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Transformation of arsenic by indigenous soil microbes as affected by phosphorus from contaminated soil of India

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ABSTRACT

Due to drinking of arsenic (As)-contaminated groundwater hundreds of millions of people in the world are at risk which makes as removal from aquatic systems of utmost importance. Highly arsenic polluted soil (16.5 mg kg-1) was used for the experiments at arsenic laboratory of BCKV, Nadia, India. Citrobacter sp. strain BC-1 significantly removed (7.6) and bioaccumulate (4.95) arsenic highest in P15As15 treatment and loss (2.9) was higher in P10As15. Similarly, Pseudomonas sp. strain BC-1 also significantly removed (7.4) and bioaccumulate (4.8) As highest in P15As15 and loss (2.8) was higher in P10As15. Percentage removal of as was 47-59%, bioaccumulation 29-38%, and loss 17-23% with Citrobacter sp. strain BC-1 and it was 47-58% (removal), 29-39% (bioaccumulation), and 17-21% (loss) with Pseudomonas sp. strain BC-1. Maximum removal and bioaccumulation of phosphorus was 37.8% and 32.1% for P10As15 in Citrobacter sp. strain BC-1. In Pseudomonas sp. strain BC-1 it was 33.1% and 27.2%, respectively for P10As15. At the same level of arsenic, increase in phosphorus significantly increased the removal and bioaccumulation of phosphorus but opposite was true during calculation in terms of percentage removal and percentage bioaccumulation. Therefore, these two promising bacterial strains can be used as bio-inoculants for bio-remediation of arsenic polluted soil in India.

1. Introduction

Environmental protection has the foremost importance in the present day life of mankind. Arsenic (As) is of anthropogenic and geogenic origin is a notorious toxic metalloid. Its pollution in the environment has been detected on a large scale in many districts of Indian state West Bengal and huge population are suffering from diseases like anomalies in the skin and carcinogenesis which leads to death (Das *et al.,* 2013). Arsenic have ability to exist in oxidation states like +5, +3, -1 in a sedimentary or aquatic system which depends on redox conditions (Attanayake *et al.,* 2015). Transformation of arsenic through biochemical process plays an important role to determine the fate in arsenic-rich treatment wastes (Sheik *et al.,* 2012). Through diverse bacterial community it canundergo different microbial transformation reactions like oxidation, methylation or reduction (Cheng *et al.,* 2015). Bacterium Pseudomonas fluorescens reduces iAsV to iAsIII microbially whereas bacterium Bacillus arsenoxydans microbially oxidize iAsIII to iAsV (Selveraj *et al.,* 2013). In soil system PO43- compete with arsenate AsV for

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sorption sites and affect as availability in soil (Jackson and Miller, 2000). In plant system, AsV is taken up via PO43- transporters. PO43- is able to reduce plant uptake of AsV which depends on the resistance characteristics of plants as well as the amounts of soluble P and as in the rhizosphere. Phosphates become more competitive over time since it is capable of slow sorption, but AsV is adsorbed more strongly than phosphate (Bradham *et al.,* 2018). As arsenic can transfer from soil system to plant system and then ultimately to animal system or human beings, it is essential to look into the process for remediation of arsenic toxicity through different biotic and abiotic technologies (Mumford *et al.,* 2012). There are a group of soil microorganisms which are capable of transforming arsenic into different forms (Srivastava *et al.,* 2012). It is well known that As+iii is more toxic than As+V on bacteria and actinomycetes. Besides, tolerance to arsenic is more in fungi than bacteria and actinomycetes, to both As+iii and As+V. Number of microorganisms show high degree of tolerance and transformation ability and these should be the target area for microbial bio-remediation (Elton, 2013). For remediation of As-contaminated soils, increasing interest in the application of bioremediation technology is growing. Approaches for microbial remediation of arsenic polluted soils can therefore be taken care off by inoculating the previously identified best elite isolates or by isolating, identifying and inoculating the efficient arsenic transforming microorganisms from arsenic affected soils or strains from type culture collection (Nemanja, 2015). Pseudomonas sp. is a gram-negative bacterium which has the prospect of rhizoremediation of organic compounds. It has not been used for arsenic removal till now. Citrobacter sp. is a gram-negative anaerobic bacterium can be used to reduce arsenic by its reductase activities. Several research works have been carried out on isolating and identifying the efficient arsenic transforming microorganisms. But no research has been carried on effect of phosphorus on bacterial transformation of arsenic. In order to fulfill the objective of the study on bacterial transformation of arsenic as influenced by phosphorus using Citrobacter sp. strain BC-1 and Pseudomonas sp. strain BC-1 bacterial strain, the experiment was conducted under controlled condition in broth to explore the possibilities of reducing arsenic contamination.

2. Materials and Methods

Highly arsenic polluted (16.5 mg kg^{-1}) composite soil sample (0-15 cm) of Gotera village under Chakdah Block in the district of Nadia, West Bengal, India (23°00'44.9"N and 88°34'59.6"E) was used for the experiments (Fig 1). The soil sample was properly labelled and brought to the laboratory for the investigation. Counting of microbial population was made just after collection of soil samples to have an effect at field condition. Soil sample was analyzed for different physicochemical properties including total and Olsens extractable. As loading in soil as well as microbial population following the standard methodology. To fulfill the objective of the investigation laboratory experiments was conducted to study the effect of different levels of phosphorus (0, 10, 15 mg L^{-1}) and arsenic (10, 15 mg L^{-1}) on bacterial transformation in broth. Two Arsenic transforming bacteria strain *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 were taken for the experiment. They were grown in broth at different concentration of phosphorus (0, 10, 15 mg L^{-1}) and arsenate (10, 15 mg L^{-1}) with the treatments combinations for each of the bacterial strains with three replications. The treatments are (T_1) P_0As_{10} – Phosphorus at 0 mg L^{-1}+Arsenic at 10 mg L^{-1}; (T_2) $P_{10}As_{10}$ –Phosphorus at 10 mg L^{-1}+Arsenic at 10 mg L^{-1}; (T_3) $P_{15}As_{10}$ – Phosphorus at 15 mg L^{-1} +Arsenic at 10 mg L^{-1}; (T_4) P_0As_{15} – Phosphorus at 0 mg L^{-1}+Arsenic at 15 mg L^{-1}; (T_5) P_{10} As_{15}– Phosphorus at 10 mg L^{-1} + Arsenic at 15mg L^{-1}; (T_6) $P_{15}As_{15}$ – Phosphorus at 15 mg L^{-1}+ Arsenic at 15 mg L^{-1}. Enumeration of total bacteria, fungi, actinomycetes and cyanobacteria (CFU) in the soil were studied by serial dilution pour plate technique. Stock solutions of 1000 mg L^{-1} arsenic were prepared by dissolving sodium arsenate (Na_2HAsO_4, 7 H_2O) in a one litre volumetric flask. A requisite volume of the arsenic solution containing 10 and 15 mg L^{-1} arsenic was applied to treatment. Stock solutions of 1000 mg L^{-1} phosphorus were prepared by dissolving potassium dihydrogen orthophosphate (KH_2PO_4) in a one litre of volumetric flask. A requisite volume of the phosphorus solution containing 10 and 15 mg L^{-1} phosphorus was applied to each treatment. The arsenic extracting solution used in this treatment was 0.5 M $NaHCO_3$ solution (sodium bicarbonate). The solution was prepared by dissolving 42 gm of $NaHCO_3$ in distilled water and volume was made up to 1 litre after adjusting the pH of the solution at 8.5 Iodine

solution of 0.002 M was used as an oxidizing agent during the colorimetric analysis of As^{V} which oxidizes As^{iii} to As^{V}. The nutrient broth used for this experiment was prepared by adding 0.5% sodium chloride, 0.3% beef extract, 0.5% peptone and distilled water. 50 ml nutrient broths were taken in a number of 100 ml capacity conical flasks made up of Borosil. Then arsenic concentrations of 0, 10, 15 mg L^{-1} as sodium arsenate were added to the prescribed treatment of each nutrient broth. Also phosphorus concentrations of 0, 10, 15 mg L^{-1} as KH_2PO_4 were added to those different treatments. Bacterial inoculation was made with 1 mL cell suspensions having density of ~ 10^8 CFU mL^{-1}. All these treated conical flask were kept in the laboratory for 14 days incubation at 30 ± 1^0C on a rotary set at 150 rpm. After 14 days of incubation, arsenic and phosphorus accumulation by cell biomass, and arsenic and phosphorus in nutrient broth upon removal of bacterial cell biomass were estimated. For estimation of bioaccumulation of arsenic and phosphorus, the harvested cell pellets obtained by centrifugation were re-suspended and washed first in 0.02 M $MgCl_2$ and after that in deionised water before drying at 65^0C for 24 hours. The dried cell pellets were digested with HNO_3. The total arsenic content was measured using atomic absorption spectroscopy (Perkin Elmer Atomic Absorption Spectrophotometer with Flow Injection Analysis System (FIAS 400) @λ_{max} ≅ 193.7 nm) and total phosphorus by using spectrophotometer. Loss of arsenic from the broth was calculated by substracting between arsenic removal in broth and bioaccumulation of arsenic in bacterial cells. Using SPSS Statistics 17.0 all statistical analyses were carried out by along with analysis of variance (ANOVA) to examine statistical significance. Statistical analysis of data was subjected to Fisher's least significant difference (LSD) test at the significance level $P < 0.05$.

3. Results and Discussion

Physico-chemical properties and microbial compositions of the arsenic affected soil were studied for better understanding of their

Figure 1. Site for soil sample collection under study

interactions and relationship and presented in Table 1 and 2. It was observed that the soil was neutral (pH 7.5) in reaction and non saline. The soil was in medium range of available phosphorus (24.9 mg/kg), low in organic carbon (4.7 g/kg), low in available potassium (0.22) and low in available nitrogen (174.0 kg/ha). The cation exchange capacity of the soil was high (22.9). Soil textural class was recorded as silty loam and the soil is a typic Haplustepts. The total arsenic loading was 16.5 mg kg^{-1} and the Olsen extractable arsenic was 4.29 mg kg^{-1}. The magnitude of arsenic contamination in our experimental soil was in higher range. Tamaki and Franken Berger (1992) reported that the mean natural content of arsenic in soils of about 5-6 mg kg^{-1} with a typical range of 1-40 mg kg^{-1} is in higher range. Biswas (2009) also observed higher range of arsenic in the soils of Nonaghata village (22°57'29.1"N and 88°34'22.4") of Haringhata Block of Nadia District, West Bengal, India which was heavily infested with arsenic and it was ranging from 8.4 to 24.3 mg kg^{-1} of total arsenic and 2.9 to 15.8 mg kg^{-1} of Olsen extractable arsenic. The bacterial population was 41.00×10^5 gm^{-1} CFUand medium in range. The cyanobacterial, actinomycetes and fungal population were in medium range (Table 2). Biswas, 2009 observed that the total population of bacteria and cyanobacteria were significantly lower in arsenic polluted soils than the non-polluted soils of West Bengal. With the increase of inherent soil arsenic loading, a decreasing trend in the population was reported by him. Two bacterial strains *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 having greater tolerance and transforming ability of arsenic were selected for observing the effect of different level of phosphorus and arsenic on arsenic transforming ability after

14 days of incubation. Removal of arsenic, bio-accumulation in cells and loss after 14 days from As^{v} enriched broth presented in Table 3.It revealed that arsenic removal (7.6) and bioaccumulation (4.98) by *Citrobacter sp.* strain BC-1 was highest in $P_{15}AS_{15}$ and loss of arsenic (2.9) was higher in $P_{10}AS_{15}$. Similarly Table 3 also showed that arsenic removal (7.4) and bioaccumulation (4.8) by *Pseudomonas sp.* strain BC-1 was highest in $P_{15}AS_{15}$ and loss of arsenic (2.8) was higher in $P_{10}AS_{15}$. Significant difference was observed among the treatments on arsenic removal and bio-accumulation in cells after 14 days of incubation.

The lowest removal was seen when phosphorus @ 0 mg L^{-1} and arsenic @ 10 mg L^{-1} (P_oA_{10}) was applied and the highest removal was observed when phosphorus @ 15 mgL^{-1} and arsenic @ 15 mg L^{-1} ($P_{15}A_{15}$) was applied. The trend was applicable for both the bacterial strains. Similar trend was also observed in case of bio-accumulation of arsenic in both the bacterial strain. No significant difference was observed between *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 in terms of removal, bioaccumulation in cells and loss of arsenic after 14 days of incubation. This depicted that both the organisms performed in similar manner in terms of transformation of arsenic at different levels of phosphorus and arsenic. On the other hand, it can be conferred that both the organisms were more or less similar efficient in terms of transformation of arsenic. A gradual increase in arsenic removal and bioaccumulation after 14 days of incubation was observed when the phosphorus content of nutrient broth increases but this increase was non-significant. Therefore, increase of phosphorus level or addition of phosphorus had no significant effect on increase in arsenic removal or bioaccumulation. The trend was observed for both the bacterial strain. On the other hand, almost in all the treatments arsenic removal or bioaccumulation was significantly higher in broth containing 15 mg L^{-1} of arsenic than 10 mg L^{-1} at the same level of phosphorus. But in terms of percentage of removal of arsenic, the opposite was true *i.e.* percent of removal was higher at 10 mg L^{-1} of arsenic broth than 15 mg L^{-1} of arsenic broth at same level of phosphorus. No significant difference was observed in percentage of arsenic loss but it was slightly higher side in 10 mg L^{-1} of arsenic broth than 15 mg L^{-1} of arsenic broth. The percentage of removal or bioaccumulation or loss of arsenic should be great concern for the study. From the Figure 2 and 3 it was observed that removal of arsenic ranges from 47 to 59%, bioaccumulation from 29 to 38% and loss from 17 to 23% with *Citrobacter sp.* and it was 47 to 58% (arsenic removal), 29-39% (bioaccumulation), and 17-21% (loss) with *Pseudomonas sp.*. Biswas (2009) observed that *Citrobacter sp.* strain BC-1 caused 17% removal, 15% bioaccumulation and 2% loss of arsenic and *Pseudomonas sp.* strain BC-1 caused 21% removal, 18% bioaccumulation and 3% loss of arsenic in 50 μg L^{-1} of broth. The higher efficiency in both the organisms in terms of percent removal, bioaccumulation and loss in the present experiment as compare to earlier findings of Biswas (2009) with the same organism might be due to presence of lower level of arsenic (10 mg L^{-1} and 15 mg L^{-1}) in our present experiment. Though it was statistically at par, but percent removal or bioaccumulation or loss of arsenic in 10 mgL^{-1} broth was in the higher state than 15 mg L^{-1} broth in the present experiment. The efficiency of the bacterial strains might be better at lower levels of arsenic.

Table 1. Physico-chemical properties of the experimental soil

Parameters	Value
Soil pH (1:2.5 :: Soil: Water suspension)	7.5
Electrical conductivity (dsm^{-1}) (1:2.5 :: Soil: Water suspension)	0.37
Oxidizable organic carbon (g/Kg)	4.7
Available Nitrogen (kg/ha)	174.0
Cation exchange capacity (cmol (p^{+}) Kg^{-1} of soil)	22.9
Sand (%)	6.1
Silt (%)	70.7
Clay (%)	23.2
Textural class	Silty loam
Soil taxonomy	Typic haplustepts
Exchangeable cation (cmol (p^{+}) Kg^{-1} of soil)	8.37
Exchangeable Ca^{++} Mg^{++} (cmol (p^{+}) Kg^{-1} of soil)	3.28
Exchangeable K^{+} (cmol (p^{+}) Kg^{-1} of soil)	0.22
Exchangeable Na^{+} (cmol (p^{+}) Kg^{-1} of soil)	0.10
Amorphous Fe (%)	0.34
Total Arsenic (As) mg/Kg	16.5
Olsen extractable arsenic (mg/Kg)	4.29
Available P (mg/Kg) (Olsen extractable)	24.9
Water holding capacity (%)	34.0

Table 2. Microbial population of experimental soil

Soil sample	Bacterial CFU* × 10^{5} gm^{-1}	Cyanobacteria CFU × 10^{2} gm^{-1}	Actinomycetes CFU × 10^{5} gm^{-1}	Fungi CFU × 10^{3} gm^{-1}
Gotera soil	41.00	40.00	25.00	28.00

*CFU – Colony Forming Unit

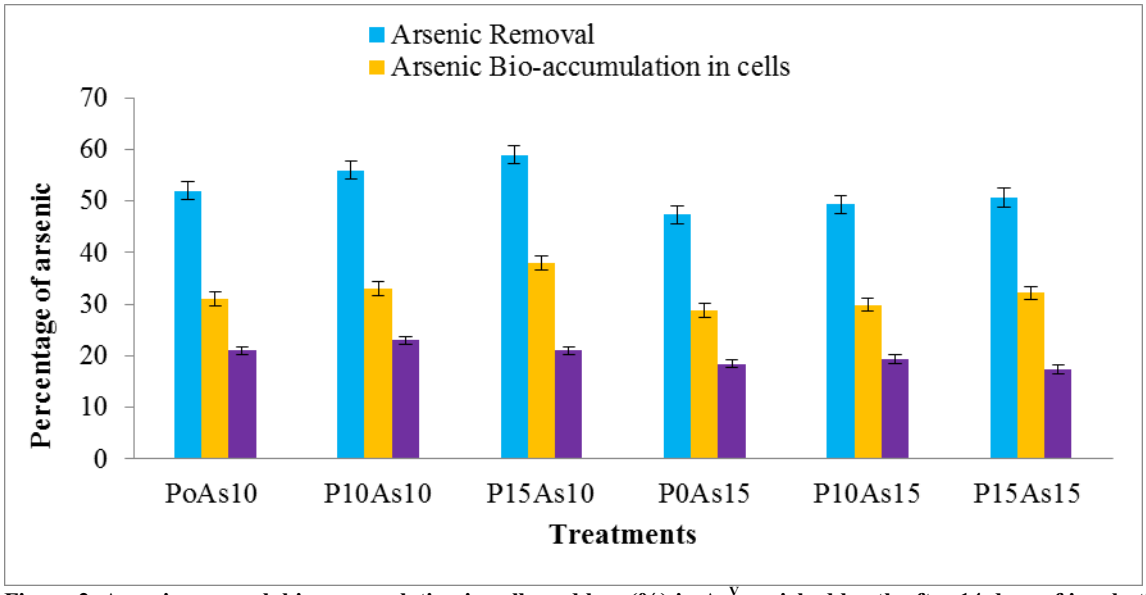

Figure 2. Arsenic removal, bio-accumulation in cells and loss (%) in As^{V} enriched broth after 14 days of incubation by *Citrobacter* sp. strain BC-1

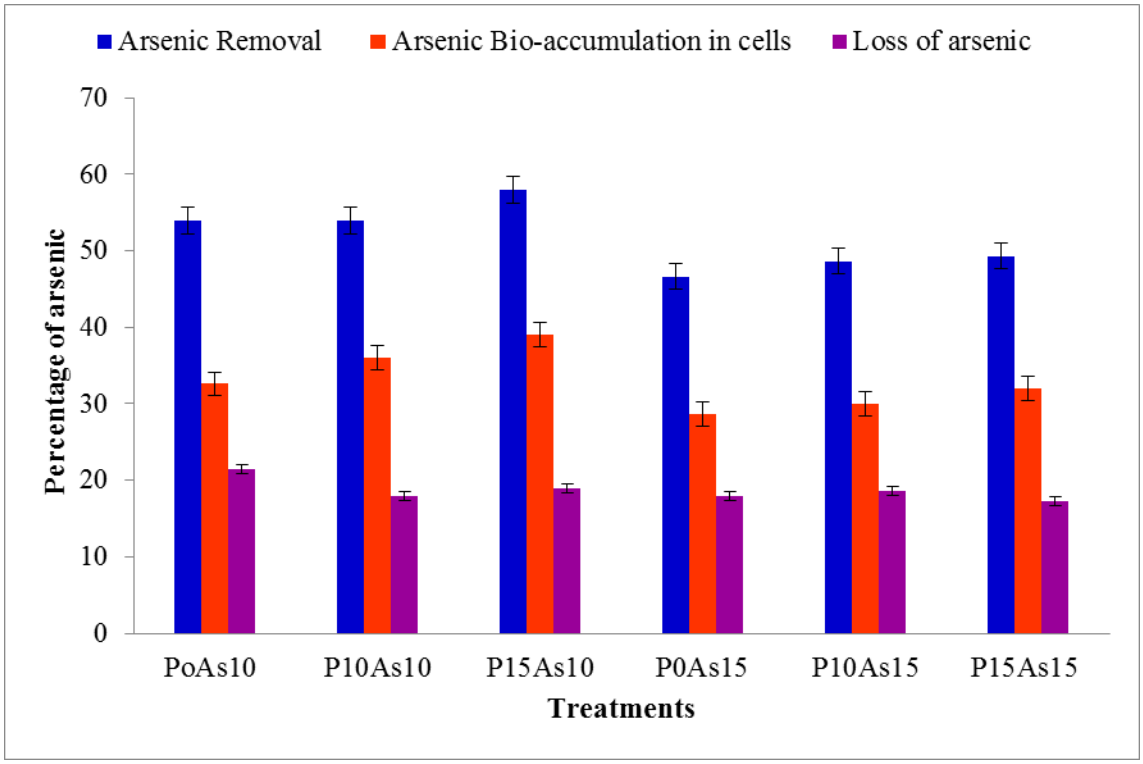

Figure 3. Arsenic removal, bio-accumulation in cells and loss (%) in As^V enriched broth after 14 days of incubation by *Pseudomonas* sp. strain BC-1

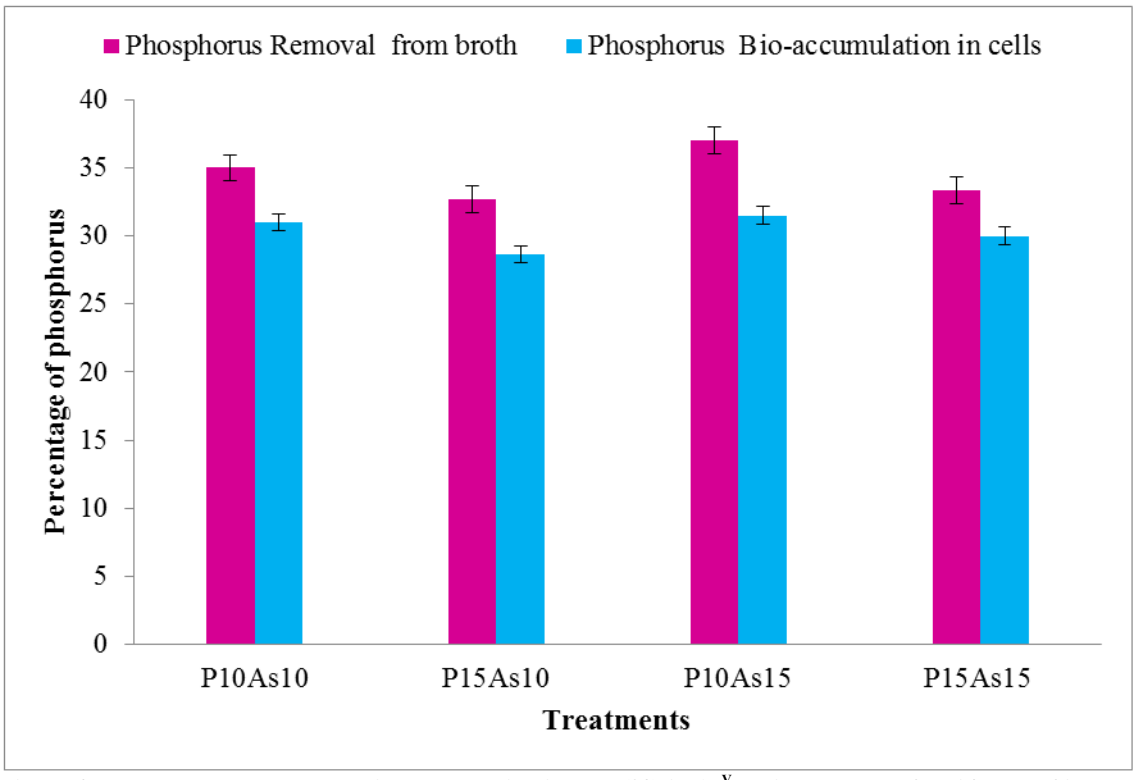

Figure 4. Phosphorus removal and bio-accumulation in cells (%) in As^V enriched broth after 14 days of incubation by *Citrobacter* sp. strain BC-1

Therefore, the critical level of arsenic concentration to be estimated for showing the highest level of arsenic removal or loss, which needs further study. After 14 days of incubation, removal of phosphorus from broth and bioaccumulation of phosphorus within bacterial cells were observed and the data are presented in the Table 4. In terms of removal and bioaccumulation of phosphorus the two bacterial isolates were similar in nature. Both the bacterial strain increased the phosphorus removal and bioaccumulation. There is no significant difference between the two bacterial isolates for the purpose. There was significant difference between all the treatments. Phosphorus removal by *Citrobacter sp.* from broth was higher in $P_{15}As_{15}$ and for *Pseudomonas sp.* strain BC-1 it was $P_{15}As_{10}$. Similarly, phosphorus accumulation in cells was higher in $P_{15}As_{15}$ by *Citrobacter sp.* strain BC-1 and $P_{15}As_{10}$ in case of *Pseudomonas sp.* strain BC-1. It is interesting to note that in case *Pseudomonas sp.* both phosphorus removal and bioaccumulation was higher in $P_{15}As_{10}$ than $P_{15}As_{15}$. This may be due to the fact that at higher concentration of arsenic the bacterial activity of *Pseudomonas sp.* strain BC-1 is hampered. But another bacterial strain *Citrobacter sp.* strain BC-1 is efficient in phosphorus removal and bioaccumulation at slightly higher concentration of arsenic ($P_{15}As_{15}$). The phosphorus removal and bioaccumulation was negligible for both the bacterial strain at P_0 level of phosphorus at both As_{10} and As_{15} which indicated that phosphorus nutrient is essential for both the microbes for removing phosphorus under highly arsenic contaminated soil. It is interesting to note that at the same level of phosphorus (P_{10} or P_{15}) in both the strain with increase in arsenic content phosphorus removal and bio-accumulation also increase. From Figure 3 and 4, in *Citrobacter sp.* strain BC-1 the maximum percentage removal of phosphorus and percentage bioaccumulation was 37.8% and 32.1% for $P_{10}As_{15}$. In *Pseudomonas sp.* strain BC-1 the maximum percentage removal of phosphorus and percentage bioaccumulation was 33.1% and 27.2% for $P_{10}As_{15}$. Results showed that increase in arsenic concentration had no significant effect on removal or bioaccumulation of phosphorus in broth for both the inoculants. At the same level of arsenic, increase in phosphorus significantly increased the removal and bioaccumulation of phosphorus but opposite was true during calculation in terms of percentage removal and percentage bioaccumulation. These depict that percent removal or bioaccumulation of phosphorus is higher at lower concentration. This pattern was similar as observed in case of arsenic. There was no significant difference between treatment and bacterial strain. Young *et al.,* 2012 revealed

Table 3. Arsenic removal from broth, bio-accumulation in cells and loss of arsenic by selected bacterial strains from As^V enriched broth after 14 days of incubation

Bacterial strains	Treatments	Arsenic Removal from broth (mg L^{-1})	Arsenic Bio-accumulation in cells (μg gm^{-1})	Loss of arsenic (mg L^{-1})
Citrobacter sp. strain BC-1	P_oAs_{10}	5.2	3.1	2.1
	$P_{10}As_{10}$	5.6	3.3	2.3
	$P_{15}As_{10}$	5.9	3.8	2.1
	P_0As_{15}	7.1	4.32	2.78
	$P_{10}As_{15}$	7.4	4.5	2.9
	$P_{15}As_{15}$	7.6	4.98	2.62
	Mean	6.5	4.0	2.5
Pseudomonas sp. strain BC-1	P_oAs_{10}	5.4	3.26	2.14
	$P_{10}As_{10}$	5.4	3.6	1.8
	$P_{15}As_{10}$	5.8	3.9	1.9
	P_0As_{15}	7.0	4.3	2.7
	$P_{10}As_{15}$	7.3	4.5	2.8
	$P_{15}As_{15}$	7.4	4.8	2.6
	Mean	6.4	4.06	2.34
Bacteria (B)	SEm(±)	0.175	0.146	0.212
	CD (P=0.05)	NS	NS	NS
Treatments (T)	SEm(±)	0.304	0.253	0.367
	CD (P=0.05)	1.330	1.058	NS
(B ×T)	SEm(±)	0.329	0.258	0.418
	CD (P=0.05)	NS	NS	NS

that *Citrobacter* sp. strain NC-1 reduced arsenate within 24 h and exhibited arsenate-reducing activity. The strain NC-1 was able to extract arsenic from contaminated soils (arsenate to arsenite) through solid-phase reduction. Selvan kumar *et al.,* 2017 found that *Citrobacter* sp. strain RPT can survive under the As stress and identified for application in bioremediation of As. Wei *et al.* 2015 reported that arsenic immobilization by *Pseudomonas* sp. strain GE-1-induced ferrihydrite can be applied as an alternative remediation strategy. Joshi *et al.* 2008 found that *Pseudomonas* sp. exhibited a maximum accumulation of 4 mg As g^{-1} (dry weight). Aksornchu *et al.* (2008) obtained 24 bacterial isolates of which 5 isolates observed higher arsenic adsorbing capacities ranging from 80.9 to 96.9%. The reports of the above findings are comparatively in higher range to that of our present observation. In our study loss of 17-23% with *Citrobacter sp.* and 17-21% (loss) with *Pseudomonas sp.* were found and the reason for the loss or removal of arsenic from the broth may possibly due to volatilization as well as microbial cell accumulation. Again volatilization of arsenic depends upon several factors *viz.* initial concentration of arsenic, substrate time and amount. Transforming arsenite and arsenate into mono, di or trimethyl arsenic through number of microorganism were reported (Qin *et al.* 2006). In our study the loss (17-23%) of arsenic from the broth by *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 was either due to methylation or otherwise and further experiments required to be conducted. In our study using different arsenic concentrations, it was revealed that the highest recorded resistance was found in both the strains. Majumder *et al.* 2013 found that approximately 37 % of As^{iii} (aerobic) and 30 % As^{V} (anaerobic) were volatilized by bacterial isolates within 3 days. In our study both the bacterial strains were resistant and effectively removed arsenic in arsenic polluted soil of India state West Bengal. This may be due to that the microbes develop various intrinsic arsenic tolerance mechanisms to sustain in the adverse environmental conditions. Another factor may be that arsenic metal resistant of the bacteria has genes located on plasmids and genetic system named ARS OPERON may be the main functional unit for arsenic resistance. Xi-Xiang *et al.* 2012 found that *Synechocysis* sp. strain PCC6803 has strong ability for arsenic accumulation and tolerance, which may be applied in the phytoremediation of aquatic arsenic. In our study, at the same level of phosphorus (P_{10} or P_{15}) with increase in arsenic content phosphorus removal and bio-accumulation also increase non-significantly. In 2011,

Table 4. Phosphorus removal from broth and bio-accumulation in cells by selected bacterial strains from As^{V} enriched broth after 14 days of incubation

Bacterial strains	Treatments	Phosphorus Removal from broth (mg L^{-1})	Phosphorus Bio-accumulation in cells (µg gm^{-1})
Citrobacter sp. strain BC-1	P_oAs_{10}	--	Negligible
	$P_{10}As_{10}$	3.5	3.1
	$P_{15}As_{10}$	4.9	4.3
	P_0As_{15}	--	Negligible
	$P_{10}As_{15}$	3.7	3.15
	$P_{15}As_{15}$	5.0	4.5
	Mean	4.3	3.8
Pseudomonas sp. strain BC-1	P_oAs_{10}	--	Negligible
	$P_{10}As_{10}$	3.15	2.8
	$P_{15}As_{10}$	4.35	4.05
	P_0As_{15}	--	Negligible
	$P_{10}As_{15}$	3.2	2.85
	$P_{15}As_{15}$	4.38	3.9
	Mean	3.7	3.4
Bacteria (B)	SEm(±)	0.055	0.046
	CD (P=0.05)	NS	NS
Treatments (T)	SEm(±)	0.077	0.065
	CD (P=0.05)	1.406	1.31
(B ×T)	SEm(±)	0.110	0.092
	CD (P=0.05)	NS	NS

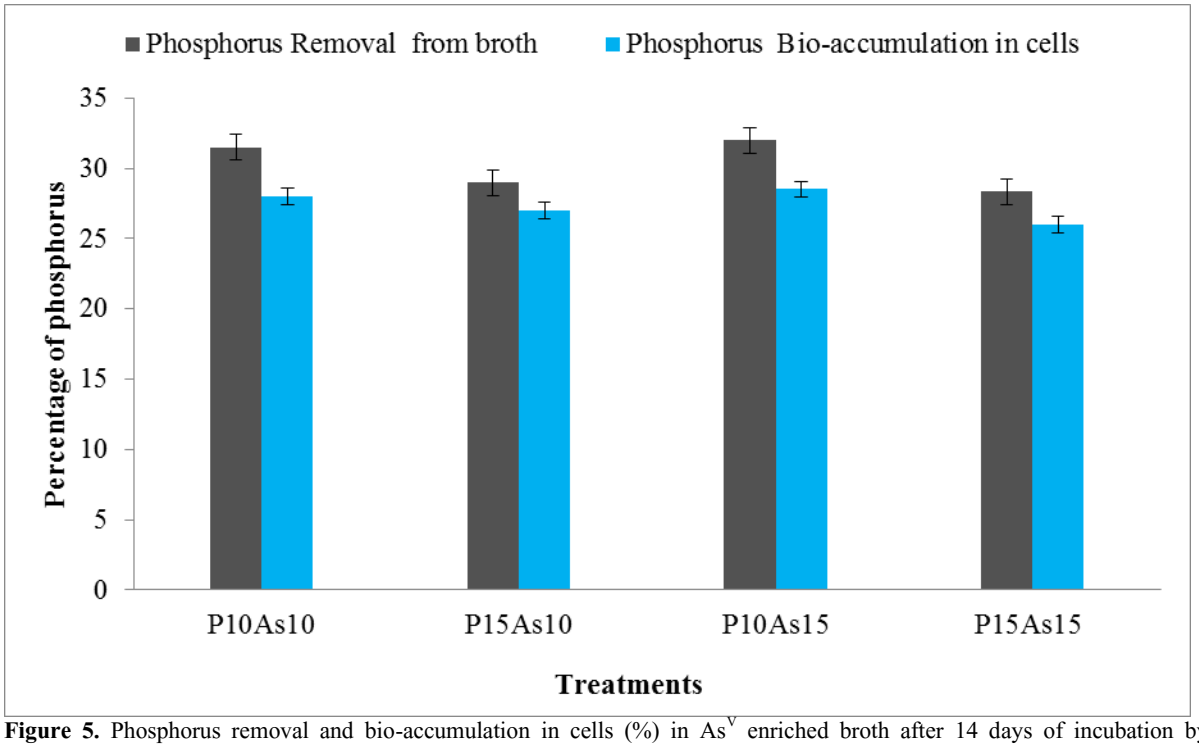

Figure 5. Phosphorus removal and bio-accumulation in cells (%) in As^{V} enriched broth after 14 days of incubation by *Pseudomonas* sp. strain BC-1

Wolfe-Simon and coworkers revealed an isolate, strain GFAJ-1, which was able to substitute arsenic for phosphorus. In our study it was found that both the bacterial strain increased the phosphorus removal and bioaccumulation. Das *et al.* 2016 reported that the increase in phosphate-extractable As in solid phase with concomitant rise in As in aqueous phase over the course of incubation further attested to the importance of reductive dissolution in promoting As release**.** Beltrano *et al.* 2013 revealed that arsenic toxicity symptoms occurred at relatively low As concentration in the plants, probably due to the low phosphorus nutritional status.

4. Conclusion

Magnitude of arsenic contamination in ground water in a sizeable area of West Bengal, India is alarming. Not only in the drinking water, accumulation of arsenic through underground irrigation water and there after intake of it through food chain is a serious concern. The bacterial inoculants *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 efficiently removed arsenic from As^{v} enriched broth after 14 days of incubation. Therefore, these two promising bacterial strains *Citrobacter sp.* strain BC-1 and *Pseudomonas sp.* strain BC-1 can be used in future to ameliorate soil arsenic.

5. References

Anna P Z. Cheng, Deeb M, Peter M G, Richard K S and M. Mark (2018). Accumulation of arsenic and lead in garden-grown vegetables: Factors and mitigation strategies. *Science of the Total Environment* 640: 273–283.

Attanayake C P, Hettiarachchi G M, Martin S, G M. Pierzynski (2015). Potential bioavailability of lead, arsenic, and polycyclic aromatic hydrocarbons in compost-amended urban soils. *Journal of Environmental Quality* 44: 930–944.

Beltrano J, Ruscitti M, Arango M C and M. Ronco (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. *Journal of Soil Science and Plant Nutrition* 13: 123-141.

Biswas T. (2009). Studies on possible mitigation option of soil arsenic through bacterial transformation. PhD. thesis submitted to Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal in the Department of Agricultural Chemistry and Soil Science.

Bradham K D, Diamond G L, Burgess M, Juhasz A, Klotzbach J M, Maddaloni M, Nelson C, Scheckel K, Serda S M, Stifelman M and D J. Thomas (2018). *In vivo* and *in vitro* methods for evaluating soil arsenic bioavailability: relevant to human health risk assessment. *Journal of Toxicology and Environmental Health, Part B* 21: 83–114.

Cheng Z, Paltseva A, Li I, Morin T, Huot H, Egendorf S, Su Z, Yolanda R, Singh K, Lee L, Grinshtein M, Liu Y, Green K K, Wai W, Wazed B and R. Shaw (2015). Trace metal contamination in New York City garden soils. *Soil Science* 180: 167-174.

Das I, Ghosh K, Das D K and S K. Sanyal (2013). Assessment of arsenic toxicity in rice plants in areas of West Bengal. *Chemical Speciation and Bioavailability* 25: 201–208.Das S, Chia-Chuan L, Jiin-Shuh J, Chuan-Chun L and Y. Huai-Jen (2016). Effects of microbially induced transformations and shift in bacterial community on arsenic mobility in arsenic-rich deep aquifer sediments. *Journal of Hazardous Material* 310: 11-19.

Eton E and Codling. (2013). Phosphorus and Arsenic Uptake by Corn, Wheat and Soybean from Broiler Litter Ash and Egg Layer Manure Ash. *Journal of Plant Nutrition* 36: 1083–1101.

Joshi D N, Patel J S, Flora S J S and K. Kalia (2008). Arsenic accumulation by Pseudomonas stutzeri and its response to some thiol chelators. *Environmental Health and Prevention Medicine* 13: 257–263

Majumder A, Kallol B, Kole S C and S. Ghosh (2013). Efficacy of indigenous soil microbes in arsenic mitigation from contaminated alluvial soil of India. *Environmental Science and Pollution Research* DOI 10.1007/s11356-013-1560-x.

Mellem J, Bijnath H and B. Odhav (2012). Bioaccumulation of Cr, Hg, As, Pb, Cu and Ni with the ability for hyperaccumulation by *Amaranthus dublis*. *African Journal of Agricultural Research* 7: 591–596.

Mumford AC, Barringer J L, Benzel W M, Reily P A and L Y. Young (2012). Microbial transformations of arsenic: mobilization from glauconitic sediments to water. *Water Research* 46: 2859-2868.

Nemanja B, Sandra S, Zoran B, Milan B, Dragan M, Rada P and C. Aleksandar (2015). Arsenic in Agricultural Soils of a Historically Mined and Industrial Region of Southern Serbia and Northern Kosovo: Bioavailability and Uptake by Plants Species *Zea mays* L. and *Solanum tuberosum* L. *Soil and Sediment Contamination* 24: 656–674.

Selvankumar T, Radhika R, Mythili R, Arunprakash S, Srinivasan P, Govarthanan M, Kim. Hyunook (2017). Isolation, identification and characterization of arsenic transforming exogenous endophytic *Citrobacter* sp. RPT from roots of *Pteris vittata*. *3 Biotech* 7: 264.

Selvaraj K, Sevugaperumal R and V. Ramasubramanian (2013). Phytoremediation of arsenic chloride by Indian mustard (*Brassica juncea*). *Indian Journal of Fundamental and Applied Life Science* 3: 184–191.

Sheik C S, Mitchell T W, Rizvi F Z, Rehman Y, Faisal M, Hasnain S, McInerney M Z and L R. Krumholz (2012). Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PLoS ONE* 7(6): e40059.

Srivastava S, Verma P C, Singh A, Mishra M, Singh N and Sharma N and Singh N. (2012). Isolation and characterization of *Staphylococcus* sp. strain NBRIEAG-8 from arsenic contaminated site of west Bengal. *Applied Microbiology and Biotechnology* 95(5): 1275–1291.

Tamaki S and J. Frankenberger (1992). Environmental biochemistry of arsenic. *Review of Environmental Contamination and Toxicology* 124: 79-110.

Wolfe-Simon F, Blum J S, Kulp T R, Gordon G W, Hoeft S E, Pett-Ridge J, Stolz J F, Webb S M, Weber P K, Davies P C, Anbar A D and R S. Oremland (2011). A bacterium that can grow by using arsenic instead of phosphorus. *Science* 332(6034): 1163-6.

Xiu W, Guo H, Liu Q, Liu Z, Zou Y and B. Zhang (2015). Arsenic Removal and Transformation by *Pseudomonas* sp. Strain GE-1-Induced Ferrihydrite: Co-precipitation Versus Adsorption. *Water Air and Soil Pollution* 226: 167. DOI 10.1007/s11270-015-2408-4.

Xi-Xiang Y, Wang L H, Bai R, Huang H and S. Guo-Xin (2012). Accumulation and Transformation of Arsenic in the Blue-Green Alga *Synechocysis* sp. PCC6803. *Water Air and Soil Pollution* 223: 1183–1190.

Young C C, Akinori N, Kweon J and K. Shintaro (2012). Isolation and characterization of an arsenate-reducing bacterium a 1 nd its application for arsenic extraction from contaminated soil. *Journal of Industrial Microbiology & Biotechnology* 39(1): 37-44