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Effect of bentonite on arsenic uptake by beet leaf cultivar Pusa Bharti grown on contaminated soil

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ABSTRACT

Greenhouse experiments were conducted to assess the effect of bentonite amendment on arsenic uptake by beet leaf (*Beta vulgaris* L. bar. *bengalensis* Roxb.) cultivar Pusa Bharti. Application of bentonite to the contaminated soil increased biomass yield from 0.24 (control) to 0.44 g pot^{-1} @ 0.25% bentonite amended soil (T_3) at first harvest and 0.65 g pot^{-1} @ 0.5% bentonite amended soil (T_4) after second harvest over the two years. The bio-accumulation factor (%) of arsenic varied from 13.60 in control (T_1) to 3.77 (T_3) in soil amended with 0.25% bentonite at first harvest and reduced by 62.4% in the second harvest. The hazard quotient was significantly reduced below 0.50 because of 0.25 and 0.50% bentonite application after first and second harvest. The arsenic concentration in plants was reduced to 0.49 from 1.77 mg kg^{-1} in first harvest, while the values were 0.50 and 1.39 mg kg^{-1} in 0.5% amended and control soil, respectively after second harvest. Importantly, clay amendments effectively reduced the labile arsenic content up to 54.8 and 58.5% in soil during first and second harvest, respectively. Soil pH was raised significantly only in 0.5% clay amended soil (7.89) as compared to control (6.70) after complete crop harvest. The effect of bentonite application @ 0.25 and 0.5% was statistically at par in most of the parameters. Hence, it may be recommended that application of bentonite @ 0.25% may be useful to reduce the arsenic uptake by beet leaf as well as its immobilization in polluted soil.

Key words: Arsenic, bentonite, hazard quotient, immobilization, beet leaf.

INTRODUCTION

Vegetable cultivation has become important to ensure the nutritional security of ever growing population in the world. At present, it is very common in the marginal lands of developing countries of the world. On the other hand, worldwide 170 million people are exposed to arsenic (As) contamination. The contamination of water occurs due to the dissolution of minerals like arsenopyrites from parent materials, geochemical reactions, and biological activities or from anthropogenic sources such as the leaching of manmade arsenic compounds from smelting of metal ores, and wood preservatives (Shevade and Ford, 11). Arsenic contamination of groundwater and soil caused by those materials pose a great threat to vegetable cultivation by application of contaminated irrigation water. Consumption of arsenic contaminated drinking water may cause kidney, urinary tract, liver, skin and rectum cancers in humans. Non-carcinogenic diseases related to arsenic exposure are hypertension, diabetes mellitus, cerebrovascular and cardiovascular systems, and dysfunction of respiratory system (Thomas *et al.*, 13). Thus, arsenic contaminated soils affect the food quality and safety by risk of biomagnifications and bioaccumulation in human food chain because it is poorly biodegradable (Mihaltan *et al.*, 7).

Therefore, a strong immobilization technique is required to reduce the transfer of arsenic from soil to crop and crop to human food chain. Clay minerals are one of the adsorbents of arsenic, which act as good amendment due to its ease availability, non-toxic, cost effective and large specific surface area (McBride and Martinez, 5; Peremolov *et al.*, 9). The probable mechanism of arsenic immobilization in soil is through adsorption. Application of such clay minerals to soil for arsenic alleviation, health risk assessment of arsenic intake through vegetables grown on contaminated soil is an underexplored area of research. In view of that, bentonite clay mineral was selected as immobilizing agent for remediation of arsenic contaminated soil.

MATERIALS AND METHODS

Smectite clay mineral in the form of bentonite was evaluated to check the bioavailability of arsenic from soil to crop and arsenic uptake by beet leaf. Bentonite clays were purchased from S D Fine-Chem Ltd., Mumbai, India. The arsenic contaminated soil used for pot experiment was collected (0-15 cm depth, order: Inceptisols) from Mitrapur, West Bengal, India. The soil is slightly acidic to neutral (pH = 6.49) having electrical conductivity (EC) 0.26 dS m^{-1}, organic

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carbon (OC) 4.50 g kg^{-1}, total soil arsenic content 14.10 mg kg^{-1} and available arsenic content 3.60 mg kg^{-1}. Pot culture experiments were conducted during winter 2016 and 2017 with beet leaf cultivar Pusa Bharti as a test crop and to study the residual effect of clay amendment, under greenhouse conditions. Air-dried, grounded, 2 mm sieved 4 kg of contaminated soil was used in each pot. Bentonite clay mineral was applied to the soil at 4 levels (T_1 = control (0%) where no bentonite was applied, T_2 = 0.125%, T_3 = 0.25%, and T_4 = 0.50%) with three replications. Recommended dose of fertilizer 240:120:120 mg pot^{-1} NPK was added to soil. Half dose of nitrogen and full dose of phosphorus and potassium was applied as basal and remaining nitrogen was added 30 days after sowing. The plants were thinned to 4 seedlings per pot after germination. 60% water holding capacity of the soil was maintained during the course of study. Two cuttings were taken at 30 day interval and concentration of arsenic in the digested samples was determined with Inductively Coupled Plasma Mass spectroscopy (ICP-MS). Available soil arsenic was extracted by Olsen's reagent (0.5 *M* $NaHCO_3$, pH = 8.5) and total soil arsenic was determined by HF (Hydrofluoric acid) digestion method (McLaren *et al.*, 6) and arsenic in the filtrate was analyzed by ICP-MS. The non-carcinogenic risk of consumption of beet leaf grown on arsenic contaminated soil amended with bentonite was characterized by hazard quotient (HQ). Hazard quotient is defined as the ratio of the average daily dose (ADD; mg^{-1} kg^{-1} day^{-1}) of As to their reference dose (RfD; mg^{-1} kg^{-1} day^{-1}) (Kumararaja *et al.*, 3). For arsenic, RfD is 0.0003 mg kg^{-1} body weight day^{-1} (Ramirez-Andreotta *et al.*, 10). Statistical analysis of data over the two years was done using pool analysis.

RESULTS AND DISCUSSION

Beet leaf has high nutritional value as a leafy vegetable. The leaf and shoot biomass dry weight usually show plants' capability to resist the adverse environmental impact like temperature stress, moisture stress, heavy metal contamination *etc*. In the present study, the amount of clay mineral had a significant contribution to the high biomass yield of beet leaf grown in an arsenic contaminated soil. Plant dry biomass yield increased with increasing the amount of clay application. After first harvest at 30 DAS (days after sowing), highest yield was obtained under T_3 (@ 0.25% bentonite ameneded soil) and T_4 treatments (@ 0.5% bentonite amended soil) after second harvest (30 DAS) over the two years as compared to the control T_1 (contaminated soil). The biomass yield was found to be increased from 0.24 in T_1 (control) to 0.44 g pot^{-1} in T_3 at first harvest over the two years (Table 1). However, at second harvest, the highest biomass yield was increased by 62.5% by application of bentonite @ 0.5% compared to the unamended soil over the two years. However, there was no significant difference in yield was observed between the two experimental years. The higher biomass yield indicated that application of bentonite improved the plant growth by arsenic adsorption/ immobilization in soil. The probable mechanism might be due to increase in alkalinity, which helped to adsorb arsenic through physical adsorption on Si-O and Al-O groups on the edges of clay particles (Su *et al.*, 12; Mar *et al.*, 4).

Bioaccumulation factor is the ratio of metal concentration in plant to that in the soil or the effectiveness of a plant in concentrating the pollutant into aerial part. This parameter assessed the efficiency of bentonite to immobilize arsenic in soil (Table 2). The bioaccumulation factor (%) of arsenic varied from 13.60 in control (T_1) to 6.41 (T_2), 3.77 (T_3), and 4.60 (T_4) in soil ameneded with 0.125, 0.25 and 0.5 % bentonite, respectively at first harvest over the two years. At second harvest, bioaccumulation factor reduced by 34.01% (T_2), 56.7% (T_3), and 62.4% (T_4) over the control (T_1) by application of 0.125, 0.25, and 0.5% bentonite, respectively, pooled over the two years. But there was no significant difference observed in bioaccumulation factor between the two experimental years during both the harvesting times. The results clearly indicated that bioaccumulation factor decreased with increasing the amount of clay addition to the arsenic contaminated soil. Very small bio-accumulation factor (%) of beet leaf had been shown in control as well as bentonite treated plot because the factor was calculated in terms of dry weight basis, which is far more less than the fresh weight. Similar report was also obtained by Gaw *et al*. (1) when they used

Table 1. Effect of different levels of bentonite on beet leaf biomass yield (g pot^{-1}) after 1st and 2nd harvest pooled over two years (2016 and 2017).

Year	Biomass yield 1st harvest	Year	Biomass yield 2nd harvest
1st	0.34	1st	0.50
2nd	0.37	2nd	0.56
$CD_{0.05}$	0.08	$CD_{0.05}$	0.14
Treatment		Treatment	
T_1	0.24	T_1	0.40
T_2	0.35	T_2	0.44
T_3	0.44	T_3	0.61
T_4	0.38	T_4	0.65
$CD_{0.05}$	0.12	$CD_{0.05}$	0.19

Table 2. Effect of different levels of bentonite on bioaccumulation factor (%), hazard quotient and arsenic concentration (mg kg^{-1} dry weight) in beet leaf after 1^{st} and 2^{nd} harvest pooled over two years (2016 and 2017).

Year	Bioaccumulation factor		Hazard quotient		Arsenic concentration	
	1^{st} harvest	2^{nd} harvest	1^{st} harvest	2^{nd} harvest	1^{st} harvest	2^{nd} harvest
1^{st}	7.23	5.68	0.74	0.57	0.92	0.73
2^{nd}	6.96	8.29	0.91	0.62	0.88	0.95
$CD_{0.05}$	3.06	4.82	0.44	0.08	0.35	0.49
Treatment						
T_1	13.60	11.32	1.64	0.98	1.77	1.39
T_2	6.41	7.47	0.75	0.60	0.78	0.90
T_3	3.77	4.90	0.49	0.43	0.49	0.57
T_4	4.60	4.26	0.41	0.39	0.55	0.50
$CD_{0.05}$	1.76	3.05	0.30	0.23	0.22	0.40

lettuce and radish to decontaminate the horticultural soils from sigmaDDT, arsenic and heavy metals like cadmium, lead, and copper. Hence, beet leaf showed good arsenic uptake capacity as a test crop grown in arsenic contaminated soil.

Consumption of arsenic contaminated food materials is one of the important pathways to affect the human being. Along with food intake, it is also possible that incidental ingestion and inhalation of dust containing arsenic may be a significant pathway of exposure (Huq *et al.*, 2). To assess the arsenic immobilization efficiency of bentonite clay mineral, health risk assessment on vegetable consumption from clay amended soil and contaminated soils, hazard quotient was calculated using standard protocol (Kumararaja *et al.*, 3). The results suggested that (Table 2) hazard quotient was significantly reduced from 1.64 in control (T_1) to 0.75 (T_2), 0.49 (T_3), 0.41 (T_4) in clay amended soil when bentonite was applied @ 0.125, 0.25 and 0.5%, respectively, after first harvest over the two years. At second harvest, the hazard quotient was reduced by 36.7, 54.1 and 58.2% over the control when bentonite applied @ 0.125, 0.25 and 0.5%, respectively, over the two years. Although, there was no significant difference in HQ values during the two experimental years at both the harvesting times, however it was clear that HQ values were lower in all the treatments during second harvest. The reduction in hazard quotient on application of bentonite might be due to reduced metal uptake by the beet leaf due to its adsorption/immobilization. Values of HQ equal to or more than 1 indicates that consumption of food materials may be hazardous to human being due to intake of arsenic (Ramirez-Andreotta *et al.*, 10). Consumption of leafy vegetables constitutes only food materials, which contribute to arsenic uptake in human food chain. If other sources of contamination like drinking water, groundwater for irrigation is taken into account, then HQ of 0.50 may be considered as safe limit in risk assessment of contaminated soil. Therefore, according to this limit, the unamended soil as well as 0.125% bentonite treated soil may still be hazardous to human being after first and second harvest. Moreover, the control soil having hazard quotient of 1.64 and 0.98 after first and second harvest, respectively, might be unsafe for growing any vegetable on this soils and need application of amendments for safe and sustainable crop production.

Bioavailability of arsenic depends on its uptake by plants and forms of arsenic. The concentration of arsenic in above ground portion of beet leaf was significantly reduced by bentonite application to the contaminated soil (Table 2). Application of bentonite reduced the arsenic concentration in beet leaf to 0.78 mg kg^{-1} (T_2), 0.49 mg kg^{-1} (T_3) and 0.55 mg kg^{-1} (T_4) from 1.77 mg kg^{-1} (T_1) in control soil at first harvest and the values for second harvest were found to be 1.39 mg kg^{-1} (T_1) in control soil and 0.90 mg kg^{-1} (T_2), 0.57 mg kg^{-1} (T_3), 0.50 mg kg^{-1} (T_4) when bentonite was applied @ 0.125, 0.25 and 0.5%, respectively to experimental soil. However, arsenic concentration in plants in the soils of T_3 and T_4 was statistically at par during both the harvesting time over the two experimental years. Hence, Application of bentonite @ 0.25% to the soil might have served the purpose in practical sense. The bioavailability of arsenic depends on its concentration in soil solution and its rate of release from soil solids. The arsenic concentration in the leafy portion of beet leaf decreased considerably due to immobilization of arsenic in soil after the application of clay amendment to soil.

The labile fraction (available form) of arsenic were significantly reduced over the two years by

Table 3. Effect of different levels of bentonite on available and total arsenic in soil (mg kg^{-1}) after 1st and 2nd harvest, and soil pH (1: 2.5 soil: water; measured after completion of crop cycle) pooled over two years.

Year	Available arsenic in soil after		Total arsenic in soil after		Soil pH after crop cycle
	1st harvest	2nd harvest	1st harvest	2nd harvest	
1st	2.83	2.50	12.63	12.69	7.22
2nd	2.98	2.44	12.47	11.53	7.31
$CD_{0.05}$	0.85	0.67	0.91	2.72	0.26
Treatment					
T_1	4.05	3.67	13.48	12.47	6.70
T_2	3.66	3.14	12.20	12.11	7.23
T_3	2.08	1.62	12.74	12.23	7.18
T_4	1.83	1.52	11.78	11.64	7.89
$CD_{0.05}$	1.32	1.19	1.88	2.03	0.80

bentonite application to the soil after each harvest (Table 3), respectively. Similarly, the labile fraction of arsenic also reduced by 9.6 , 48.6 and 54.8% in T_2, T_3, and T_4, respectively over the control (T_1) after first harvest and the corresponding values are 14.4, 55.8 and 58.5%, respectively over the control after second harvest over the two years. Most importantly, after both the harvesting time, total arsenic content in soil did not change significantly over the two years with bentonite application as compared to control soil (Table 3). This is might be due to the effectiveness of bentonite clays to immobilize arsenic in the solution phase. Similarly, no significant difference in labile and total arsenic content was found in between the two experimental years. Application of bentonite clay minerals reduced the labile fraction of arsenic to a great extent, which might be due to larger surface area of clay minerals and their high adsorption capacity in the solution phase.

Application of bentonite clay minerals also raised the soil pH from 6.70 in control to 7.23, 7.18, and 7.89 in T_2, T_3, and T_4, respectively after the final harvesting of beet leaf but the increase was not statistically significant between all the treatments as compared to control except T_4 (Table 3). Only application of bentonite @ 0.5% could significantly raise the soil pH as compared to control over the two years. Smectite dominant clay minerals like bentonite adsorb maximum arsenic in the pH range of 6 to 8 (Mohapatra *et al.*, 8; Mar *et al.*, 4). The raise in pH may be one of the important mechanism of reduced availability and strong adsorption of arsenic in bentonite amended soil.

Arsenic is held in soil through their parent materials, chemical adsorption on clay, organic matter or may be through biological adsorption. Addition of bentonite increased the chemical sorption of arsenic and reduced its mobility and rate of release in soil as a result of strong adsorption with clay particles. The results showed that the effect of bentonite application @ 0.25 and 0.5% was statistically at par in most of the cases for arsenic immobilization (Table 4). Hence, it may be recommended that application of bentonite @ 0.25% may be useful instead of applying @ 0.50% to the contaminated soil. The method described enables application of bentonite to soil reduced the bioavailability of arsenic and thereby reduced the risk of vegetable consumption grown on contaminated soil.

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Table 4. Statistical ANOVA of pool analysis of different parameters of beet leaf during the year 2016 and 2017.

Parameter	BAF 1st harvest	BAF 2nd harvest	HQ 1st harvest	HQ 2nd harvest	As in plant 1st harvest	As in plant 2nd harvest	Biomass yield 1st harvest	Biomass yield 2nd harvest	As (t) in soil 1st harvest	As (t) in soil 2nd harvest	Available As in soil 1st harvest	Available As in soil 2nd harvest	Soil pH
Year df	1	1	1	1	1	1	1	1	1	1	1	1	1
Treatment df	3	3	3	3	3	3	3	3	3	3	3	3	3
Year × Treatment df	3	3	3	3	3	3	3	3	3	3	3	3	3
Error (a) df	2	2	2	2	2	2	2	2	2	2	2	2	2
Error (b) df	9	9	9	9	9	9	9	9	9	9	9	9	9
Year ss	0.49	40.97	0.16	0.002	0.009	0.284	0.0004	0.006	0.14	8.16	0.131	0.006	0.03
Treatment ss	360.50	185.04	5.72	1.29	6.67	2.98	0.12	0.27	9.75	2.17	22.38	21.1	4.30
Year × Treatment ss	14.09	16.58	0.13	0.01	0.212	0.148	0.004	0.001	3.97	1.22	0.50	0.117	0.47
Error (a) ss	6.07	15.08	0.13	0.004	0.078	0.156	0.004	0.012	0.536	4.791	0.465	0.289	0.043
Error (b) ss	16.27	49.22	0.49	0.27	0.27	0.860	0.076	0.190	18.60	21.76	9.25	7.41	3.37
Year Ms	0.45	40.98	0.16	0.002	0.01	0.28	0.0004	0.006	0.14	8.16	0.13	0.01	0.03
Treatment Ms	120.17	61.68	1.91	0.43	2.22	0.99	0.04	0.09	3.25	0.72	7.46	7.03	1.43
Year × Treatment Ms	4.70	5.53	0.04	0.003	0.07	0.05	0.0013	0.001	1.32	0.41	0.17	0.04	0.16
Error (a) Ms	3.04	7.54	0.06	0.002	0.04	0.08	0.002	0.006	0.27	2.40	0.23	0.14	0.02
Error (b) Ms	1.81	5.47	0.05	0.03	0.03	0.10	0.01	0.02	2.07	2.42	1.03	0.82	0.37
Year 'F' cal	0.15	5.43	2.55	1.21	0.24	3.63	0.20	0.93	0.52	3.40	0.56	0.04	1.45
Treatment 'F' cal	66.5	11.3	35.4	14.3	75.15	10.18	4.75	4.29	1.57	0.30	7.26	8.54	3.83
Year × Treatment 'F' cal	2.60	1.01	0.80	0.11	2.39	0.52	0.17	0.01	0.64	0.17	0.16	0.05	0.42
Year 'F' tab	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Treatment 'F' tab	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86
Year × Treatment 'F' tab	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86

No. of replications taken = 3; df = degrees of freedom; ss = Sum of square; Ms = Mean square; cal = calculated value; tab = tabulated value; BAF = Bioaccumulation factor; HQ = Hazard quotient; Arsenic = As; As (t) = Total arsenic in soil; Soil pH (Soil = solution = 1:2.5) measured after completion of crop cycle; CD ($P_{0.05}$) values for each parameters are given in each table.

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