Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 11	No. 3	pp. 439-446	2012

Original Research Paper

Microbial Growth and Arsenic Tolerance Ability as Influenced by Inherent Arsenic Loading in Polluted Soils of West Bengal

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 17/1/2012
Accepted: 4/3/2012

Key Words:
Arsenic
Tolerance limit
Microorganisms
Polluted soil

ABSTRACT

The effect of inherent arsenic loading on microbial growth in polluted soils of Haringhata block in the district of Nadia, West Bengal as well as the arsenic tolerant ability of the composite cultures of the said soils in different concentrations of arsenate (As^{V}) and arsenite (As^{III}) at different hours of incubation were studied. Total arsenic loading in the affected soils ranged from 4.70 to 16.56 mg/kg and to that of Olsen extractable arsenic (available arsenic) from 0.74 to 2.98 mg/kg. Total and available arsenic loading adversely affected the bacterial and cyanobacterial population, but not the fungi and actinomycetes. Significant negative correlations were obtained between total soil arsenic and bacterial population (r = -0. 798** in CFU and -0.800** in MPN method), available arsenic and bacterial population (r = -0.870** in CFU and -0.783** in MPN), total soil arsenic and cyanobacterial population (r = -0.853**) as well as available arsenic and cyanobacterial population (r = -0.857**). As^{III} was more toxic than As^{V}, the growth of the composite cultures appeared up to 20,000 mg/L in As^{V} enriched broth and up to 500 mg/L in As^{III} enriched broth in some of the soils after 168 hours of incubation. On an average, with increase in incubation period, arsenic tolerance ability increased and microbial growth appeared at the higher levels of As concentration. Microbial growth appeared at higher concentration of As^{V} and As^{III} with those soils having comparatively higher inherent As loading.

INTRODUCTION

Arsenic is one of the most toxic elements with diverse chemical behaviour in the natural environment. The widespread arsenic contamination in groundwater in different parts of West Bengal and Bangladesh is well known. The permissible limit (WHO) of arsenic in drinking water is 10 µg/L and maximum acceptable concentration (MAC) is 50 µg/L but the magnitude in affected areas of West Bengal is much more (50-3700 µg/L) than the permissible limits (Mandal et al. 1996). Indiscriminate use of arsenic contaminated groundwater for irrigation, particularly in boro rice resulting continuous accumulation of the toxic metalloid into soils of West Bengal. Studies revealed that the total and Olsen extractable arsenic, which constitute the soil As pool amenable to plant uptake varied from 3-24 mg/kg and 3-16 mg/kg, respectively, in the affected soils of West Bengal, which are comparatively higher than those reported for the soils of several other countries (Ghosh et al. 2004, Laha et al. 1911, Majumder & Kole 2011). Major crops such as cereals and legumes grown in arsenic contaminated fields accumulate substantial amounts of arsenic in their edible parts that may pose serious health hazards (Hug & Naidu 2005).

Soil is a living system with dynamic population of heterogeneous types of macro and microorganisms. Arsenic has also a direct influence on reduction of soil microbial population (Hiroki 1993, Mahimairaja et al. 2005, Laha et al. 2011). At higher arsenic level, reduction in microbial population and inhibition of soil enzymatic activities were recorded (Van Zwieten et al. 2003). But, many microbial communities have the capacity to adapt As-contaminated environment by developing resistance and tolerance ability (Smith et al. 1998, Salam et al. 2009, Huang et al. 2010). Some of them are capable of transforming arsenic into less toxic form through different mechanisms (Smith et al. 2001, Krumova et al. 2008, Cavalca et al. 2010). Therefore, an attempt has been made to find out the relationship between inherent soil arsenic loading and microbial population as well as the maximum tolerance limit of arsenic by composite cultures with a view to isolate the efficient arsenic transforming microorganisms from the arsenic affected soils, in future.

MATERIALS AND METHODS

Five soil samples (S_1-S_5) of highly arsenic contaminated area of Nonaghata village of block Haringhata and one sample (control-S_6) from Regional Research Station Gayeshpur of Bidhan Chandra Krishi Viswavidyalaya, in the district of Nadia, West Bengal were collected for the experiment. The study was conducted under controlled condition in the Ar-

senic Laboratories of the University (Niche Area of Excellence and NAIP-4 at Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya). The soil samples were immediately used without drying for microbial study, to have an effect of field conditions, keeping along sets for estimation of moisture content to express the result on dry weight basis. Air dried, 2 mm sieved soil samples were analysed for different physico-chemical properties as described by Jackson 1967. All the soil samples were typic haplustepts. Olsen extractable arsenic was determined by Olsen method as described by McLaren et al. (1998).

Olsen extractable arsenic and total arsenic: Soils were extracted with Olsen reagent (0.5 M $NaHCO_3$; pH 8.5) (soil : extractant :: 1 : 10 w/v) and filtered. The leachate was diluted to 50 mL. Ten mL of the aliquot was taken in 50 mL volumetric flask, 5 mL of concentrated HCl and 1 mL of mixed reagent [5% KI (w/v) +5% ascorbic acid (w/v)] were added to it, kept for 45 minutes to ensure complete reaction and the volume was made up to 50 mL. The resultant solution was analysed in a PerkinElmer Atomic Absorption Spectrophotometer with Flow Injection Analysis System (FIAS 400) @ λ_{max}@193.7 nm where the carrier solution was 10% v/v HCl, the reducing agent (to ensure all As species be reduced to AsH_3 and to be measured against a calibration with standard As^{+3} solution) was 0.2% $NaBH_4$ in 0.05% NaOH. For total arsenic, soils were digested by a tri-acid mixture of HNO_3, $HClO_4$ and H_2SO_4 (10:4:1) and filtered through Whatman No. 42 filter paper. The digest was diluted to 50 mL. The same procedure was then followed as that of Olsen extractable arsenic.

Microbial population of the soils: Enumeration of total bacteria, actinomycetes, fungi and cyanobacterial population in the soil samples were done by serial dilution-pour plate technique. The bacterial population was enumerated both by CFU and MPN methods. Nutrient agar medium for bacteria (Parkinson et al. 1971), starch casein medium for actinomycetes (Kuster & Williams 1964), Martin's Rose Bengal Streptomycin agar medium for fungi (Martin 1950) and modified Chu's-10 medium for cyanobacteria (Sufferman & Morris 1964) were used for enumeration of the microorganisms.

Tolerance limit of arsenic by composite soil cultures: To study the maximum tolerance limit of arsenic by composite soil cultures, 1 mL each of serial diluted soil suspensions were poured aseptically in 100 mL conical flask containing 50 mL sterilized nutrient broth (Parkinson et al. 1971) with different concentrations of arsenate (As^V) : 0, 50, 100, 500, 1000, 5000, 10000, 15000, 20000, 25000 mg/L and arsenite (As^{III}) : 0, 10, 50, 100, 200, 300, 400, 500, 700, 1000 mg/L and incubated at 30 ± 1°C. The arsenic salts were sodium arsenate (As^V) and arsenic trioxide (As^{III}). The appearance of microbial growth after 24, 48, 96 and 168 hours of incubation were studied by observing the turbidity (optical density at 600nm) using Systronics digital spectrophotometer (Model 106).

RESULTS

Physico-chemical properties of the experimental soils are presented in Table 1. pH of the soils ranged from 7.0-7.5, E.C. from 0.14-0.17 ds/m, oxidisable organic C from 4.23 - 5.10 g/kg, available N from 126-180 kg/ha, available P_2O_5 from 48.5-60.0 kg/ha and available K_2O from 133-146 kg/ha. The variation in pH, E.C., organic C, available N, P_2O_5 and K_2O amongst the soils were non-significant. The total soil As and Olsen extractable As content (available) of the polluted soils (S_1-S_5) were significantly higher than control soil of Gayeshpur (S_6) excepting the S_2 for available As. Among polluted soils, total As content differ significantly among themselves with no significant difference between S_1 and S_3. The S_5 soil content, the highest total and available As, differ significantly with all the others. The total As in polluted soils ranged from 4.70-16.56 mg/kg and available As ranged from 0.74-2.98 mg/kg, whereas in control soil of Gayeshpur, it was 1.19 mg/kg and 0.15 mg/kg respectively.

Table 1: Physico-chemical properties of experimental soils.

Soil sample	Soil pH	EC (dS/m)	Oxidizable Organic C (kg/ha)	Available N (kg/ha)	Available p_2O_5 (kg/ha)	Available K_2O (kg/ha)
S_1	7.5	0.15	4.25	156	48.5	142
S_2	7.0	0.14	4.56	165	60.0	138
S_3	7.5	0.16	5.10	180	56.0	135
S_4	7.3	0.15	5.03	172	53.5	133
S_5	7.1	0.17	4.23	173	58.5	146
S_6	7.2	0.15	4.50	160	58.0	140
Average	7.3	0.15	4.61	168	55.7	139
SEm (±)	0.06	0.01	0.12	3.6	1.3	1.5
CD (P=0.05)	NS	NS	NS	NS	NS	NS

Table 2: Arsenic loading and microbial population of the experimental soils.

Treatment	Total As (mg/kg)	Olsen As (mg/kg)	Bacterial CFU* $\times 10^5$ g^{-1}	Bacterial MPN** $\times 10^5$ g^{-1}	Actinomycetes CFU $\times 10^5$ g^{-1}	Fungi CFU $\times 10^5$ g^{-1}	Cyanobacteria CFU $\times 10^5$ g^{-1}
S_1	7.89	1.62	54.7	67.3	24.3	26.3	66.0
S_2	4.70	0.74	78.0	94.0	31.3	30.7	84.7
S_3	7.61	1.69	69.0	108.0	28.3	29.0	71.3
S_4	10.70	2.13	47.7	71.3	26.7	38.3	47.0
S_5	16.56	2.98	44.0	56.0	26.3	27.0	41.0
S_6	1.19	0.15	91.3	137.0	32.3	36.3	135.0
SEm(±)	0.36	0.19	6.7	1.3	4.5	5.3	7.5
CD (P=0.05)	1.06	0.61	20.9	3.9	NS	NS	23.6

*CFU - Colony forming unit; **MPN - Most probable number

Table 3: Correlation coefficient between soil arsenic and microbial population.

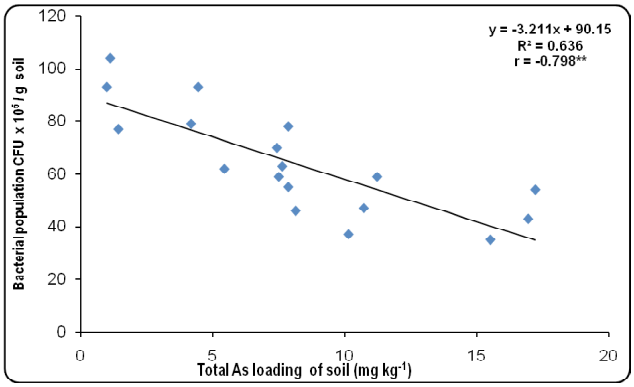
Correlation coefficient between	Equation	*r* value
Total soil arsenic vs Bacteria CFU	y= - 3.211x + 90.15	-0.798**
Total soil arsenic vs Bacteria MPN	y= - 4.896x + 128.4	-0.800**
Total soil arsenic vs Actinomycetes CFU	y= - 0.449x + 31.86	-0.326
Total soil arsenic vs Fungi CFU	y= - 0.445x + 34.89	-0.230
Total soil arsenic vs Cyanobacteria CFU	y= - 5.761x + 120.8	-0.853**
Olsen Extractable Arsenic vs Bacteria CFU	y= - 17.80x + 91.72	-0.870**
Olsen Extractable Arsenic vs Bacteria MPN	y= - 24.19x + 126.4	-0.783**
Olsen Extractable Arsenic vs Actinomycetes CFU	y= - 2.312x + 31.80	-0.330
Olsen Extractable Arsenic vs Fungi CFU	y= - 2.008x + 34.39	-0.204
Olsen Extractable Arsenic vs Cyanobacteria CFU	y= - 29.39x + 119.7	-0.857**

** Significant at 1% level

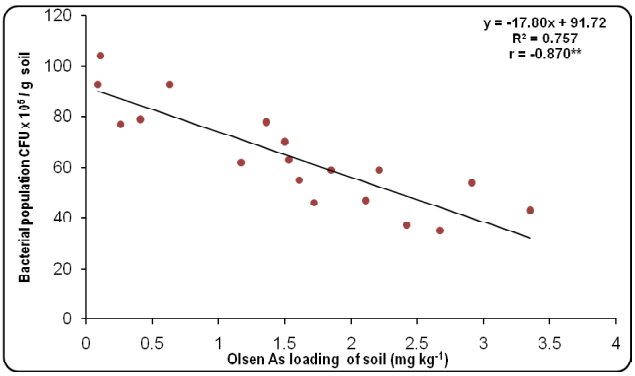
The total number of bacteria, actinomycetes, fungi and cyanobacteria of experimental soils are presented in Table 2. The CFU and MPN values for bacteria were found to vary from 44×10^5-91.3×10^5 and 56×10^5-137×10^5 per gram dry soil respectively. The highest number of bacterial population (both CFU and MPN) was observed in Gayeshpur soil (91.3×10^5 and 137×10^5 per gram dry soil), which was free from arsenic pollution. The lowest number of bacterial population was observed in S_5 (44×10^5 per gram dry soil). The highest cyanobacterial population (CFU) was observed in Gayeshpur soil (135×10^2 per gram dry soil), and the lowest in S_5 (41×10^2 per gram dry soil). Bacterial and cyanobacterial populations were significantly lower in arsenic polluted soils of Nonaghata than the non-polluted Gayeshpur soil. For fungi and actinomycetes the population variation among the soils was non-significant.

A decreasing trend in the population of the organisms with increase of inherent soil As loading was observed. The relationship between microbial population and As loading of both total and available fractions is presented in Table 3 and Fig. 1. Significant negative correlations (r = -0.798** and -0.800**) were obtained between total soil As and CFU of bacteria as well as with MPN, showing the linear relationship with the equations y = - 3.211x + 90.15 and y = - 4.896x + 128.4. Similar to total As, significant negative correlations between available As and bacterial population (CFU and MPN) were also found (r = -0.870** and -0.783**) with the linear relationship y = -17.80x + 91.72 and y = - 24.19x + 126.4. Significant negative correlations (r = 0.853** and r = - 0.875**) were also obtained between soil As loading (total and available) and cyanobacteria population, showing the linear relationship with the equations y = - 5.761x + 120.8 and y = - 29.39x + 119.7. Although CFU of actinomycetes and fungi were negatively correlated with total and available As of the soils (-0.326, -0.230 and -0.330, -0.204 respectively), none of these were statistically significant.

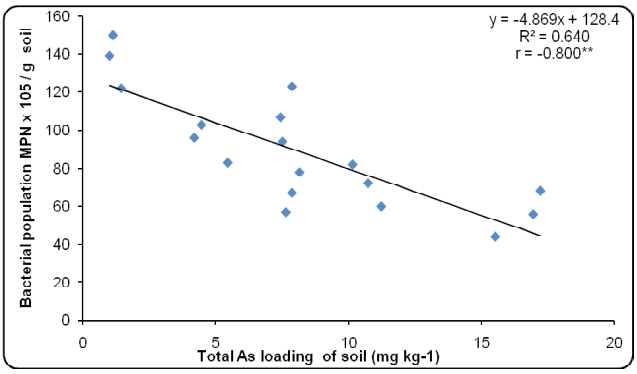
Appearance of microbial growth in As^V and As^{III} enriched nutrient broth at different concentrations with different arsenic affected soil dilutions are shown in Fig. 2 and Fig. 3 respectively. On an average, it was observed that with increase in incubation period, microbial growth appeared at the higher concentrations. From Fig. 2, it was observed that growth appeared within 24 hrs in all the soil samples enriched with 50 mg/L of As^V. In soils S_1 and S_5 growth also appeared in 100 mg/L arsenate enriched broths within 24



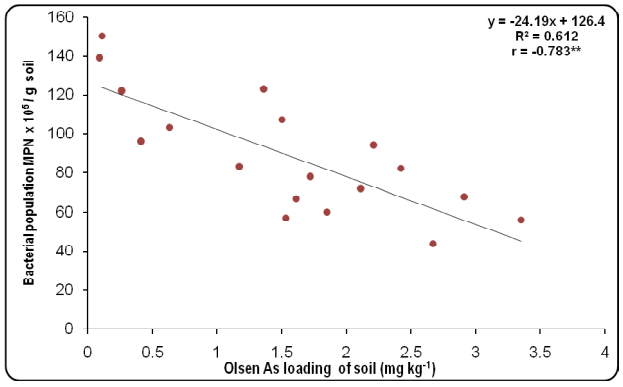
Total As and bacterial population (CFU)



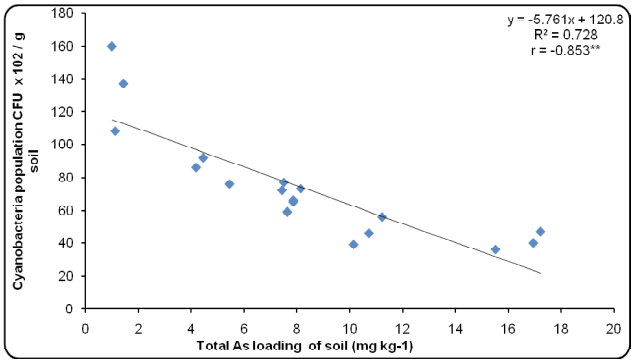
Olsen As and bacterial population (CFU)



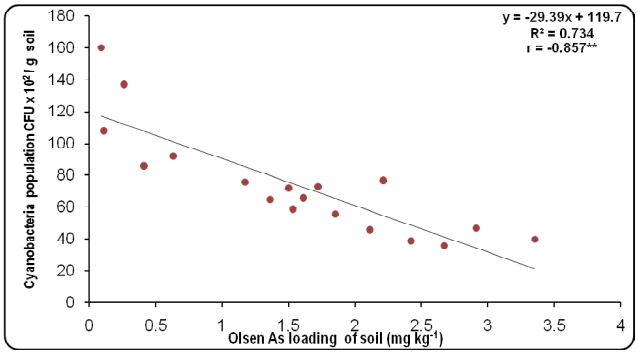
Total As and bacterial population (MPN)



Olsen As and bacterial population (MPN)



Total As and cyanobacteria population (CFU)



Olsen As and cyanobacteria population (CFU)

Fig. 1: Relationship between inherent arsenic loading in polluted soils and microbial population.

hours of incubation. At 48 hours incubation, growth appeared in all the soil cultures at 1,000 mg/L As^V, along with the maximum limit of 15,000 mg/L As^V in S_5 followed by S_3 and S_4 at 10,000 mg/L. After 96 hours incubation, growth appeared in all the soil cultures up to 15,000 mg/L excepting S_2, where it was up to 10,000 mg/L. At 168 hours incubation, maximum tolerance limit up to 20,000 mg/L arsenate was observed in the soils S_1, S_4 and S_5 and up to 15,000 mg/L in the soils S_2, S_3 and S_6.

It was observed from Fig. 3 that in arsenite enriched broth; growth appeared in all the composite cultures up to 10 mg/ L of As^{III} at 24 hours of incubation. At 48 hours of incubation, growth was seen up to 50 mg/L in four soil cultures S_1, S_3, S_4 and S_6. Growth appeared at the highest level of 100 mg/L in S_5 and growth was restricted at 10 mg/L of arsenite in S_2. At 96 hours of incubation, microbial growth was observed in all the soil cultures up to 100 mg/L of As^{III}. After 168 hours, microbial growth was observed at the highest level of 500

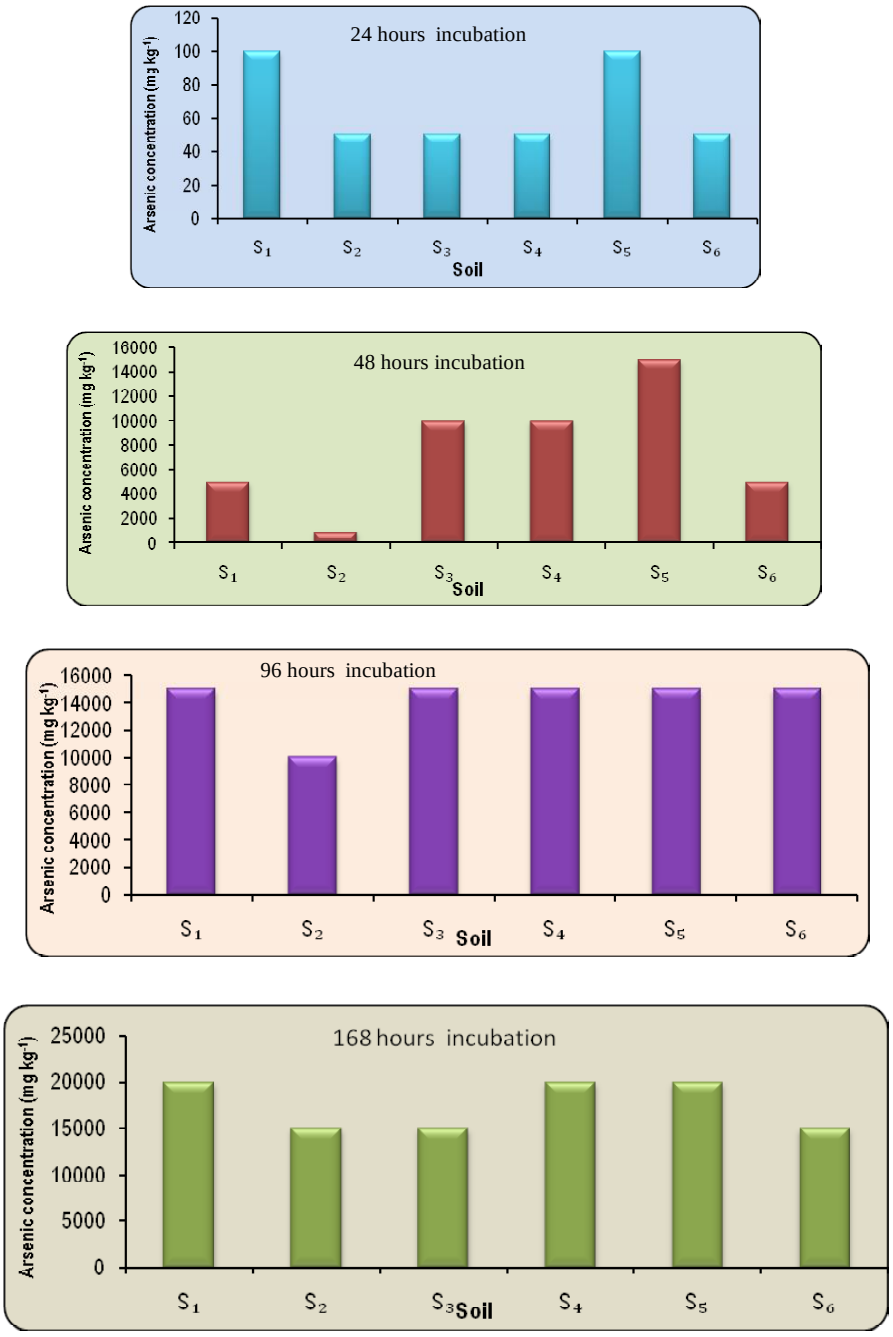


Fig. 2: Appearance of microbial growth with composite soil cultures in nutrient broth enriched with different levels of As^V.

mg/L in two soil cultures S_3 and S_5, while for other four samples, it was up to 100 mg/L of arsenite enriched broth.

DISCUSSION

Data revealed that the soils were neutral in reaction and non-saline. The soils were in medium range of oxidisable organic C, low in available N, moderate in available P_2O_5 and low in available K_2O. Total As loading in the polluted soils ranged from 4.70-16.56 mg/kg, whereas, in control soil of Gayeshpur, it was 1.19 mg/kg. Available As in the polluted soils ranged from 0.74-2.98 mg/kg, while in control soil it was 0.15 mg/kg. Soil arsenic values in As-affected areas of West Bengal were reported earlier to be in the similar range. Total As was reported, ranging from 3.2 to 24.3 mg/kg,

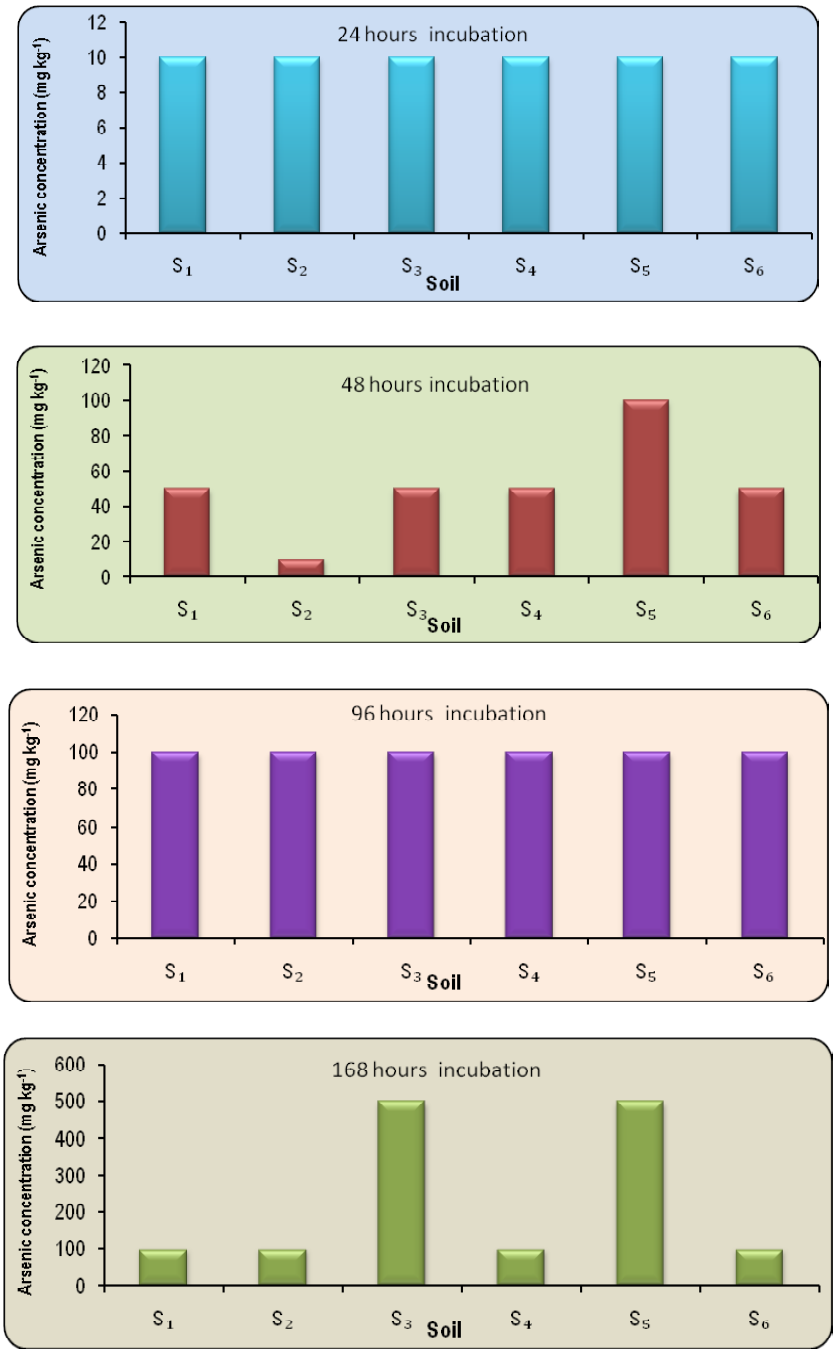


Fig. 3: Appearance of microbial growth with composite soil cultures in nutrient broth enriched with different levels of As^{III}.

whereas, available As was between 2.9 and 15.8 mg/kg (Ghosh et al. 2004). Therefore, data revealed that the experimental soils from Nonaghata were highly arsenic affected and require a serious attention for remediation. On the other hand, in Gayeshpur soil, As content was close to average concentration estimated in the continental crust (1.5 to 2.0 mg/kg) (Smith et al. 1998) and therefore, can be considered as safe.

A decreasing trend in the population of the organisms with increase of inherent soil As loading was recorded. Bacterial and cyanobacterial population were significantly lower in As polluted soils of Nonaghata than the non-polluted Gayeshpur soil. For fungi and actinomycetes the population variation among the soils was not significant. Significant negative correlations between soil arsenic loading (to-

tal soil As, available As) and bacterial (CFU, MPN) and cyanobact-erial population, and non-significant correlations between soil As loading (total and available) and fungal and actino-mycetes population were also observed. Soil organic C, N, P and K play a major role in increasing microbial proliferation in soil. But in the experimental soils, variation in soil nutrients including organic C was non-significant. From the above observations, it can be interpreted that the decrease in bacterial and cyanobacterial population in polluted soils of Nonaghata village in comparison to that of Gayeshpur soil was due to the presence of higher quantities of As in the former, and arsenic loading contributed detrimental role for decreasing the population. However, the fungal and actinomycetes populations were not so badly affected. A decrease in population of microbes with high concentration of soil As has also been recorded by earlier workers (Bisessar 1982, Van Zwieten et al. 2003). Hiroki 1993 shown that As^{III} is more toxic to bacteria and actinomycetes than As^{V} and that fungi not only display a higher tolerance to As^{III} than bacteria and actinomycetes but also show the same tolerance to both As^{V} and As^{III}. The CFU counts of bacterial isolates were comparatively lower than that of MPN. The reasons for lower CFU count than MPN might be due to slower growth of the said organisms in solid medium as also observed and explained by Kual et al. (2001).

On an average, it was observed that with increase in incubation period, arsenic tolerance ability increased and microbial growth appeared at the higher levels of As concentration. After 96 hours of incubation, almost all the composite cultures showed the tolerance ability and exhibited their growth up to 15,000 mg/L of As^{V} and 100 mg/L for As^{III}. The three soil cultures S_1, S_4 and S_5 showed the maximum tolerance limit up to 20,000 mg/L of As^{V} and two soil cultures S_3 and S_5 exhibited the highest level of tolerance up to 500 mg/L of As^{III} at 168 hours incubation. Data revealed that microbial growth appeared at higher concentration of As^{V} and As^{III} with those soils having comparatively higher inherent As loading, particularly the S_5 soil. This might be due to the development of As tolerance and resistant ability of the inherent soil microorganisms as explained by Smith et al. (1998). The soil having higher As loading might be used as a potential source of isolating As tolerant and transforming microorganisms. Between the two species of arsenic, growth appeared up to 20,000 mg/L at As^{V} enriched broth and to that of 500 mg/L for As^{III} enriched broth after 168 hours of incubation. From the above observation, it can be interpreted that As^{III} was much more toxic than As^{V} for the soil microorganisms and it supported the earlier findings (Ji & Silver 1992, Hiroki 1993, Omerland & Stolz 2005, Qin et al. 2006).

CONCLUSION

From the above observations it can be inferred that inherent As loading of the soils had a direct adverse effect on microbial population, particularly towards bacteria and cyanobacteria. Composite soil microbial cultures from As polluted areas could tolerate and proliferate in As enriched media as high as 20,000 mg/L of As^{V} and 500 mg/L of As^{III}. Therefore, the soils showing better growth of microorganisms at higher concentration of both As^{V} and As^{III} enriched broth might be the potential source of As tolerant and arsenic transforming microorganisms, could be used for isolating the efficient As transforming microorganisms and hence, might be the efficient tools for remediation of the hazardous metalloid.

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