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Delineation of Soil Arsenic and Observe Arsenic Tolerance Limit of Bacteria Isolated from Nadia, West Bengal

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ABSTRACT

Background: Arsenic, a carcinogenic metalloid polluting terrestrial and aquatic ecosystem throughout the Globe. Not only human or animals, microbes like bacteria or fungi also affected by arsenic toxicity and in high arsenic (As) contaminated pockets, crop productivity decreases due to restricted proliferation of beneficial microbes.

Methods: To fulfil the objectives of present study, soil samples were collected from four different arsenic affected blocks of Nadia district in the November 2020, which are analysed for arsenic content and bacterial count apart from physicochemical or nutrient status in Directorate of research, Bidhan Chandra Krishi Viswavidyalaya (BCKV) Kalyani, WB.

Result: Results revealed that plant available (3.28 mg kg^{-1}) and total arsenic content (16.46 mg kg^{-1}) was highest at Chakda block with a positive significant corelation (r=0.88) between plant available and total arsenic. Available arsenic showed a negative regression curve with bacterial colony forming unit (cfu), which was polynomial in nature. Tolerance limit of bacteria was observed by adding As_2O_3 salt in bacterial broth in varied amount (100-5000 ppm), where bacteria showed a decreasing population with increasing As content in both cfu count and optical density (OD) test with a R^2 value 0.92. Experiment results specify that bacteria isolated from highly As contaminated soil showed more resistance to As toxicity, might be due to exclusion or natural selection mechanism, while bacteria isolated from less contaminated soil died due to enzymatic disruption and genotoxicity.

Key words: Arsenic toxicity, Bacteria, Reduce growth, Soil available arsenic, Tolerance limit.

INTRODUCTION

Arsenic, a highly toxic carcinogenic substance with diverse chemical properties was discovered by a German alchemist Alberts Magnas in1250 (Rahaman *et al.,* 2020). Basically, it is a metalloid that has raised serious environmental and human health concerns recently (Bag *et al.*, 2019). Most abundant forms of arsenic in the environment are As^{+3} and As^{+5}, of which As^{+3} is more toxic and relatively more mobile than As^{+5} (Jones, 2007). Among inorganic forms under reduced condition, As^{+3} is the dominant form, while As^{+5}, is prevalent in oxygenated environment and they can change their valence states depending on soil pH and redox conditions. The toxicity pattern of different forms and oxidation states of Arsenic is TMA (-3)>MMA(3) >DMA(3) >As^{+3}>> As^{+5}>MMA(5) >DMA(5) (Gong *et al*., 2001).

Arsenic enters into terrestrial and aquatic ecosystem through a combination of geological and anthropogenic processes. Arsenic contamination is reported in over 70 countries throughout the World in which, 20 countries are severely affected including Bangladesh, India, China, Brazil, Chili, United States of America (Sanyal, 2019). In the severity of Arsenic toxicity, Bangladesh ranks first followed by India. Among the 28 states of India, lower Gangetic plane especially West Bengal is most severely affected by arsenic contamination (Rahman *et al.*, 2001). Analysing inner scenario Sanyal, (2019) reported arsenic contamination in ground water of West Bengal is distributed over 3417 villages in 111 blocks of 12 districts. Among these 12 districts, Malda, Murshidabad, North 24-parganas, South 24-parganas, Nadia, Howrah, Hooghly and Kolkata have Arsenic levels above 300 µg l^{-1}, which is much higher than the safe limits of Arsenic in drinking water stipulated as per WHO guidelines (50 µg L^{-1}).

Not only for drinking water, arsenic became even a threat for irrigation water also, because of continuous use of arsenic contaminated irrigation water may enhance its concentration in edible parts of crops (Khanam *et al*., 2021).

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Latter consequences are little bit horrifying, because it will certainly enter in our food chain through edible parts of plant or water and ultimately increase the content of arsenic in our body leading to different types of health issues like keratosis, melanosis, backfoot and ultimately cancer (Rahman *et al.,* 2001).

On the other hand, one of the most important components of soil is microorganisms, which make it living and dynamic (Jacoby *et al.*, 2017). Microorganisms primarily enhance the plant growth by decomposing organic matter, increasing the availability of nutrients and in so many ways. Among different types of microorganisms, bacteria is most dominated one followed by fungi in soil (Rousk *et al.*, 2010). It is observed that high concentration of arsenic may affect microbial population, whereas reverse result also found, where arsenic concentration is reduced by native microbial population. Researches also proved that among all these microorganisms, bacteria preformed best in reducing As content of soil and plant. Present investigation on study on arsenic tolerance level of bacteria was conducted to know the tolerance level of soil bacteria upto which it can withstand As toxicity.

MATERIALS AND METHODS

Study area

Nadia district of West Bengal is recently considered as the highest arsenic affected zone of our country (Fig 1). Four different blocks *viz*., Haringhata, Krishnanagar, Ranaghat, Chakda of Nadia district, WB, India had been selected for the present study Surface soil samples (0 to 15 cm) taken from five different sites of each four blocks. Soil samples were collected from low land rice fields with GPS.

Soil samples collected from the experimental sites, properly levelled and brought into the directorate of research laboratory, Bidhan Chandra Krishi Viswavidyalaya (BCKV). Half portion of each soil samples was stored in the refrigerator for microbial work and other half were air-dried, ground to pass through a 2-mm sieve, stored in polythene packets for analysis,. Counting of bacterial population was carried out just after collection of soil samples.

Physical and chemical analysis of soil samples

Some physical properties like particle size distribution, textural class, bulk density and water holding capacity were.

The soil pH and EC were determined in soil-water suspension (soil: water :: 1: 2.5) (Jackson, 1973).

Oxidizable soil organic carbon (OC) was following the Walkley-Black wet digestion method (Walkley and Black, 1934). The available nitrogen content of soil was determined through modified Kjeldhal method (Subbiah and Asija, 1956). Available phosphorus by Olsen method. Available potassium by neutral normal Amonium acetate (soil: extractent :: 1:10) (Brown and Warncke 1988).

Soils are extracted with Olsen reagent (0.5M $NaHCO_3$, pH 8.5; Soil: extractant : : 1: 10) and filtered for estimating available As in soil (Khanam *et al.*, 2021). HNO_3 acid digestion method (Rahman *et al*., 2007) was followed to estimate total As content in soil.

Bacterial population study

Isolation of Bactria from soil is done by Thronton's Agar media (Thornton,1922).

Serial dilution and pore plate technique (Sanders, 2012) is followed for isolating bacteria from soil. Bacterial growth was determined by the turbidity of bacterial broth in spectrophotometer for their turbidity in 600nm wavelength. Then after five days plating was done with Throntons ager medium for counting of colony forming unit.

RESULTS AND DISCUSSION

Physical properties of the soil

Results (Table 1) of mechanical analysis revealed that all the soils collected from Nadia district have high clay and textural class varies from silty clay to sandy loam. Soils collected from Chakda have highest clay (49.8%) but lowest sand (3.5%),whereas soils collected from Haringhata were high in sand (61.2%) but poor in clay (18.2%). Similar results were found by Bhattacharya *et al.* (2009), which showed high clay content (70%) in Chakda block making it suitable for rice cultivation. Bulk density (BD) was found highest in case of Haringhata soils (1.49 g cm^{-3}) may be due to high

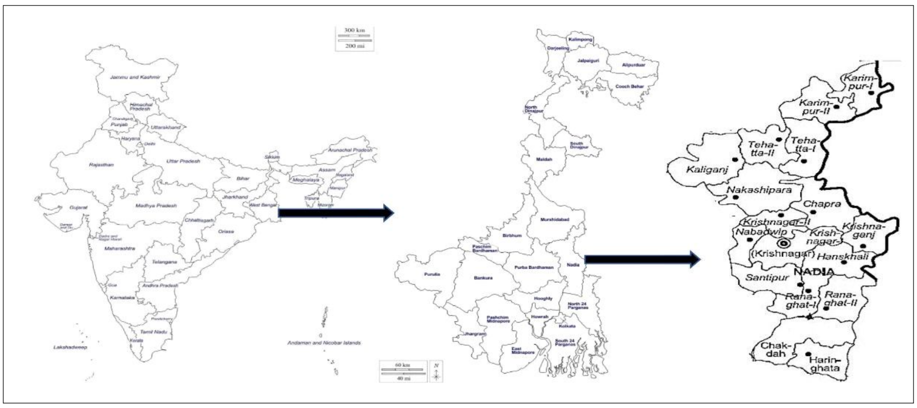

Fig 1: Sampling areas in Nadia district of West Bengal, India.

sand content, while lowest BD was recorded in the soils of Chakda block (1.23 g $cm^{3\ -1}$) probably due to higher clay content and diversified farming (Mashiane *et al.,* 2023). water holding capacity (WHC) varies between 48% to 38.3% and found highest in Chakda block due to high clay content (Table 2).

Chemical properties of soil

The chemical properties of the experimental soils have been analysed and tabulated in Table 3. Chemical properties *viz*., pH, EC, oxidizable organic carbon, available Nitrogen, Phosphorus and Potassium, which may controls the availability of arsenic, have been analysed following standard procedure. pH of soil samples varied from 6.34 to 7.53, which is more or less in neutral range, which aligns with results of Ghosh *et al*. (2021). According to Borah *et al.* (2020) pH of soil in this lower Gangetic plain is neural (6.90), which also supports the magnitude of pH in the current research. Electrical conductivity of soil varies between 0.14 to 0.36 dS m^{-1}, which is much lower than critical value (4dS m^{-1}) and similar to Ghosh *et al.* (2021) finding showing non-saline property of soil. The mean oxidizable organic carbon (OC) content was 0.48%, which is low to medium, with a range of 0.43 to 0.56%. Nitrogen content varies from 147 to 222 Kg ha^{-1}, showing low content of nitrogen in soil and phosphorus content varied from 19 to 36 Kg ha^{-1}, which represents the magnitude of P is usually high. Potassium content in the tested soils was medium, which ranged from 133 to 195 kg ha^{-1} with average of 161.20 kg ha^{-1}. Available N, P, K content of this area was similar to the findings of Mondal *et al*. (2022).

Olsen extractable (available) and total As in soil

Olsen extractable or available arsenic directly affect plants and microorganisms (Brandt *et al.,* 2023). Soil collected from arsenic affected belts of Nadia district showed high Olsen extractable arsenic content (Fig 2) with mean value 1.89±0.62 mg kg^{-1}. Mishra *et al.* (2022) reported that mean concentration of available. As in Nadia district is 1.45 mg kg^{-1}, which is similar to the current study. Among four different blocks, Chakda showed highest available As ranged from 1.74 to 3.28 mg kg^{-1} with a mean value 2.57±0.73 mg kg^{-1},

Table 1: Location of soil sampling sites with soil chemical properties.

Block	Sample number	Latitude (°N)	Longitude (°E)	pH	EC (dS/m)	Oxidizable organic carbon (%)	Available N Kg ha^{-1}	Available P Kg ha^{-1}	Available K Kg ha^{-1}
Haringhata	H_1	22.9725	88.5684	6.80	0.33	0.46	168	21	151
	H_2	22.9787	88.5525	7.23	0.31	0.51	195	23	157
	H_3	22.9397	88.5758	7.38	0.34	0.48	181	22	152
	H_4	22.9760	88.5647	7.15	0.36	0.50	184	25	155
	H_5	22.9275	88.5748	6.92	0.28	0.53	201	31	158
Chakda	C_1	23.0205	88.5832	7.21	0.32	0.54	209	33	188
	C_2	23.0258	88.5983	6.51	0.27	0.61	222	36	198
	C_3	23.0158	88.5814	7.53	0.31	0.58	215	34	185
	C_4	23.0002	88.5778	7.42	0.35	0.56	218	31	192
	C_5	23.0140	88.5634	7.36	0.33	0.63	235	41	195
Ranaghat	R_1	23.1934	88.5105	7.10	0.26	0.50	175	30	138
	R_2	23.2051	88.5273	6.87	0.25	0.51	180	26	135
	R_3	23.2060	88.5339	7.16	0.23	0.56	212	31	146
	R_4	23.2020	88.5408	6.34	0.14	0.49	173	29	133
	R_5	23.1944	88.4912	6.53	0.21	0.54	206	32	139
Krishnanagar	K_1	23.3921	88.4986	6.81	0.30	0.43	166	24	166
	K_2	23.3341	88.4628	6.73	0.25	0.40	160	21	172
	K_3	23.3556	88.4745	6.56	0.24	0.37	147	19	161
	K_4	23.3759	88.4850	6.75	0.31	0.50	178	26	157
	K_5	23.4001	88.4620	7.20	0.36	0.53	202	28	164

Table 2: Physical characteristics of the experimental soil.

Blo block	Particle size distribution (%)			Texture	Bulk density	WHC
	Sand	Silt	Clay		(g $cm^{3\ -1}$)	(%)
Chakda	3.50	46.70	49.80	Silty clay	1.23	48.00
Haringhata	61.20	20.30	18.50	Sandy loam	1.49	38.30
Ranaghat	41.00	28.00	31.00	Silty clay loam	1.32	46.00
Krishnanagar	46.00	19.00	35.00	Silty loam	1.41	45.70

which is similar to the findings of Sinha and Bhattacharyya, (2012) whereas, lowest magnitude recorded in Krishnagar with average As content 1.18±0.55 mg kg^{-1}. Total soil arsenic content in soil shows similar pattern (Fig 3) , where highest As content recorded in Chakda (13.34±3.63 mg kg^{-1}), while lowest magnitude was recorded in Krishnanagar (4.20±2.01 mg kg^{-1}). Total As concentration in soil generally varies from 0.1 to 10 mg kg^{-1} but Shrivastava *et al*. (2014) recorded average magnitude 9.5 mg kg^{-1} for the specific area, which is quite similar to that of present finding.

Corelation of As with different parameters

Values from Table 3 is a significant positive correlation between soil available As and pH (r^2=0.708**). According to Singh and Srivastava (2020) bio-availability and mobility of arsenic depends on soil pH. Results revealed that (Table 4) there was positive corelations between Olsen extractable As and EC (r^2=0.318), OC (r^2=0.301), N (r^2=0.296), P (r^2=0.205), K (r^2=0.359) though these values are not statistically significant. Total as in soil showed positive and significant corelation with soil pH (r^2=0.575) and Olsen extractable As (r^2=0.875**) content at significance level $p<0.01$. Strong positive corelation also found between total As and organic carbon content in soil (r^2=0.502) with available N content (r^2=0.463) also but at 0.05 level of significance ($p<0.05$). Dobran and Zagury (2006) also found similar types of correlation between As content and organic carbon present in soil. Corelation coefficient (r^2) between total soil As and EC, P and K observed positive but not significant with correlation coefficient (r^2) magnitudes 0.356, 0.351 and 0.313 respectively.

Available arsenic content and bacterial population of soil and their relationship

Available arsenic content in soil and bacterial cfu population has been represented in Fig 4 and 5. Bacterial population has been represented by colony forming units (cfu) of bacteria and the readings are taken after 3 days of incubation. Highest bacterial population of incubation was observed in K_5 soil sample of Krishnagar block (44×10^5/g of dry soil), which might be due to low available As ultimately not hampering the natural proliferation of bacteria. Lowest bacterial population recorded in soil sample C_5 of Chakda (12×10^5 g^{-1} of dry soil) , where high contamination of arsenic of 3.275 mg kg^{-1}. Similar type of result shown by Majumder *et al.* (2013), where they have found decrease in bacterial population due to high nutrient content. Results also showed that bacterial population after 3 days of incubation and Olsen arsenic content was negatively correlated. A decreasing trend in bacterial population with increasing

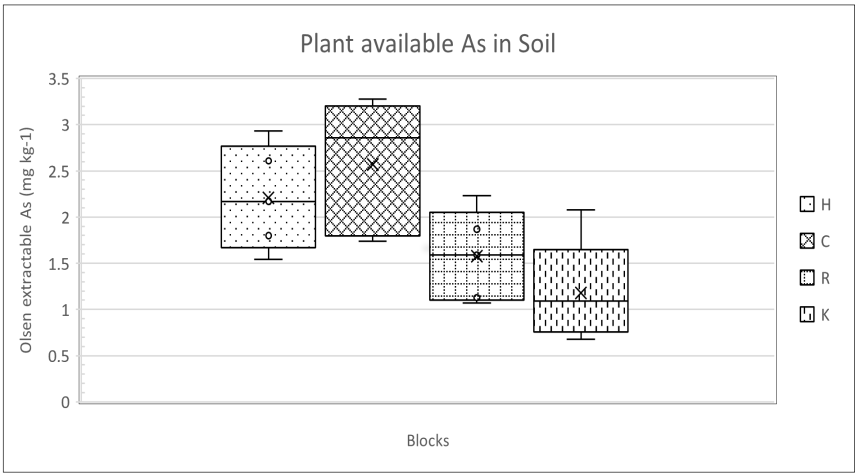

Fig 2: Available soil arsenic distribution in four blocks of Nadia, West Bengal.

Table 3: Correlation between different chemical properties.

Correlation	pH	EC	OC	N	P	K	Olsen ex. As	Total As
pH	1							
EC	0.708**	1						
OC	0.413	0.194	1					
N	0.485*	0.266	0.958**	1				
P	0.245	-0.013	0.903**	0.874**	1			
K	0.363	0.495*	0.418	0.548*	0.462*	1		
Olsen ex. As	0.508*	0.318	0.301	0.296	0.205	0.359	1	
Total As	0.575**	0.356	0.502*	0.463*	0.351	0.313	0.875**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

inherent arsenic loading was observed and equation, which shows relation between Olsen extractable As content and bacterial growth y = $-4.983x_3 + 32.352x_2 - 68.309x + 56.545$ with r^2 value 0.62 (Fig 4), where x is available As content in soil. The equation represents a third order polynomial or a cubical relationship of Olsen extractable As with bacterial growth. Hassan *et al.* (2022) also found similar type of polynomial correlation between bacterial growth and heavy metal concentration. From lower As to comparatively higher As values, bacterial growth drastically reduces due to high toxic effects of As, after that with higher As values, the bacterial population remains similar or constant might be due to development of arsenic resistance in bacteria. Highest concentration of As shows further gradual decrease of bacterial count signifying that registrant bacteria also cannot withstand such amount of As toxicity. Arsenic is a toxic metalloid that can inhibit the microbial growth, hence resulting low microbial population. A decrease in microbial population with increase in As content has been recorded by Van Zwieten *et al.* (2003). Decrease in bacterial population with increasing As content in soil might be due to enzymatic inhibition, disruption of cellular respiration, DNA rupture and toxin exclusion mechanism of bacteria (Pal *et al.,* 2022).

Tolerance level of bacteria

Bacterial broth treated with arsenic salt (As_2O_3) and kept for five days in incubation chamber and then OD value of solutions were taken under spectrophotometer (λ=600 nm). In lower As concentration of 100 ppm, the OD values were highest in bacterial broth isolated from soils of Krishnagar block *i.e.*, K_1 gave the highest (1.427), which signifies that broth was totally turbid due to high bacterial growth. But for all soils, OD value sharply decreases with increase in As concentration in broth. At 2500 ppm arsenic broth for most of the soils except Chakda become transparent as the bacterial growth stops due to high as toxicity.

The OD value ranges from 0.036 to 0.018, which signifies there is no bacterial growth in the broth. In case of soils collected from Chakda block, OD values were low at lower arsenic concentration than Ranaghat and Krishnanagar but were not sharply decrease with increase in arsenic concentration. Results showed that for C_4 and C_5 sample the OD values were 0.228 and 0.211 respectively in 4000 ppm arsenic solution which signifies the bacteria in C_4 and C_5 sample can tolerate As concentration up to 4000 ppm. Regression equation between As content and OD value was y = -0.522ln(x) + 1.4292 with high negative

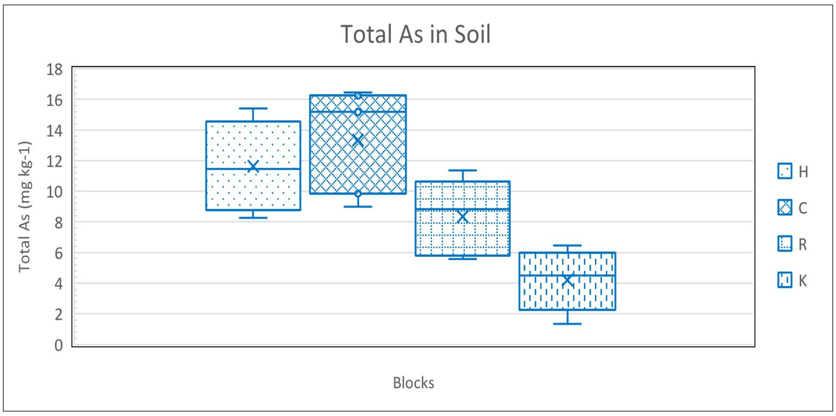

Fig 3: Total soil arsenic distribution in four different blocks of Nadia.

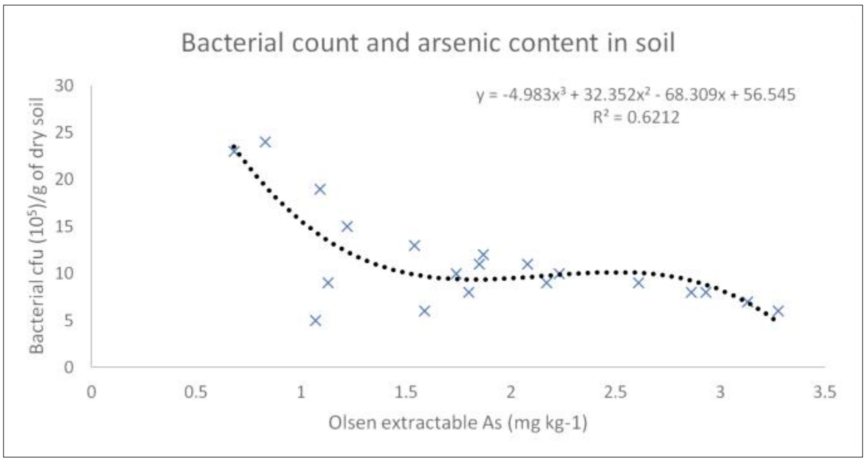

Fig 4: Relation between available As in soil and bacterial cfu count (mg kg^{-1}).

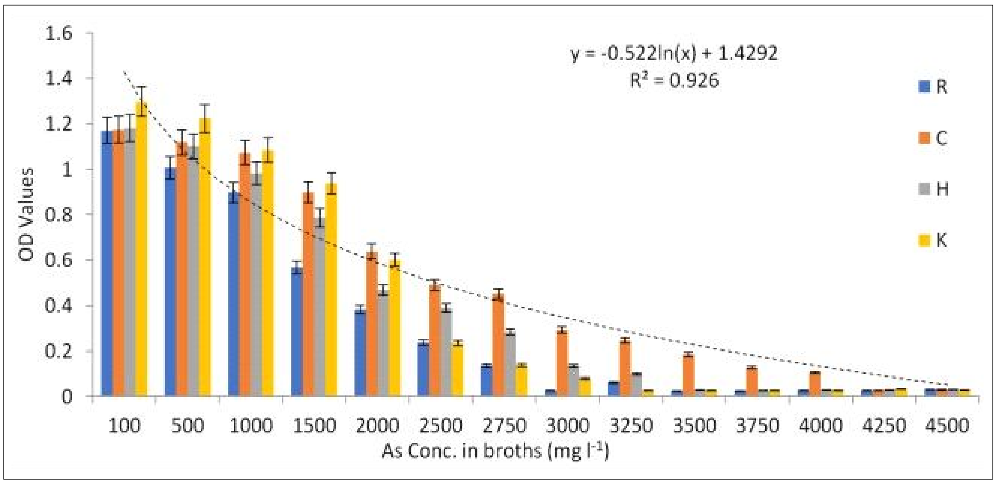

Fig 5: Tolerance level of bacteria against variable as concentration in broth.

regression coefficient (R^2 = 0.926), where y is the OD value measured in spectrophotometer and x represents As content in broth (Fig 5). It was observed that bacterial population decreases in a logarithmic rate with increasing As content which might be the of cellular toxicity due to higher concentration of arsenic (Mairaj *et al*., 2022). Tolerance level of bacteria is reported 10-100 µ mols for arsenite which is similar to the current study (Rehman *et al.,* 2010).

CONCLUSION

Arsenic content both total and available recorded higher than permissible limit in most of the sites, though the highest concentration was recorded in the soils of Chakda district. It can be concluded that soil total arsenic has a direct positive correlation with Olsen extractable arsenic at 0.01 level of significance but with other physicochemical parameters no such strong relation was found. Number of bacterial showed a third order polynomial regression with soil available As having R^2 value -0.93. Deta further revealed that bacterial growth mostly restricted beyond 2500 mgl^{-1} As concentration in broth due to enzymatic inhibition and genotoxicity though the bacteria isolated from Chakda block showed As tolerance upto 4000 mgl^{-1} or ppm. Present study concluded that due to continuous exposure of soil arsenic, bacteria found in the Chakda block become somewhat registrant to As through natural selection or exclusion mechanism. Bacteria present in the soils of other blocks become suspectable to arsenic as limited exposure to this toxin and the growth was drastically reduced with increase in As content of the broth. Although after a certain concentration all type of bacteria is killed by arsenic toxicity.

Conflict of interest

This statement declares whether the authors do not have any conflict of interest (financial or personal) for the research.

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