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Effect Of Biofertilizer And Dairy Waste On Growth Response And Removal Of Arsenic From Soil Using *Vetiveria Zizanioides*

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ABSTRACT

Phytoextraction can provide an effective in situ technique for removing heavy metals from polluted soils. The study reported in this paper was undertaken to evaluate the growth response of *Vetiveria zizanioides* in arsenic (As) contaminated soils and its ability to remove As. The plant has been recently identified to be tolerant to high As concentration and have great potential in remediating As contaminated soils. This has been demonstrated by conducting pot culture experiment. The soil spiked with different levels of As i.e. 0, 500, 1000, 1500 and 2000 mg kg^{-1} was amended with organic amendments like dairy waste, mycorrhizae and biofertilizer (Azotobacter) and another set without amendments was also kept for comparison to assess the effect of amendments on arsenic removal. The plants were harvested after six months and their growth and As accumulation in roots and shoots were estimated. The results from this study indicate that the plant exhibited high tolerance to As toxicity in the soils and normal growth was attained upto 500 mg kg^{-1} when amended with dairy waste, mycorrhizae and Azotobacter. The plant was not survived at 500 mg kg^{-1} without amendments. This indicate that the soil amendments invoked greater root growth and enhanced the phytoextraction process relative to all other treatments. At higher concentrations viz. 1000, 1500 and 2000 mg kg^{-1} with and without amendments, the plant was not survived. The accumulation of As was much greater in roots (185.4 mg kg^{-1}) than in shoots (100.6 mg kg^{-1}). The As level in the polluted soil was reduced from 500 mg kg^{-1} to 214 mg kg^{-1} after six months. The removal of As level in the rhizosphere was found to be 57%. Microbial population was not affected in the As contaminated soil amended with dairy sludge, mycorrhizae and Azotobacter. These results indicate that *Vetiveria zizanioides* could possibly be used with success for removal of As from contaminated soils.

KEYWORDS

Arsenic; Spiked soils; Dairy Waste; Mycorrhizae; Azotobacter; Phytoextraction; *Vetiveria zizanioides*.

INTRODUCTION

Arsenic has long been identified as a carcinogen, and its elevated concentration in an ecosystem is of great concern from public health point of view and the environment. Arsenic contamination in soil results from various human activities including mining, combustion, wood preservation and pesticide application. There are tens of thousands of arsenic contaminated sites world wide with arsenic concentrations as high as 26.5 gm kg^{-1}[4]. In India, arsenic pollution in groundwater is mainly reported in the state of West Bengal in India. Some of the most affected districts having arsenic in drinking water in the West Bengal are South 24 paraganas (conc. 0.06 – 3.2 ppm), North 24 Paraganas (conc. 0.06 – 128 ppm), Malda (0.05 – 1.434 ppm), Nadia (0.05 – 1.0 ppm) and Barshaman (conc. 0.10 – 0.50 ppm). This pollution is mostly reported from the intermediate water depth of 20 – 80m below ground level. Arsenic contaminated soil is one of the major sources of arsenic in drinking water. Therefore, cleaning up of these contaminated sites becomes an urgent issue[11].

Practically, remediation of contaminated soil by washing or cut off wall method requires high investment. Phytoremediation, which is a form of ecological engineering, has emerged as an alternative mechanism that has proven to be effective and relatively inexpensive. Phytoremediation is the use of vegetation for in-situ treatment of contaminants such as heavy metals and pesticides in soil and water. The ideal characteristics of plants species to be used to remove the toxic contaminants from soil should have high biomass, short life span and are able to tolerate and accumulate high concentrations of the contaminants[9]. Several studies have shown that higher level of arsenic accumulates in the root compared to the leaf and stem. Moreover, the uptake of arsenic and other heavy metals for remediation in neutral pH soil, high clay level and soil where organic matter content is available, would be difficult[12].

Vetiver is a kind of perennial grass with strong ecological adaptability and large biomass and is easy to manage and grow under different soil conditions. Its various characteristics makes it an ideal for controlling the environmental pollution. Its roots reach to 3 – 4m depth in the first year. It has commercial value and is non-food grass. The application of *Vetiveria zizanioides* was first developed by the World Bank for soil and water conservation in India in the 1980s (Srisatit et al., 2003). It has been reported that the total dry weight of *Vetiveria zizanioides* grown in 250 mg As/kg soil significantly decreased and arsenic accumulated more in the leaf[14].

Several reports have shown that organic amendments improve the physico-chemical and microbiological properties of mine spoil dumps and result in fast establishment of vegetation on stressed sites. Juwarkar (1999) reported that organic amendments of diversified origin (pressmud, ETP, sludge and farm yard manure) can be used for phytoremediation of coal mine and metal mine spoil dumps[1]. In this study, efforts are made to increase the growth and survival of *Vetiveria zizanioides* by using organic amendments like dairy waste and microbial inoculants viz. Mycorrhizae and Azotobacter through pot culture experiments and arsenic removal from contaminated soil.

MATERIALS AND METHODS

Pot culture experiment

The pot culture experiments were conducted at NEERI, India using the soil spiked with arsenic. Vetiveria zizanioides was grown in pots containing the soil spiked with $Na_2HAs_2O_4$. The *Vetiveria zizanioides* was grown in five different concentrations of arsenic i.e. 0, 500, 1000, 1500 and 2000 mgAs / kg soil with organic amendments like dairy waste, mycorrhizae and biofertilizer (Azotobacter) and without organic amendments for six months (180 days). Thereafter, plants were harvested and the arsenic accumulation in roots and leaves was assessed.

Different treatments of amendments, microbial inoculants and arsenic metal

Pot culture studies were conducted to ascertain the phytoremediation of arsenic contaminated soils with *Vetiveria zizanioides* having different treatments with organic amendments (Dairy waste, Mycorrhizae and Azotobacter). *Vetiveria zizanioides* was planted in pots with different concentrations of arsenic. Different treatments screened under pot culture studies were as follows: -

T1: Uncontaminated soil, 0 mg/kg

T2: Arsenic contaminated soil, 500 mg/kg (without amendment)

T3: Arsenic contaminated soil (500 mg/kg) + 50 tons/ha dairy waste + mycorrhizae + Azotobacter strains

T4: Arsenic contaminated soil, 1000 mg/kg (without amendment)

T5: Arsenic contaminated soil (1000 mg/kg) +50 tons/ha dairy waste + mycorrhizae Azotobacter strains

T6: Arsenic contaminated soil, 1500 mg/kg (without amendment)

T7: Arsenic contaminated soil (1500 mg/kg) + 50 tons/ha dairy waste + mycorrhizae + Azotobacter strains

T8: Arsenic contaminated soil, 2000 mg/kg (without amendment)

T9: Arsenic contaminated soil (2000 mg/kg) + 50 tons/ha dairy waste + mycorrhizae + Azotobacter strains

Preparation of the soil spiked with arenic

The uncontaminated soil was collected from NEERI's premises and spiked with toxic concentrations of arsenic metal i.e. $Na_2HAs_2O_4$. Spiking was done to increase the concentration of the different concentrations of metal in the soil. Ratio of 1 Kg of soil / liter of solution was used based on the hydraulic conductivity of the spiked soil. Each pot was filled with 10 kg of arsenic soil. The plant material i.e. *Vetiveria zizanioides* was cleaned and cut into 35 cm long pieces, planted and nursed for one month. During this nursing period, the arsenic spiked soil was analyzed for various physico-chemical and microbiological properties as per the standard procedure[8] while the microbial population of arsenic spiked soil was analyzed as per the methods of Norland, 1991 and Page et al., 1982 and expressed in the terms of colony forming units (CFU/g).

Growth observations

The survival of the plants was periodically observed and data with respect to height, root length and biomass (wet and dry weights) were recorded for each plant. The data were analyzed by using randomized block design at 5% confident level.

Arsenic accumulation

After having recorded the growth parameters, plants were cleaned, cut and the roots and leaves were separated. To get the stable dry weight every part was put into an oven at 60^0C for three days. Both wet and dry weights were recorded. All dried parts were grounded and mixed thoroughly and then digested as per US EPA – 3030, 1982 method. The samples were analyzed for arsenic content by using ICP-AES. Arsenic concentration in each part of the plant was calculated and defined as mg arsenic per kg of dry weight.

RESULTS AND DISCUSSIONS

Effect of different blends of dairy waste, mycorrhizae and biofertilizer strains on physico-chemical and microbiological properties of arsenic contaminated soil after six month of plantation

The results presented in TABLE 1 and 2 showed that the T3 treatment (i.e. arsenic spiked soil (500 mg/kg) + dairy waste @ 50 t/ha + mycorrhizae + biofertilizers) was found to be the most responsive treatment, which favoured improvement in physical and chemical properties. The organic carbon content of the soil increased from 0.43 to 0.86% respectively. The results presented in TABLE 3 showed that in treatment T3, maximum arsenic percentage reduction of 66.8%. The plants was not survived after six months of plantation in 1000, 1500 and 2000 mg/kg of arsenic contaminated soil. Thus, the plant could tolerate arsenic concentration upto 500 mg/kg and above this concentration it becomes phytotoxic to plant.

The results presented in TABLE 4 showed that the microbial population with respect to bacteria, fungi and actinomycetes also increased in T3 treatment as compared to as such arsenic contaminated soil. This indicates that the addition of organic amendments viz. dairy waste, mycorrhizae and microbial inoculants favoured improvements in microbial population. Mycorrhizal fungi have been associated with plants growing on heavy metal contami-

TABLE 1: Physical characteristics of different ameliorative treatments of arsenic contaminated soil after six months of planting

Plant species	Treatments	Bulk density, g/cc	Maximum water holding capacity, %	Porosity, %	Sand, %	Silt, %	Clay, %	Texture class
At the time of plantation								
Vetiveria zizanioides	T1	1.19	61.80	52.27	27	26	47	Clay
	T2	1.21	55.20	50.13	28	26	46	Clay
	T3	1.20	57.70	51.80	28	26	46	Clay
After six months of plantation								
Vetiveria zizanioides	T1	1.19	61.90	53.47	27	26	47	Clay
	T2	1.14	64.84	54.71	28	26	46	Clay
	T3	1.09	61.55	54.74	27	25	46	Clay

TABLE 2: Chemical characteristics of different ameliorative treatments of arsenic contaminated soil after six months of planting

Plant species	Treatments	pH	EC, mS/cm	CEC, meq/100g	Organic carbon, %	Total nutrients, %		
						N	P	K
At the time of plantation								
Vetiveria zizanioides	T1	7.90	0.19	55.27	0.45	0.096	0.072	0.157
	T2	6.60	0.48	51.13	0.42	0.063	0.060	0.165
	T3	6.70	0.42	52.19	0.43	0.078	0.068	0.162
After six months of plantation								
Vetiveria zizanioides	T1	7.70	0.21	53.27	0.48	0.090	0.070	0.156
	T2	6.76	0.48	52.36	0.40	0.018	0.015	0.160
	T3	7.77	0.87	53.12	0.86	0.020	0.015	0.175

TABLE 3: Total metal content of different ameliorative treatments of arsenic contaminated soil after six months of planting

Plant Species	Treatments	Heavy Metals (mg kg^{-1})							
		Cr	Zn	Pb	Cd	Mn	Fe	Cu	As
At the time of plantation									
	T1	47.0	48.2	31.2	10.6	800.5	35000.0	50.8	3.6
Vetiveria zizanioides	T2	49.6	56.3	33.4	11.6	823.6	35015.5	50.6	500.0
	T3	48.6	53.6	32.6	11.2	820.6	35010.2	50.2	500.0
After six months of plantation									
	T1	45.0	45.6	29.2	9.6	790.6	34990.0	48.2	2.80
Vetiveria zizanioides	T2	47.2	54.2	30.3	10.6	815.6	35000.5	48.0	495.0
	T3	31.4	29.6	20.5	6.4	530.4	34450.2	26.4	214.0

nated soil (Shetty et al., 1994; Chaudry et al., 1998) and play a possible role in arsenic hyperaccumulation (Ma et al., 2001). Similar observations are observed under present study.

Growth observations

During the experimental period, *Vetiveria zizanioides* was found to be highly tolerant and survived in the soil spiked with arsenic up to 500 mg As/kg with organic amendments such as dairy waste, Mycorrhizae and Azotobacter (T3) and grew well with respect to plant height, root length and biomass (Figures 1, 2 & 3). In case of treatments T5, T7 and T9 with organic amendments as well as T4, T6 and T8 without organic amendments, *Vetiveria zizani oides* could not tolerate and survive because of high arsenic concentrations. It was found that the growth

TABLE 4: Microbiological characteristics of different ameliorative treatments of arsenic contaminated soil after six months of planting

Plant species	Treatments	Bacteria, CFU/g	Fungi, CFU/g	Actinomycetes, CFU/g	Azotobacter, CFU/g	Rhizobium, CFU/g
At the time of plantation						
Vetiveria zizanioides	T1	$17x10^5$	$26x10^3$	$43x10^3$	$23x10^3$	$21x10^3$
	T2	$20x10^4$	$35x10^2$	$40x10^2$	$10x10^2$	$15x10^2$
	T3	$35x10^4$	$60x10^2$	$55x10^2$	$20x10^2$	$22x10^2$
After six month of plantation						
Vetiveria zizanioides	T1	$30x10^5$	$35x10^3$	$62x10^3$	$40x10^3$	$34x10^3$
	T2	$28x10^4$	$50x10^2$	$52x10^2$	$21x10^2$	$28x10^2$
	T3	$25x10^5$	$35x10^3$	$28x10^3$	$21x10^3$	$35x10^3$

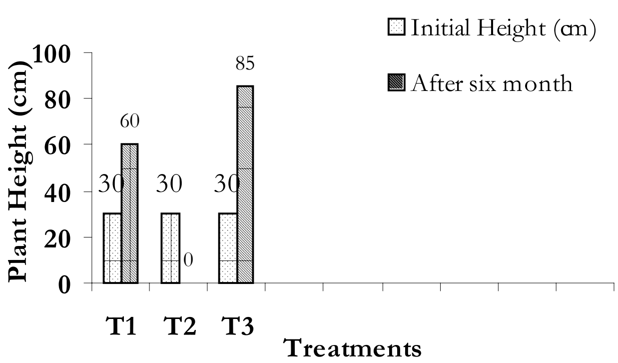

Figure 1: Growth performance of *Vetiveria zizanioides* planted under pot culture experiments with arsenic contaminated soil after six month of planting

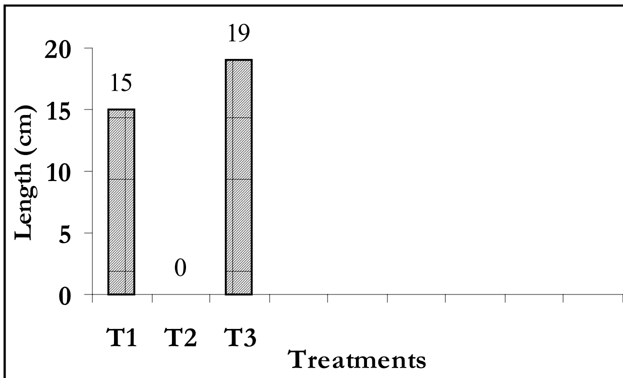

Figure 2: Root length of *Vetiveria zizanioides* planted under pot culture experiments with arsenic contaminated soil after six month of planting

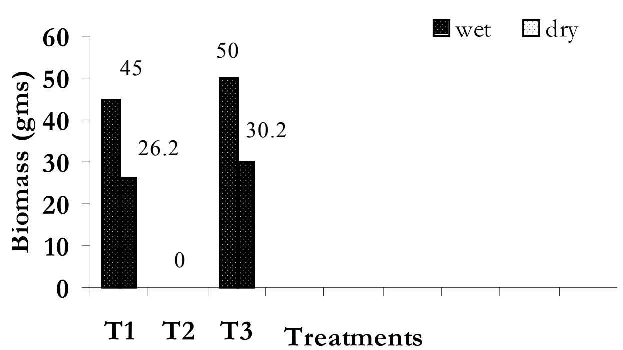

Figure 3: Biomass (Wet and dry weight) of *Vetiveria zizanioides* after removal of plant planted under pot culture experiments after six month of plantation

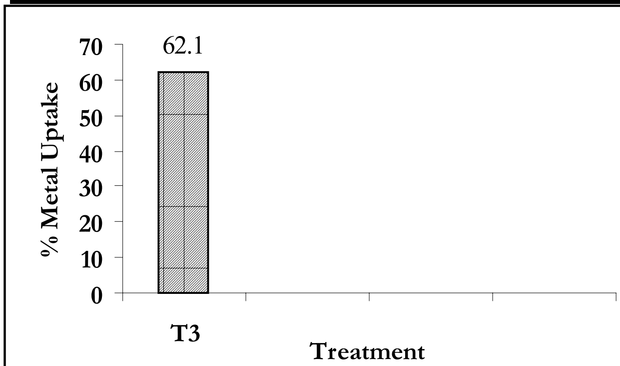

Figure 4: Percentage metal uptake by *Vetiveria zizanioides* planted under pot culture with arsenic contaminated soil after six month of planting

of plants in the terms of the height, root length was significant between the arsenic treated soils and control at 5% confident level. The results reported here thus confirmed that *Vetiveria zizanioides* was highly tolerant and thus could grow in the soil spiked with arsenic up to 500 mgAs/kg due to addition of organic amendments such as dairy waste, Mycorrhizae and Azotobacter. Earlier results reported that the plant could tolerate upto 250 mgAs/kg of arsenic by Truong (2000) and Srisatit (2003).

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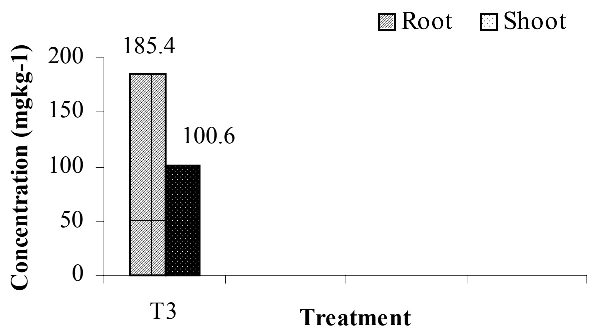

Figure 5: Metal uptake by *Vetiveria zizanioides* in root and shoots planted under pot culture with arsenic contaminated soil after six month of planting

Arsenic accumulation of *Vetiveria zizanioides*

Arsenic accumulation was found in all parts of the *Vetiveria zizanioides* at 500 mg As/kg concentration of the spiked soil (T3). The total arsenic accumulation by *Vetiveria zizanioides* was 286 mg/kg in 500 mg As/kg arsenic spiked soil and organic amendments with dairy waste, Mycorrhizae and Azotobacter (Figure 4). Trough (1999) also reported the similar distribution of metal in the respective parts of the plants. The amount of arsenic accumulation in roots (185.4 mg As/kg) was higher than that in leaves (100.6 mg As/kg) as shown in figure 5. Similar finding was also reported by Srisatit et al., (2003). Shiralipour (2002) reported that plant with compost amendments removed <8.15% As from AAC (Artificially arsenic contaminated soil). The result presented in TABLE 3 conducted through pot culture experiments showed that in treatment T3, maximum arsenic percentage reduction of 66.8%.

CONCLUSION

Vetiveria zizanioides could tolerate and grow well up to 500 mg/kg arsenic spiked soil. An improvement in physico-chemical properties and microbial population was due to the addition of organic amendments (dairy waste, Mycorrhizae & Azotobacter). In the case of 1000, 1500 & 2000 mgAs/kg spiked soil, *Vetiveria zizanioides* could not tolerate and survived even with the organic amendments. The present study indicates that plant height; root length, metal uptake and arsenic efficiency were higher in 500 mg As/kg contaminated soil due to the organic amendments (dairy waste, Mycorrhizae and Azotobacter). The arsenic accumulation in root was higher than that in the leaves of *Vetiveria zizanioides*. It is recommended that *Vetiveria zizanioides* could be used to remove arsenic from contaminated soil. However, further research should be carried out to increase the arsenic uptake rate of *Vetiveria zizanioides.*

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