobic bacteria which are able to take energy for their growth from oxidation of arsenite. But arsenic has a direct influence on reduction of soil microbial population [8, 9]. At higher arsenic level, reduction in microbial population and inhibition of soil enzymetic activities are observed [10]. Studies revealed that the total arsenic concentration of soils varied from 3.2 mg kg $^{-1}$ to 24.3 mg kg $^{-1}$ in the affected soils of West Bengal [7]. Therefore, an attempt has been made to find out the relationship between arsenic concentration of the soils with the soil microbial population viz. total bacteria, actinomycetes and fungi.

## Materials and Methods

Twenty one soil samples from different arsenic contaminated areas of Nadia District, West Bengal were collected for the experiment. The study was conducted under controlled condition in the Arsenic Laboratory of Bidhan Chandra Krishi Viswavidyalaya – The Niche Area of Excellence. The soil samples were immediately used without drying for microbial population study, to have an effect of field condition, keeping along sets for intimation of moisture content to express the result on dry weight basis. Air dried 2 mm sieved soil samples were analyzed for total arsenic.

**Estimation of total soil arsenic**

Soils were digested using tri-acid mixture of nitric acid, perchloric acid and sulphuric acid at the ratio 10:4:1, filtered by whatman no. 42 filter papers. The digest was diluted to 50 ml. Then arsenic content was measured by the method as described by Majumder *et al*. [7]. 10 ml of the aliquot was taken in 50 ml volumetric flask, 5 ml of concentrated HCl and 1 ml of 5% potassium iodide and 1 ml of 5% ascorbic acid were added to it, kept for 45 minutes to ensure complete reduction and finally volume was made upto 50 ml. The resultant solution was analysed in a Parkin Elmer Atomic Absorption Spectrometer with Flow Injection Analysis system (FIAS 400) @ λ max 193.7 nm where the carrier solution was 10% $^{v}/_{v}$ HCl as the reducing agent (to ensure all As species be reduced to $ASH_3$ and to be measured against a combination with standerd $As^{+3}$ solution) was 0.2% $NaBH_4$ in 0.05% Na OH.

**Microbial population of the soils**

Ennumeration of total bacteria, actinomycetes and fungi (colony forming unit – CFU) of the fresh soil samples were done by serial dilution spread plate method. The plates were incubated at $37^0$C temperature and colonies were counted after 2 days incubation.

Nutrient Agar Medium was used for bacterial population count, containing 3.0 gl$^{-1}$ beef extract, 5.0 gl$^{-1}$ peptone, 5.0 gl$^{-1}$ sodium chloride and 15 gl$^{-1}$ agars, the pH of the medium was adjusted to 7.

Asparagine Mannitol Agar medium was used for counting of actinomycetes, containing 1.0 gl$^{-1}$ dipotassium hydrogen phosphate, 0.5 gl$^{-1}$ potassium nitrate, 0.2 gl$^{-1}$ magnesium sulphate, 0.1gl$^{-1}$ cacium chloride, 0.1 gl$^{-1}$ sodium choride, trace amount of ferric chloride, 0.5 gl$^{-1}$ asparagine, 0.1 gl$^{-1}$ mannitol, 15 gl$^{-1}$ agar and pH of the medium was adjusted to 7.4.

Czapex Dox Agar medium was used for counting of fungi containing 3.0 gl$^{-1}$ sodium nitrate, 1.0 gl$^{-1}$ dispotassium hydrogen phosphate, 0.50 gl$^{-1}$ magnesium sulphate, 0.50 gl$^{-1}$ potassium chloride, 0.01 gl$^{-1}$ ferrous sulphate, 0.3 gl$^{-1}$ sucrose, 15 gl$^{-1}$ agar and pH of the medium was adjusted to 7.

## Results and Discussion

Total arsenic content of the soils as well as the total number of bacteria, actinomycetes and fungi are presented in table 1. From this study the data (Table 1) revealed that the total arsenic concentration of the polluted soils ranged from 7.35 to 20.00 mg kg$^{-1}$. This result is in keeping with the earlier reports [7]. The bacterial population was found to vary from 44x10$^{-8}$ to 203x10$^{-8}$, and actinomycetes from 10 x10$^{5}$ to 61 x10$^{5}$ and fungi from 4x10$^{5}$ to46x10$^{5}$ CFU per gram soil (Table 1). Total soil As content and microbe populations were highly significant with bacteria -0.802$^{**}$, actinomycetes -0.763$^{**}$ and fungi -0.732$^{**}$ (Table 2).

Table 1 Values with different alphabets are significantly different from each other according to duncan test (P=0.05). Each value is a mean of three replicates.

From this study we saw that bacterial, actinomycetes and fungal population decresed significantly with increasing arsenic concentration of the soils. The relationship between soil arsenic concentration and microbial population is presented in table 1. It was found that significant negative concentrations were obtained between total soil arsenic and bacterial population (-0.802$^{**}$), total soil arsenic and actinomycetes population (-0.763$^{**}$), and total soil arsenic and fungal population (-0.732$^{**}$) as compared to the lower arsenic concentration of the experimental soil. From the above observation it was found that bacteria, actinomyetes and fungal population already affected by the presence of higher quantities of arsenic in soils. Similar observations were repeated by earlier workers [10, 11]. Hiroki [8] shown that $As^{+3}$ is more toxic to bacteria and actonomycetes than $As^{+5}$ and fungi not only display a higher tolarence to $As^{+5}$ than bacteria and actinomycetes; but also showed the same tolerance to both $As^{+5}$ and $As^{+3}$. However in the present investigation, Arsenic speciation was not studied.

**Table-1: Microbial population as influenced by total arsenic of the soils**

| Soil Sample No. | Total As (mg /kg) | Microbial Population (CFU/g) (Average of 2 replications) | | |
|---|---|---|---|---|
| | | Bacteria (x $10^8$) | Actinomycetes (x $10^5$) | Fungi (x$10^5$) |
| $S_1$ | 7.25 ± 2.53 $^{f}$ | 121.00 ± 6.68 $^{c}$ | 32.00 ± 5.36 $^{bc}$ | 32.00 ± 6.55 $^{b}$ |
| S2 | 7.82 ± 4.37 $^{ef}$ | 132.00 ± 7.85 $^{b}$ | 38.00 ± 3.54 $^{b}$ | 31.00 ± 6.74 $^{bc}$ |
| S3 | 7.55 ± 4.16 $^{ef}$ | 138.00 ± 8.77 $^{b}$ | 38.00 ± 3.93 $^{b}$ | 30.00 ± 5.36 $^{bcd}$ |
| S4 | 8.09 ± 3.16 $^{def}$ | 140.00 ± 7.19 $^{b}$ | 40.00 ± 5.83 $^{b}$ | 30.00 ± 3.96 $^{bcd}$ |
| S5 | 8.36 ± 4.25 $^{def}$ | 93.00 ± 5.68 $^{d}$ | 40.00 ± 3.91 $^{b}$ | 31.00 ± 6.66 $^{bc}$ |
| S6 | 8.64 ± 3.85 $^{def}$ | 90.00 ± 8.48 $^{de}$ | 39.00 ± 5.09 $^{b}$ | 30.00 ± 6.27 $^{bcd}$ |
| S7 | 10.55 ± 4.43 $^{cdef}$ | 90.00 ± 7.27 $^{de}$ | 40.00 ± 4.74 $^{b}$ | 32.00 ± 4.79 $^{b}$ |
| S8 | 11.14 ± 3.28 $^{cdef}$ | 85.00 ± 6.83 $^{de}$ | 28.00 ± 5.26 $^{cd}$ | 22.00 ± 6.24 $^{cde}$ |
| S9 | 12.00 ± 3.21 $^{bcdef}$ | 81.00 ± 6.21 $^{def}$ | 20.00 ± 4.99 $^{defg}$ | 21.00 ± 6.16 $^{de}$ |
| S10 | 12.99 ± 3.13 $^{abcdef}$ | 79.00 ± 4.65 $^{efg}$ | 27.00 ± 5.73 $^{cde}$ | 19.00 ± 3.61 $^{ef}$ |
| S11 | 13.65 ± 3.63 $^{abcdef}$ | 70.00 ± 6.03 $^{fgh}$ | 23.00 ± 3.26 $^{def}$ | 19.00 ± 6.81 $^{ef}$ |
| S12 | 14.60 ± 4.31 $^{abcde}$ | 66.00 ± 7.52 $^{h}$ | 22.00 ± 5.40 $^{def}$ | 19.00 ± 3.57 $^{ef}$ |
| S13 | 14.57 ± 3.63 $^{abcde}$ | 68.00 ± 6.87 $^{gh}$ | 18.00 ± 4.90 $^{fghi}$ | 18.00 ± 6.23 $^{ef}$ |
| S14 | 14.99 ± 3.71 $^{abcd}$ | 69.00 ± 6.43 $^{fgh}$ | 16.00 ± 5.25 $^{fghi}$ | 11.00 ± 5.67 $^{fg}$ |
| S15 | 15.00 ± 4.57 $^{abcd}$ | 69.00 ± 8.16 $^{fgh}$ | 15.00 ± 4.67 $^{fghi}$ | 10.00 ± 5.83 $^{fg}$ |
| S16 | 15.13 ± 2.73 $^{abcd}$ | 55.00 ± 4.97 $^{i}$ | 11.00 ± 3.55 $^{hi}$ | 10.00 ± 4.10 $^{fg}$ |
| S17 | 17.02 ± 4.60 $^{abc}$ | 53.00 ± 6.81 $^{i}$ | 10.00 ± 3.08 $^{i}$ | 6.00 ± 0.98 $^{g}$ |
| S18 | 19.00 ± 4.26 $^{ab}$ | 52.00 ± 7.93 $^{i}$ | 21.00 ± 3.06 $^{def}$ | 5.00 ± 1.39 $^{g}$ |
| S19 | 19.99 ± 3.73 $^{a}$ | 50.00 ± 7.34 $^{i}$ | 19.00 ± 5.44 $^{efgh}$ | 5.00 ± 1.34 $^{g}$ |
| S20 | 20.00 ± 4.68 $^{a}$ | 44.00 ± 5.39 $^{i}$ | 12.00 ± 4.95 $^{fghi}$ | 4.00 ± 1.61 $^{g}$ |
| S21 | 0.00 ± 0.00 $^{g}$ | 203.00 ± 3.42 $^{a}$ | 61.00 ± 4.33 $^{a}$ | 46.00 ± 6.33 $^{a}$ |
| S.Em± | 2.100 | 3.840 | 2.738 | 2.973 |
| CD (P=0.05) | 6.002 | 10.976 | 7.826 | 8.498 |

**Table-2: Correlation between total soil as and microbial population**

| Correlation between | | Bacteria | Actinomycetes | Fungi |
|---|---|---|---|---|
| Total_As | "r" value | -0.802** | -0.763** | -0.732** |
| | Significant | 0.000 | 0.000 | 0.000 |
| | Number | 63 | 63 | 63 |

*.Correlation is significant at the 0.05 level; **. Correlation is significant at the 0.01 level

From the present investigation, it can be inferred that inherent arsenic concentration of the soils had a direct adverse effect on microbial population both for total bacteria, actomycetes and fungi.

## CONCLUSION

Microbial population is effected by soil arsenic. Inherent arsenic concentration of soil had a direct adverse effect on soil bacteria, actinomycetes and fungal population. Increasing arsenic concentration in soil means the decreasing of soil microbial population. The soil which had no arsenic contamination comparatively had high microbial population than other soils. So the present investigation shows arsenic free soil has high microbial population than arsenic contaminated soil.

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