Archive of SID

Int. J. Environ. Sci. Tech., 4 (3): 339-344, 2007
ISSN: 1735-1472
© Summer 2007, IRSEN, CEERS, IAU

Effect of amendment on phytoextraction of arsenic by *Vetiveria Zizanioides* from soil

[1]S. K. Singh; [1]A. A. Juwarkar; [1]S. Kumar; [1]J. Meshram; [2]*M. Fan

[1]National Environmental Engineering Research Institute (NEERI), Kolkata, Zonal Laboratory, 1-8, Sector 'C', East Kolkata, Area Development, Project, P.O: East Kolkata Township, Kolkata -700 107, West Bengal, India

[2]School of Materials Science and Technology, Georgia Institute of Technology, 30332, USA

Received 1 November 2006; revised 15 February 2007; accepted 12 May 2007; available online 20 June 2007

ABSTRACT: The present study was undertaken to evaluate the growth response of *Vetiveria zizanioides* amended with organic amendments to arsenic (As) in contaminated soils and its ability to sequester As. The test results indicate that the plants exhibited high tolerance to As in the soils and their normal growth continued even though As concentration reached 500 mg/kg. However, when As concentrations in soils were in the range of 1000~2000 mg/kg the plants could not survive no matter whether the soils were amended. The accumulation of As in roots (185.4 mg/kg) was higher than that in shoots (100.6 mg/kg). The As level in the contaminated soil was reduced from 500 mg/kg to 214 mg/kg after six months of As phytoextraction. Microbial population was not affected in the As contaminated soil amended with dairy sludge, *mycorrhizae* and *Azotobacter.*

Key words*: Arsenic, Dairy waste, Mycorrhizae, Azotobacter, Phytoextraction, Vetiveria zizanioides*

INTRODUCTION

Arsenic has long been identified as a carcinogen, and its elevated concentration in an ecosystem threatens public health and environmental safety. 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 worldwide with arsenic concentrations as high as 26.5 mg/kg (Hingston, *et al.*, 2001). In India, groundwater arsenic pollution problems mainly exist in the state of West Bengal in India. The highest arsenic concentration in water in the state is as high as 128 ppm, which is mainly caused by the arsenic contaminated soils in the area. Remediation of contaminated soil is one of the major methods to lower the arsenic concentration in groundwater (Pteris, *et al.,* 2003). Remediation of contaminated soil by conventional technologies requires high investment. Phytoremediation, based on ecological engineering mechanism, has emerged as an alternative approach to effective and inexpensive removal of arsenic in contaminated soils. Phytoremediation uses vegetation for *in-situ* treatment of contaminants such as heavy metals and pesticides in soil and water. The ideal plants to be used for phytoremediation should have high biomass productivity, short life span, and high tolerance and accumulation capacity of high concentrations of contaminants (Raskin, *et al.*, 1997; Tlustos, *et al.*, 1998). Vetiver, a type of perennial grass with strong ecological adaptability and large biomass productivity, is easy to manage and grow under different soil conditions, which makes it an ideal phytoremediation candidate for controlling environmental pollution. With the support of the World Bank, *Vetiveria zizanioides* has been used for soil and water conservation in India (Truong and Baker, 1998). It has been reported that the total dry weight of *Vetiveria zizanioides* grown in 250 mg-As/kg soil significantly decreased due to the high accumulation of arsenic in their different parts, especially in leaves (Truong and Baker, 1998). Therefore, it is necessary to find cost-effective methods for the improvement of the growth of *Vetiveria zizanioides* within contamination environment, thus enhancing their contaminants phytoremediation capacities. To this end, the organic amendments, *Mycorrhizae* and *Azotobacter*, were explored in the present study to improve the growth and survival of *Vetiveria zizanioides* for better arsenic removal from contaminated soil.

*Corresponding Author Email: *mfan3@mail.gatech.edu*
Tel./Fax: 404-385-6725

www.SID.ir

Archive of SID

MATERIALS AND METHODS

Cultivation of pot culture

The pot culture experiments were conducted at the National Environmental Engineering Research Institute (NEERI) in India using the soil spiked with arsenic. *Vetiveria zizanioides* was grown in pots containing the soil spiked with $Na_2HAs_2O_4$ in five different concentrations of arsenic, 0, 500, 1000, 1500 and 2000 mg-As/kg soil with organic amendments including dairy waste, *mycorrhizae* and biofertilizer (*Azotobacter*) and without organic amendments for six months. Thereafter, plants were harvested and the arsenic accumulations in roots and leaves were assessed.

Treatment of amendments, microbial inoculants and arsenic

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 listed 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 arsenic

Ratio of 1 Kg of soil / 1 L 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 by using standard procedure (Piper, 1966) while the microbial population of arsenic spiked soil was analyzed by using the standard methods and expressed in the terms of colony forming units (CFU/g).

Observation of plant growth

The plants tested were periodically observed and data with respect to their heights, root lengths and biomasses were recorded accordingly. The data were analyzed by using Randomized Block Design at 5% confident level.

Measurement and calculation of arsenic content

The cut roots and leaves of cleaned plants were separately dried in an oven at 60 °C for three days. The weight changes of the two parts of the plant before and after drying were recorded. All the dried parts were grounded and mixed thoroughly and then digested by using USEPA methods 3030 and 1982. The samples were analyzed for arsenic content with an ICP-AES. Arsenic concentration in each part of the plant was calculated in term of mg arsenic per kg of dry weight of root or leaves.

RESULTS

Effect of different blends of dairy waste, mycorrhizae and biofertilizer strains on properties of arsenic contaminated soil

The effects of dairy waste, mycorrhizae and biofertilizer strains physico-chemical and microbiological properties of arsenic contaminated soils are presented in Tables 1 to 3. Tables 1 to 3 show that the T3 treatment (i.e. arsenic spiked soil (500 mg/kg) + dairy waste at 50 t/ha + mycorrhizae + biofertilizers) was found to be the most responsive one, beneficial to the improvement in physical and chemical properties of the soil. The organic carbon content of the soil increased from 0.43 to 0.86%. The results presented in Table 3 showed that under treatment T3 conditions, the maximum arsenic reduction percentage was 66.8%. The plants died after six months of plantation in 1000, 1500 and 2000 mg/kg of arsenic contaminated soil. The plant could survive when arsenic concentration in soil was 500 mg/kg. The results presented in Table 4 showed that the microbial population with respect to bacteria, fungi and actinomycetes increased under T3 treatment conditions for the arsenic contaminated soil. This fact

www.SID.ir

Archive of SID

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 contaminated soil (Shetty, *et al.*, 1994; Chaudry, *et al.*, 1998; Ma, *et al.*, 2001), which is confirmed with the observation of the present study that it plays a possible important role in arsenic hyperaccumulation.

Growth observations

During the experimental period, *Vetiveria zizanioides* was found to be highly tolerant to arsenic and survived in the soil spiked with arsenic up to 500 mg As/kg when organic amendments such as dairy waste, *Mycorrhizae* and *Azotobacter* were used, and grew well with respect to plant height, root length and biomass as shown in Figs. 1 to 3.

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
	T4	1.22	54.13	49.32	28	25	47	Clay
	T5	1.20	56.00	50.01	27	26	47	Clay
	T6	1.24	52.30	48.32	28	25	47	Clay
	T7	1.23	53.32	48.93	28	26	46	Clay
	T8	1.25	50.29	47.32	29	25	46	Clay
	T9	1.23	51.65	47.77	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
	T4	1.20	65.25	54.52	27	26	47	Clay
	T5	1.18	63.30	59.58	28	24	47	Clay
	T6	1.21	54.99	56.54	28	25	47	Clay
	T7	1.10	68.10	55.20	28	26	46	Clay
	T8	1.24	55.79	53.63	29	25	46	Clay
	T9	1.10	62.04	54.44	28	26	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
	T4	6.53	0.56	49.35	0.39	0.056	0.059	0.170
	T5	6.62	0.48	50.54	0.40	0.072	0.065	0.165
	T6	6.48	0.60	48.36	0.38	0.040	0.056	0.172
	T7	6.52	0.55	49.14	0.39	0.052	0.062	0.167
	T8	6.45	0.63	48.93	0.37	0.030	0.050	0.176
	T9	6.40	0.56	49.96	0.38	0.039	0.060	0.170
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
	T4	6.73	1.09	51.20	0.38	0.016	0.013	0.163
	T5	6.90	1.01	52.13	0.48	0.018	0.015	0.170
	T6	6.56	1.19	50.08	0.39	0.016	0.013	0.164
	T7	6.75	1.12	51.20	0.43	0.017	0.010	0.169
	T8	6.44	1.32	49.80	0.36	0.014	0.014	0.164
	T9	6.68	1.26	50.60	0.39	0.015	0.018	0.171

www.SID.ir

Archive of SID

In case of treatments T5, T7 and T9 with organic amendments as well as treatments T4, T6 and T8 without organic amendments, *Vetiveria zizanioides* could not tolerate and survive due to the high arsenic concentrations under the given test conditions. Figs. 1 to 3 indicate that there were significant differences with a 5% confidence level between arsenic treated soils and controlled soils in the aspect of the growth of plants in the terms of the height and root length.

Arsenic accumulation of Vetiveria zizanioides

Arsenic accumulation was found in all parts of the *Vetiveria zizanioides* which grew in the environment with 500 mg As/kg soil (T3). The total arsenic accumulation in *Vetiveria zizanioides* reached 286 mg/kg after a six-month period of growing in the soil containing 500 mg As/kg along with the use of the organic amendments with dairy waste, *Mycorrhizae* and *Azotobacter* as shown in Fig. 4.

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)							
		Cr	Zn	Pb	Cd	Mn	Fe	Cu	As
At the time of plantation									
Vetiveria zizanioides	T1	47.0	48.2	31.2	10.6	800.5	35000.0	50.8	3.6
	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
	T4	49.5	53.1	32.5	10.6	822.1	35019.5	50.2	1000.0
	T5	48.9	52.6	31.6	10.2	818.6	35006.2	49.8	825.0
	T6	48.2	51.9	32.9	12.3	813.5	35023.5	50.3	1500.0
	T7	47.9	51.6	32.4	11.8	809.6	35011.2	49.5	1350.0
	T8	48.2	52.6	32.1	11.3	809.2	35002.5	50.9	2000.0
	T9	47.6	52.1	31.8	10.9	805.6	34996.2	49.6	1750.0
After six months of plantation									
Vetiveria zizanioides	T1	45.0	45.6	29.2	9.6	790.6	34990.0	48.2	2.80
	T2	47.2	54.2	30.3	10.6	815.6	35000.5	48.0	490
	T3	31.4	29.6	20.5	6.4	530.4	34450.2	26.4	214
	T4	46.5	50.6	28.3	9.6	808.7	34980.2	48.6	910.5
	T5	34.2	32.4	24.2	5.4	540.6	34460.2	29.6	812.5
	T6	44.6	48.6	29.9	9.0	800.2	34976.2	48.5	1380
	T7	39.4	40.2	27.2	10.7	610.7	34530.6	36.2	1225.5
	T8	45.7	51.0	30.2	10.2	798.6	34970.5	48.3	1825
	T9	40.6	41.6	26.4	7.0	630.4	34590.6	40.2	1680

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	$35x10^4$	$60x10^2$	$55x10^2$	$20x10^2$	$22x10^2$
	T4	$15x10^4$	$25x10^2$	$29x10^2$	$85x10^1$	$5x10^2$
	T5	$30x10^4$	$50x10^2$	$46x10^2$	$12x10^2$	$15x10^2$
	T6	$60x10^3$	$13x10^2$	$15x10^2$	$60x10^1$	$65x10^1$
	T7	$80x10^3$	$42x10^2$	$30x10^2$	$86x10^1$	$5x10^2$
	T8	$45x10^3$	$9x10^2$	$7x10^2$	$45x10^1$	$40x10^1$
	T9	$68x10^3$	$25x10^2$	$20x10^2$	$65x10^1$	$85x10^1$
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	$25x10^5$	$35x10^3$	$28x10^3$	$21x10^3$	$35x10^3$
	T4	$26x10^4$	$39x10^2$	$45x10^2$	$4x10^2$	$10x10^2$
	T5	$80x10^4$	$3x10^3$	$2x10^3$	$30x10^2$	$30x10^2$
	T6	$70x10^3$	$65x10^2$	$25x10^2$	$81x10^1$	$75x10^1$
	T7	$3x10^3$	$65x10^2$	$52x10^2$	$2x10^2$	$25x10^2$
	T8	$56x10^3$	$20x10^2$	$18x10^2$	$52x10^1$	$51x10^1$
	T9	$81x10^3$	$32x10^2$	$35x10^2$	$85x10^1$	$94x10^1$

www.SID.ir

Archive of SID

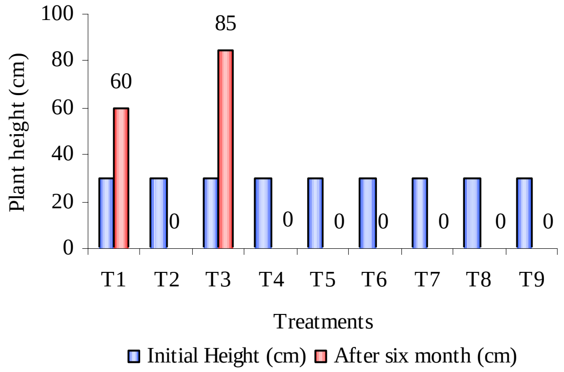

Fig. 1: Growth performance of *Vetiveria zizanioides* planted under pot culture experiments with arsenic contaminated soil after six month of planting

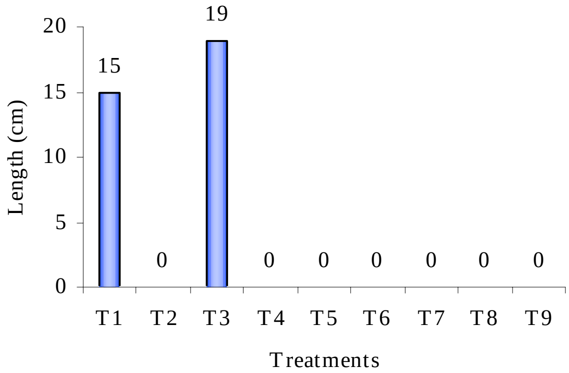

Fig. 2: Root length of *Vetiveria zizanioides* planted under pot culture experiments with arsenic contaminated soil after six months of planting

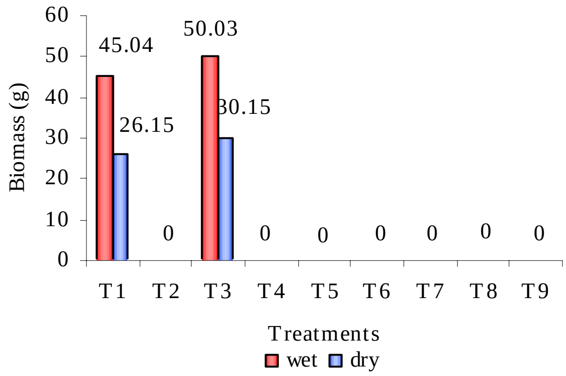

Fig. 3: Biomass (Wet and dry weight) of *Vetiveria zizanioides* after removal of plant planted under pot culture experiments after six month of plantation

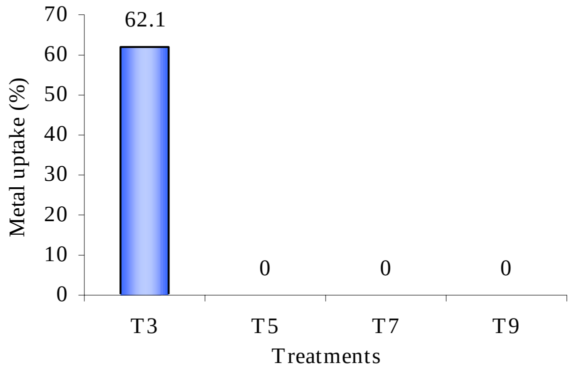

Fig. 4: Percentage arsenic uptake by *Vetiveria zizanioides* planted under pot culture with arsenic contaminated soil after six month of planting

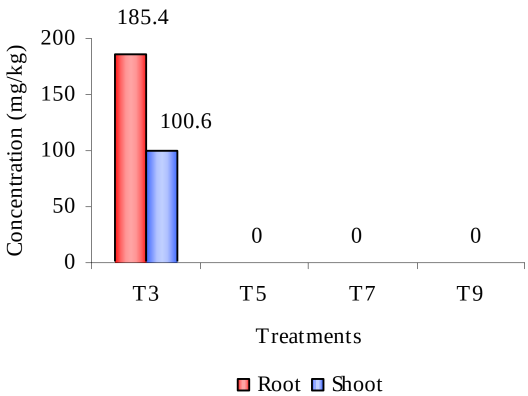

Fig. 5: Metal uptake by *Vetiveria zizanioides* in root and shoots planted under pot culture with arsenic contaminated soil after six months of planting

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 Fig. 5. Pteris, *et al.* (2003) reported that the plant with compost amendments only removed 8.15% arsenic from their artificially arsenic contaminated soil. Obviously, the arsenic removal efficiency obtained from the present research was higher than the one reported by Pteris, *et al.* (2003).

DISCUSSION AND CONCLUSION

Vetiveria zizanioides could tolerate and grow well in the soil environment with 500 mg As per 1 kg of soil, which is attributed to the improvement in physico-chemical properties and microbial population of soil, resulting from the addition of organic amendments (dairy waste, *Mycorrhizae and Azotobacter*).

www.SID.ir

Archive of SID

Vetiveria zizanioides with the help of organic amendments could be used to remove arsenic from contaminated soil even though further research should be carried out to enhance the arsenic uptake rate of *Vetiveria zizanioides*.

ACKNOWLEDGEMENT

The authors acknowledge the kind support and encouragement from Dr. Sukumar Devotta.

REFERENCES

Chaudry, T.M.; Hayes, W.J.; Khan, A.J.; Khoo, C.S., (1998). Phytoremediation focusing an accumulator plants that remediate metal contaminated soils. Aust J. Ecotoxicol, **4**, 37-51.

Hingston, J.A.; Collins, C.D.; Murphy, R.J.; Lester, J.N., (2001). Leaching of chromated copper arsenate wood preservatives: a review. *Environ. Pollut.* **111**(1), 53-66.

Ma, L.Q.; Komar, K.M.; Tu, C.; Zhang, W.; Cai, Y.; Kennelley, E.D., (2001). A fern that hyperaccumulates arsenic. Nature 409, 579.

Piper, C.S., (1966). Soil and Plant analysis. Hans. Pubs., Bombay, 401.

Pteris, L.; Cao, X.; Ma, L.Q.; Shiralipour, A., (2003). Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator. Environ. Pollut., **126**(2), 157-167.

Raskin, I.; Smith, R.D.; Salt, D.E., (1997). Phytoremediation of metals: Using plants to remove pollutant from the environment. Curr. Opin. Biotech., **8**, 221-226.

Shetty, K.J.; Hetrick, B.A.D.; Figge, D.A.H., (1994). Effect of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ. Pollut., **86**, 181-188.

Tlustos, P.; Pavlikova, D.; Balik, J.; Szakova, J.; Hanc, A.; Balikova, M., (1998). The accumulation of arsenic and cadmium in the plants their distribuition. Rotilina Vyroba **44**, 465-469.

Truong, P.; Baker, D., (1998). Vetiver grass system for environment protection. Tech. Bull., **1**, 1-6.

AUTHOR (S) BIOSKETCHES

Singh, S. K., Scientists, Environmental Biotechnology Division, National Environmental Engineering Research Institute Nehru Marg, Nagpur 440 020, India. Email: *skr_singh@neeri.res.in*

Juwarkar, A. A., Scientists, Environmental Biotechnology Division, National Environmental Engineering Research Institute, Nehru Marg, Nagpur 440 020, India. Email: *aa_juwarkar@neeri.res.in*

Kumar, S., Scientist, National Environmental Engineering Research Institute (NEERI), Kolkata Zonal Laboratory, 1-8, Sector 'C', East Kolkata, Area Development Project, P.O.: East Kolkata Township, Kolkata -700 107, West Bengal, India. Email: *s_kumar@neeri.res.in*

Meshram, J., Reader, Department of Chemistry, RTM Nagpur University, 440001, India.
Email: *j_meshram@gmail.com*

Fan, M. School of Materials Science and Technology, Georgia Institute of Technology, 30332, USA.
Email: *mfan3@mail.gatech.edu*

This article should be referenced as follows:
Singh, S. K.; Juwarkar, A. A.; Kumar; S., Meshram, J.; Fan, M., (2007). Effect of amendment on phytoextraction of arsenic by Vetiveria Zizanioides from soil. Int. J. Environ. Sci. Tech., ***4*** *(3), 339-344.*

www.SID.ir