J.Soil.Nature. 1 (2): 23-29 (July 2007)

A STUDY ON ARSENIC DECONTAMINATING CYANOBACTERIA OF AN ARSENIC AFFECTED SOIL

RIFFAT SHAHEEN[1], R. MAHMUD[2] AND J. SEN[3]

[1] Department of Food and Nutrition, Dhaka University, Bangladesh, [2]Faculty of Agriculture, Shinshu University, Japan, [3]Department of Soil Science, Bhidhan Chandra Agricultural University, India.

Accepted for publication: 24 May 2007

ABSTRACT

Shaheen, R., Mahmud, R. and Sen, J. 2007. ***A Study on Arsenic Decontaminating Cyanobacteria of an Arsenic Affected Soil.*** **J. Soil. Nature. 1(2): 23-29**

The present work was undertaken to study the arsenic (As) tolerance limits and decontamination efficiency of some cyanobacteria from an As contaminated soil of Gotera, West Bengal; under laboratory cultural condition. The soil harboured a considerably large number of cyanobacterial cells, 280000 cells g-1 with the dominating species of Genera, Aphanothece (unicellular), Phormidium (non-heterocystous filamentous form), Nostoc, Anabaena, Cylindrospermun and Calothrix (heterocysts bearing filamentous form). In liquid culture condition, most of the cyanobacterial species were able to tolerate 1000 ppm of added As in the form of Na_2HAsO_4 . However, their tolerance limit to As varied with increasing its concentrations. Only two species viz. Nostoc sp-1 and Phormidium sp. would be able to survive at 10,000 ppm of As concentration, though in solid culture condition their tolerance limit to As concentration were much less. A Cyanobacterial culture could bioaccumulate about 142.83 ppm of As and influence to volatilization loss was accounted about 937.80 ppm. Inoculation of high concentration As resistant cyanobacteria reduced water-soluble As content both in partially sterilized and non-sterilized soil by about 67% and 50% respectively. Such As decontaminating activity of them were also to be found when they were inoculated in presence of the addition of 250 ppm, 500 ppm and 1000 ppm As to soil. In partially sterilized soil, with added As, such reduction of water soluble As content by their inoculation were about 17.6, 12.7 and 16.7% respectively over control values and that in non sterilized soil were 7.7, 7.0 and 7.7% respectively. The results of this investigation clearly demonstrated that cyanobacteria has high tolerance ability to As concentrations and can able to decontaminate As from liquid culture media as well as from intracellular accumulation and influencing volatilization loss.

Keywords: Arsenic affected soil, arsenic tolerance, cyanobacteria,

INTRODUCTION

Arsenic (As), a toxic element widely distributed, in the earth crust. Recently, the widespread As contamination in groundwater in different parts of West Bengal, located predominantly in seven districts adjoining the river Bhagirathi, as well as the contiguous districts in Bangladesh, is of great concern. This toxin affects a large number of people of this region. The problem seems to be triggered off by large-scale withdrawal of groundwater for agricultural irrigation during the lean period of January to April when the groundwater recharge is at its minimum (Mandal et al., 1996). However, the surface water bodies in the affected belt have remained effectively free of the toxin, in agreement with similar observations reported elsewhere.

The concentration of As in natural surface and groundwater is generally about (1 ppb) but may exceed 1,000 ppb in mining areas or where As levels in soil are high. Groundwater is far more likely to contain high levels of As than surface water.

Inorganic As has been recognized as a human poison since ancient times and large oral doses (above 60,000 ppb in food or water) can produce death. Studies in animals show that most simple organic As compounds (such as methyl and diethyl compounds) are less toxic than the inorganic forms and that some complex organic As compounds are virtually non-toxic. However, high doses can produce some of the same effects.

The blue-green algae (Cyanophyta, Cyanobacteria) occupy a unique taxonomic position, since they combine an autotrophic mode of growth that is common to eukaryotic plant cells with a metabolic system that is generally regarded as bacterial rather than plant like.

Algae and cyanobacteria are most important so far as recycling of any particular element is concerned. cyanobacteria is almost ubiquitous in soils of new alluvial zones of West Bengal. Algae is the first organism to take up As in aqueous phase (Maeda 1983) Although cyanobacteria is susceptible to low concentration of arsenite and cytolysed at levels higher than 40 As (Ill) g-1 medium, but it cannot only tolerate high concentration of As (V) (1000 ppm) and still accumulate as high as 50,000 ug g-1 dry cell (Maeda, 1983; Maeda et al, 1985; Maeda et al, 1987).

Bioaccumulation of As by the cyanobacteria Nostoc has been identified as tool for removing As from polluted water (Maeda, 1987). Under conditions not conducive for cyanobacteria growth, particularly in the dark, cyanobacteria have been reported to excrete accumulated As (Maeda et al., 1992).

Cyanobacteria not only bioaccumulate As in the cells but also transform a part of it to TMA and no demethylation occur in cyanobacteria (Maeda et al., 1992). The present work was, therefore, undertaken to study the tolerance limits and decontamination efficiency of As of some isolates of cyanobacteria from an As contaminated soil of Gotera (Nadia district), under laboratory cultural conditions.

MATERIALS AND METHODS

Experimental Soil

Soil used in this experiment were collected from the area namely Gotera, (Chakdah Block, District Nadia, West Bengal). The site falls within the arsenic-affected zone. Composite soil sample (0- 0.15m) was collected from rice field by using soil augar. The soil sample was air- dried, powdered and sieved (20 mesh) and stored for chemical analysis. For microbiological works intact soil (after clearing root debris from the soil) was used as far as possible.

As solution preparation and determination

Sodium arsenate ($Na_2HAsO_4.7H_2O$) was used for preparing different concentration of arsenic solution. For determination of total 'Arsenic', 1 g sample of soil was digested with tri-acid (HNO_3: H_2SO_4: $HClO_4$: 10: 1: 4 by volume) solution. Using 2M HC1, the resulting digest was transferred to a 25 ml volumetric flask which is made upto volume with distilled water. The digest was then filtered through Whatman No.42 filter paper. Arsenic in the filtrate was determined by atomic absorption spectrophotometer coupled with a hydride generator unit (GBC -932 B).

Culture medium used

A modified chu – 10 medium (Safferman and Morris, 1964) with trace elements (Allen and Arnon, 1955) was used for enumeration and growing the culture of cyanobacteria (BGA). The liquid medium was solidified with 1.5% agar as and when required for experimental purposes.

Enumeration of bacteria

The most probable number technique described by Clark and Durrel (1965) was adopted for enumerating the cyanbacterial population in air dry soil. The cultures were kept in culture room at 30 ± 1°C under the illumination of 2-3 Lux light intensity by daylight fluorescent tubes for 16 hours per day for 3 weeks. The total viable bacterial populations were counted by serial dilution-pour plate method, using nutrient agar medium as the composition described earlier.

Treatment of As used in medium for experimental purposes

AS0 — Chu _ 10 media + Oppm As — solution
As1 — Chu — 10 media + l000ppm As- solution
As2 - Chu — 10 media + 5000ppm As -solution
AS3 — Chu — 10 media + 10,000ppm As –solution.

Enrichment cultures of cyanobacteria:

The soil sample, 5g portion was weighed and transferred aseptically to 100 ml sterilized Chu—10 medium with different treatments of As (As0, As1, As2, As3) contained in 250 ml conical flasks. Thereafter the flasks were incubated in the culture room at a temperature of 30 ± 1°C under the illumination of 2-3 K Lux intensity by daylight fluorescent tubes for 16 hours per day for 20 — 25 days. The cyanobacterial growth appeared in the flask were subculture in fresh medium and medium containing different treatments of arsenic and used as composite cyanobacterial flasks for further studies.

Identification of dominant cyanobacteria up to generic level

Liquid culture flasks of composite cyanobacteria obtained from each treatment were then examined under the Kerlzeiss research microscope, after mounting the cyanobacteria mass in a drop of distilled water on the microscopic slide and covering them with a thin cover slip, for characterization of the cyanobacterial species up to generic level. Most of the microscopic characters of prominence were taken into consideration and. they were identified according to Desikachary (1959).

Measurement of As decontaminating ability of cyanobacteria

One ml of culture from each untreated composite flask was inoculated aseptically in 50 ml Chu-10 medium of 10,000 ppm As concentration contained in 100 ml conical flasks. All the sets were prepared in 3 replications with control. Then, the flasks were incubated in growth chamber at 30 ± 1°C for 60 days under 16 hours light and 8 hours dark conditions. After incubation period, all the contents of each inoculated flask were filtered into separate conical flask with the help of funnel and filter paper and the

cyanobacterial mass was separated. Filter papers containing cyanobacterial mass were oven-dried for 24 hours at 60°C and kept in a desicator for cooling. After cooling weights were taken. Dry weight of cyanobacterial mass was calculated by deducting the dry weight of filter paper from the dry weight of filter paper with cyanobacterial mass. The same procedure was followed for the controls where there was no inoculation of cyanobacteria. Dried filter papers with cyanobacterial mass and only dried filter paper for controls were digested in the tri-acid mixture. All the digested samples and cyanobacterial filtrates were analyzed for As concentration with the help of AAS coupled with a hydride generator unit (GBC - 932B).

Measurement of As decontaminating ability of the isolated bacteria

The bacterial isolates were inoculated in 20 ml minimal medium (containing 5000ppm of As and incubated at 28 ± 1°C for three days. After 3 days all the inoculated cultures were centrifuged (10000 rpm for 10 minutes) except blank and bacteria were separated by decanting the medium. All the bacterial prills were collected and digested in tri-acid mixture. The concentration of As present in the bacterial filtrates, digested bacteria and in blank media was determined by using the AAS, coupled with a hydride generator unit.

Decontamination of added As from soil by cyanobacteria

An incubation study was conducted by taking 5 g portion of soil in 5″ × 1″ flat bottomed specimen tubes and subjecting them four levels of As (0ppm, 250ppm, 500ppm, l000ppm) in solution form. Then the soils were waterlogged by distilled water, the thickness of the soil layer and height of the standing water on the soil column being 1.3 ± 0.1 and 1 ± 0.1 cm respectively. Then the specimen tubes were inoculated with 1 ml of cyanobacterial culture (*Nostoc*, *Phorrnidiu*m sp.). Each level being replicated thrice and respective controls were also maintained. All the specimen tubes were incubated under daylight fluorescent tubes in the culture room for a period of one month. On expiry of the incubation period, the soil samples were analyzed for water-soluble arsenic fractions as described by (Johnston and Barnard, 1979).

RESULTS AND DISCUSSION

Physico-chemical properties of experimental soil

The physico-chemical properties of the soil are presented in Table 1, which reveal that the soil was neutral in reaction, low in electrical conductivity and contains low amount of organic carbon. The soil was high in available phosphorus (P) content, low in available potassium (K) and silt loam in nature with soil taxonomy-Typic Haplustepts. The cation exchange capacity of the soil was low, probably due to low clay and organic matter content. The amorphous Fe content and total As content of the soil was 0.22 per cent and 20.10 mg. kg- respectively.

Table 1. Physio-chemical properties of experimental soil

pH(1:2.5::SoiI:H2O)	7.50
Electrical conductivity (ds.m-1)	0.37
Oxidizable carbon (g. kg-1)	3.50
Cation Exchange Capacity {C mol (P+) kg-1}	21.90
Exchangeable K {C mol (P+) kg-1}	0.22
Exchangeable Ca ++ + Mg++ {C mol (P+) kg-1}	3.28
Olsen Extractable P2O5 (mg. kg-1)	63.20
Amorphous Fe (%)	0.22
Total arsenic (As) (mg. kg-1)	20.10
Sand(%)	6.10
Silt(%)	72.20
Clay(%)	21.70
Texture	Silt loam
Soil Taxonomy	Typic-Haplustepts

Important microbiological properties of soil

The MPN value (CFU) of cyanbacteria in air dried soil and that with the addition of 10,000 ppm As and composition of dominant genera of c (without addition of arsenic) are presented in Table 2. The results (Table 2) show that the soil harboured a considerably large number of cyanobacteriaol cells, 280000 cells g-1 with the dominating species of genera, *Aphanothece* (unicellular), *Phormidium* (non-heterocystous filamentous form) and *Nostoc, Anabaena, Cylindrospermum* and *Calothrix* (heterocysts bearing filamentous form). This indicates that the soil has the potentiality to support the favorable growth of cyanobacteria including the N2—fixing heterocystous form, might be due to its neutral pH accompanied with high content of available P which is well known important decisive factors for abundance of cyanobacterial growth in soil.

Table 2. Colony Forming Units (CFU) of cyanobacteria (BGA) and their dominating genera in Gotera soil

CFU/g soil	280000
Unicellular cyanobacteria	*Aphanothece* sp.
Non-heterocystous filaments of cyanobacteria	*Phormidium* sp.
Heterocysts bearing filamentous cyanobacteria	*Nostoc* (2 distinct sp.) *Anabaena* sp. *Cylindrospermum* sp. *Calothrix* sp.
CFU/g soil (10,000 ppm arsenic added)	330

However, addition of 10,000 ppm As as sodium arsenate ($Na_2HAsO_4.7H_20$) in the medium (modified Chu-lO) used for enumeration, drastically reduced their colony forming unit value from 280000 to 330 cells g soil, but continued growth of the species of cyanobacteria which could tolerate such a high dose of As in the medium. It suggests that there is a remarkable variation in tolerance limits of As concentration among the cyanobacterial species i.e. cyanobacteria which could tolerate such a higher concentration of As remained alive, and all other in the composite culture were cytolysed at 10,000 ppm of As concentration.

Tolerance limit of species of cyanobacteria in composite culture to As concentration

Liquid culture condition

The results of survival of the different species of cyanobacteria in their composite culture at different concentrations of As in liquid cultural conditions are presented in Table 3. The results show that at 0 ppm level of As in culture medium, the dominant genera of cyanobacterial species were *Phormidium, Nostoc* (two species N. sp,--1 and N. sp.—2), *Cylindrospermum, Anabaena, Calothrix* and unicellular *Aphanothece*. Addition of 1000 ppm As in the medium resulted death of the cells of unicellular *Aphanothece* sp. and filamentous *Cylindrospermum*.sp., indicating this level of As was toxic to them. However, the rest of the cyanobacterial species in their composition viz. *Phormidium* sp, two *Nostoc* sp., *Anabaena* sp.and *Calothrix* sp survived at this concentration of As without any cell damage. The species of three genera, excepting *Calothrix*, were also found to be able to grow at 5000 ppm level of As in the medium, indicating even this high level of As within their tolerance limit for their survival. However, at 10,000 ppm of As in the medium, only two species viz. *Nostoc* sp- 1 and *Phormidium* sp. would able to survive.

Table 3. Tolerance of different concentrations of arsenic (As) in liquid culture medium by the composite cyanobacterial genera/ species of Gotera soil.

Concentrations of arsenic in N containing Chu-lO culture medium	Composition of the growing dominant cyanobacterial genera/species
0 ppm As	*Phormidium* sp. *Nostoc* sp. (*N.* sp. -1 and *N.* sp. 22) *Anabaena* sp. *Cylindrospermum* sp. *Calothrix* sp. *Aphanothece* sp. *Phormidium* sp. *Anabaena* sp.
1000 ppm As	*Nostoc* sp. (*N.* sp. -1 and *N.* sp. -2) *Calothrix* sp. *Nostoc* sp-2
5000 ppm As	*Phormidium* sp. *Nostoc* sp *Anabaena* sp. *Nostoc* sp-1
10,000 ppm As	*Phormidium* sp.

Solid culture condition

Understanding whether the As resistant composite culture of cyanobacteria grown in liquid medium, can also able to grow in solid medium with different concentrations of As or not, the respective cultures were first grown in fresh As free medium (liquid) and then cultured in different doses of As containing solid medium for their colony formation. The results (Table 4) show that although most of them form distinct colony in agarised medium with 0 ppm As in Petri—plates but none of them formed colony in the medium with increasing doses of As except *Calothrix* sp. at 1000 ppm of As containing plates. This indicates that toxic level of As concentrations to the growth of cyanobacteria differ with the variation or change of the growing conditions. Less toxicity of the higher concentrations of As to the growth of cyanobacteria in liquid culture condition than that of the agarised solid culture condition may be attributed to the ionization or quick chemical change in the form of added As in former medium coupled with comparatively quick assimilation and release of metabolites in the medium helpful for their growth. However, further work is needed to, clarify this.

Table 4. Tolerance of different concentrations of arsenic (As) in agarised solid culture medium by the composite cyanobacterial genera/species of Gotera soil.

Concentrations of arsenic in agarised N containing Chu-lO culture medium	Distinct colony formation by cyanobacteria
0 ppm As	*Calothrix* sp. *Nostoc* sp. *Anabaena* sp. *Cylindrospermum* sp.
1000 ppm As	*Calothrix* sp.
5000 ppm As	No Colony
10,000 ppm As	No Colony

Here it could be concluded that cyanobacterial species were able to tolerate as high as 10,000 ppm of As concentration in liquid culture medium and about 1,000 ppm of As concentration in solid medium. These results were supported by the work of Maeda et al. (1983, 85, 87). They cultured the cyanobacteria in medium of 1000 ppm As (V) concentration and found that it successively tolerated such a high concentration of As.

Cyanobacteria in liquid culture

Results (Table 5) show that when As resistant cultures of cyanobacteria recultured in As free medium, washed and again inoculated in 10,000 ppm of As containing media, no growth of inoculated cyanobacteria appeared in two cases viz, media containing. 0 ppm and 1000 ppm As, might be due to

toxic effect of the high concentration of As. However, cultures obtained from 5000 ppm and 10,000 ppm of As containing media, were grown well in higher concentration of As.
On analysis for As contents, it was found that the cyanobacterial cultures accumulate as much as 142.83 ppm and 136.57 ppm of As in their body cells and that might be in organic form. The amounts of arsenic contents remain in the liquid media were 8774.53 ppm and 8370.15 ppm and the rest amounts were lost through volatilization. The amounts of volatilization loss of As due to cyanobacterial growth accounted about 528.13 ppm and 937.80 ppm over the uninoculated control value. Highest volatilization loss of As was observed with inoculation of 10,000 ppm As resistant culture of cyanobacteria. This suggests that cyanobacterial growths are able to decontaminate As from liquid media through intracellular accumulation and/or promoting volatilization loss.

Table 5. Bioaccumulation and decontamination of high dose of As (104 ppm) in liquid culture medium by the growth of cyanobacteria at 60 days incubation.

Inoculation of cyanobacterial culture and their source	Cyanobacterial Biomass As content	As in filtrate	Amount of Decontaminated or Volatilized As
*Culture of 0 ppm As	No growth of inoculated culture		
Culture of 1000 ppm As	No growth of inoculated culture		
Culture of 5000 ppm As	142.83	8774.53	528.13
Culture of 10,000 ppm As	136.57	8370.15	937.80

*Cultures were recaptured in As free-media, washed and used for inoculation.
**Volatilized As, by the growth of cyanobacteria, was computed deducting the values from control value 9451.1 ppm.

The results show that cyanobacteria could bioaccumulate arsenic intracelluraly and influence volatilization. On this aspect work was done by Maeda et al. (1983, 85, 87) who found that cyanobacteria could accumulate as high as 50,000 ug g-1 dry cell. Also Lundi (1973) observed that Oscillatoria rubescence when grown in medium containing radioactive As ion, accumulated arsenic 240 to 2800 times more than that of the concentration in the medium.

Decontamination of added As from soil by cyanobacteria

The results of the decontamination of added As from partially sterilized and non soil (As contaminated) by the activity of high arsenic resistant cyanobacteria inocula, are presented in Table 6. The results show that inoculation of As resistant cyanobacterial culture considerably reduced the water soluble As content both in partially sterilized and non-sterilized soils of Gotera during the period of incubation of one month. The magnitude of the amount of reduction of water soluble As content in soil due to inoculation of cyanobacteria, however, varied not only in respect of concentration of As added to soil but also for sterilization and non- sterilization of soil.
Inoculation without addition of As in the soil (As contaminated), reduced water soluble As contents both in partially sterilized and non-sterilized soil by about 67 and 50 per cent respectively. Addition of As 250 ppm, 500 ppm and 1000 ppm increased accordingly the water-soluble As contents in the soils. Inoculation of cyanohacterial culture reduced such contents of As in partially sterilized soil by about 17.6, 12.7 and 16.7 percent respectively. The corresponding reduction of water soluble As contents in non-sterilized soil due to cyanobacterial inoculation were comparatively low i.e. 7.7, 7.0 and 7.7 per cent respectively over their inoculated counter parts. The low reduction of water soluble As contents in non-sterilized soil due to inoculation of cyanobacteria might be accompanied with the comparatively low survival of inoculated one in presence of uninoculated indigenous population.

Table 6. Decontamination of added arsenic (As) in sterilized and non –sterilized Gotera soil (As affected) due to inoculation of high As resistant cyanobacterial inocula.

Concentration of arsenic added	Inoculation	Sterilized soil	Non-sterilized soil
		As content in soil (mg.kg-1)	As content in soil (mg.kg-1)
0 ppm	UI	0.003	0.002
	I	0.001	0.002
	UI-I	0.002 (66.66%)	0.001 (50.00%)
250 ppm	UI	77.821	74.278
	I	64.157	68.548
	UI-I	13.664(17.55%)	5.73 (7.71%)
500 ppm	UI	171.317	164.968
	I	149.487	153.358
	UI-I	21.830(12.74%)	11.610 (7.03%)
1000 ppm	UI	516.857	513.298
	I	430.557	473.668
	UI-I	86.300(16.69%)	39.630(7.72%)

Here, UI =Uninoculate and I = Inoculate.

Reference

Allen, M.B. and Arnon, D.I. (1955) Studies on nitrogen fixing blue- green algae I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. Pl. physiol., 30: 366-372

Clark, F.E. and Durrell, L.W. (1965) In: Methods of Soil Analysis (part-2), Agronomy series No.9 (Black, C.A.e.d.), Amer. Soc. Agron, P. 1506.

Desikachary, T.V. (1959). Cyanophyta. Indian Council of Agricultural Research, New Delhi.

Johnston, S.E. and Barnard, W.M. (1979). Comparative effeectiveness of fourteen solutions for extracting arsenic from Western New York soils. Soil Sci. Soc. Am. J., 43: 304-308.

Lundi, G. (1973). The analysis of fat and water soluble arseno-organic compounds in marine and Luineferic alga. Acta Chen. Scande., 27: 1586-94.

Maeda, S. (1983). Chemical soecies of trace elements and the biotransformation in organism. Kagaku, 37: 606-610.

Maeda, S.; Kumar,K.; Maeda,M.; Higashi, S. and Takashita (1987). Bioaccumulation of arseic by fresh water algae (Nostoc sp) and the application to the removgal of inorganic arsenic from an aqueous phase. Appl. Organomet. Chem., 1: 363-370.

Maeda, S.; Nakashima, S.; Takeshita, T. and Higashi, S. (1985). Bioaccumulation of arsenic by fres water algae and the application to the removal of inorganic arsenic from aqueous phase. Part ll. By Chlorella vulgaris islated rom arsenic-polluted envirnment. Sep. Sci. Tech., 20: 153-161.

Maeda, S.; Ohki, A.; Miyahara, K.; Higashi, S. and Naka, K. (1992). Metrabolism of methylated arsenic compound by arsenic-resistant bacteria (Klebsiella oxytoa and Xanthomonas sp) Appl. organomet. Chem., 6: 415-420.

Mandal, B.K.; Chowdhury, T.R.; Samanta, G.; Basu, G.K.; Chowdhury, P.P.; Chanda, C.R.; Lodh, D.; Karan, N.K.; Dhara, R.K.; Tamili, D.K.; Das, D.; Saha, K.C. and chakraborty, D. (1996). Arsenic in groundwater inseven districts of west Bengal, India-the biggest arsenic calamity in the world. Curr. Sci., 70 ; 976-986.

Safferman, R.S. and Morris, M.E. (1964). Growth characteristics of the blue-green algal virus L. J. Bacteriol. 88: 771-775.