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EDTA enhances lead uptake and facilitates phytoremediation by vetiver grass

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Abstract: *Vetiver grass (Vetiveria zizanioides) has strong and dense root system and is a potential phytoremediator plant since it can tolerate a wide range of climatic conditions and grow well in soils contaminated with heavy metals. Soil was artificially contaminated by lead (20 mgl^{-1}) during field trials. Four concentration of EDTA (Ethylene diamine tetra acetic acid-disodium salt) solution i.e. 0, 3, 5 and 10 mmol kg^{-1} were added to soil prior to harvesting, to study the influence of EDTA solution on phytostabilization by vetiver grass. Results showed that the concentration of lead in roots of vetiver is significantly increased after EDTA solution (5 mmol kg^{-1}) application. However, high concentration of EDTA (10 mmol kg^{-1}) does not show such significant increase. The toxicity of highly contaminating metal did not affect the growth of vetiver grass significantly but a slight decrease in parameters studied was noticed. No stress symptoms were observed in vetiver plants. Results of present study reveal that vetiver could be considered as a potential phytoremediator for lead contaminated site.*

Key words: *Vetiver grass, Lead, EDTA, Phytostabilization, Phytoremediation*
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Introduction

Contamination of soils by heavy metals is of widespread occurrence as a result of human, agricultural and industrial activities. Among heavy metals, lead is a potential pollutant that readily accumulates in soils and sediments. Apart from natural weathering processes, lead contamination of the enviornment has resulted from industrial activities *viz.* mining and smelting processes, agricultural activities *viz.* application of insecticide and municipal sewage and sludges and urban activities *viz.,* use of lead in gasoline, paints and other materials (Sharma and Dubey, 2005). As many of the lead pollutants are indispensable for modern human life, soil contamination with lead is not likely to decrease in the near future (Yang *et al.,* 2000; Akinolaand and Ekiyoyo, 2006; Phetsombat *et al.,* 2006). Significant increases in the lead content of cultivated soils has been observed near industrial areas. Once in water, it enters the food chain and adversely affects flora and fauna (Singh *et al*., 2005; Pandey *et al.,* 2007). Conventional cleanup technology is generally too costly and often harmful to desirable soil properties. More recently, due attention has been given to the development of a plant based technology for remediation of heavy metal contaminated soils (Chaney *et al*., 1997). It is a novel and potentially inexpensive green technology for sustainable remediation of surface soils contaminated with heavy metals (Salt *et al*.,1998; Barocsi *et al*., 2003; Baek *et al.,* 2006) and is on average tenfold cheaper than other physical, chemical or thermal remediation methods (Hooda, 2007). It includes six different remediation methods, out of these, phytostabilization and phytoextraction are two techniques that are particularly used for *in situ* phytoremediation of heavy metals (Suresh and Ravishankar, 2004). Two strategies one involving chelating agents and the other involving genetic engineering are being developed to increase phytoextraction of metals with higher biomass or by transgenic plants. Although the total lead concentration in metal contaminated soils may be high, the phyto-available lead fraction (water soluble and exchangeable) is usually very low due to the strong association of lead with organic matter, Fe-Mn oxides and clays and precipitation as carbonates, hydroxides and phosphates (Shen *et al.,* 2002; Sharma and Dubey, 2005). Synthesized chelating agents or complexing agents *viz.* EDTA, HEDTA and NTA were applied to metal contaminated soil to increase the mobility and bioavailability of metals in soils (Hooda, 2007) and also to increase the amount of heavy metals accumulated in plants (Chiu *et al*., 2005). However, at high concentration they can also be toxic to plants (Cooper *et al*., 1999).

Vetiver grass (*Vetiveria zizanioides* L. Nash) is a plant with considerable promise for soil and water conservation as well as phytoremediation application because of its dense root system and wide climatic tolerance to extreme environmental conditions (Xia, 2004; Lavania *et al*., 2004) including soil pH values between 3.0-10.5 and temperature form 14-55^{o}C. Previous studies indicate that the use of vetiver grass coupled with the use of chelating soil amendments such as EDTA, has considerable potential for the use as a remedial strategy for lead contaminated soil (Lai and Chen, 2004) such as those associated with firing ranges (Wilde *et al.,* 2005) and lead/zinc mine tailings (Yang *et al.,* 2003; Chiu *et al.,* 2006).

The present study was undertaken to elucidate the effect of EDTA soil amendment for phytoremediation of lead contaminated site by vetiver grass.

Materials and Methods

A field plot trial was designed in Botanical Garden of Department of Plant Science, the well leveled clods and weed free field was prepared to ensure adequate plant stand and early vigour.

Prior to conducting the experiments a surface soil sample was collected from the plot and was used for basic characteristics analysis (Table 1). The surface soil (0-20 cm) of the contaminated site was sampled, air dried under room temperature and ground to passed through a 2 mm sieve. The soil's pH value was determined using glass electrodes in a soil : water ratio of 1:1 (McLean, 1982). The soil particle size distribution was analysed using the pipette method (Gee and Bauder, 1986).

Organic carbon content was determined by Walker-Black wet combustion method (Nelson and Sommers, 1982). Cation exchange capacity (CEC) was determined by using the ammonium acetate method (Rhodes, 1982). Total N (Micro-Kjeldhal method), available P (molybdenum blue method after samples were extracted with Na_2CO_3) and exchangeable K (after samples were extracted with NH_4OAc) were also determined (Kapur and Govil, 2000).

$Pb(NO_3)_2$ solution (20 mg l^{-1}) was added to soil to control the total concentration of 1000 mg Pb kg^{-1}. Treated soils was irrigated at bi-weekly intervals with tap water in order to maintain optimum water level to enable the heavy metal salt to reach a steady state. Vetiver tillers (clonal progeny of local population) were planted as 4 tillers per clump at 60x25 cm spacing and was cut at 30 cm high before planting. Four concentration of EDTA (disodium salt) *i.e.* 0, 3, 5 and 10 mmol kg^{-1} soil were added to contaminated soil. The growth conditions and observed symptoms of toxicity displayed by plants were also recorded. Tested plants were harvested 7 days after EDTA solution was added to soil. Harvested plant were oven dried at 80^0C for 24 hr and weighed.

Lead accumulation (mg kg^{-1} DW) in different parts of vetiver and soil was determined by Atomic Absroption Spectroscopy (GBC Avanta S, AAS. Australia) after samples were digested with concentrated HNO_3 + $HClO_4$ (AOAC, 1990). One way ANOVA was carried out to compare the means of different treatments at 5% level of significance.

Results and Discussion

Effects of lead on plant growth: Survival rate of vetiver grass in different treatments after planting for 15 days and its coverge after planting for 3 months are listed in Table 2. Survival rate and cover are quite similar to as that of control indicating that external lead did not influence the growth of vetiver grass. It can also be seen from the data that vetiver produced new tillers much earlier and their growth was faster. It increased 21 new tillers per four clumps in control indicating that vetiver had strong endurance to infertility (Xia *et al*., 1999). Data clearly indicates that all plants showed normal development without signs of heavy metal stress, when no EDTA was added to the soil (Lai and Chen, 2004). However, high concentration of lead resulted in decrease of growth and biomass of vetiver grass. Similar results were also reported by Chantachon *et al*. (2004) when vetiver grass was grown in lead contaminated soil.

Effects of EDTA treatments on lead uptake by vetiver grass: Lead uptake data are summerised in Table 3. A significant difference was found between concentration of lead in different parts of vetiver grown in contaminated soil in comparison to its concentration in plants grown in non contaminated soil (control). Lead concentration in roots of vetiver (374.90 mg kg^{-1} DW) grown in contaminated soil was about 69 times higher than in non-contaminated soil.

Application of chelating agent EDTA, prior to harvest, significantly increased the amount of lead phytoextracted relative to untreated control plants. Concentration of lead in the roots (374.90 mg kg^{-1} DW) was significantly increased upto 1034 mg kg^{-1} DW, only by adding 5 mmol kg^{-1} EDTA solution. However, 10 mmol kg^{-1} EDTA solution did not show such enormous increase in the lead

Table - 1: Physical and chemical properties of soil

Soil texture	Sandy loam*
Soil pH	5.23
Organic carbon (g kg^{-1})	27.23
CEC (Cmol(+) kg^{-1})	10.28
Total N (g kg^{-1})	1.0
Available P (g kg^{-1})	0.43
Exchangeable K (g kg^{-1})	0.68
Total Pb (mg kg^{-1})	13.27

*(Sand 47.4%, Slit 30.2% and Clay 22.1%)

Table - 2: Effects of lead on plant growth

	Control	Pb + 0 EDTA
Survival rate (%)	99 ± 1	96 ± 2
Cover (%)	89	88
Tiller no./4 clumps	21	19
Plant height (cm)	187.0	150.0
Biomass (g m^{-2})	906.9	876.0

Table - 3: Effects of EDTA treatments on lead uptake (mg kg^{-1} DW) and its distribution in different parts of vetiver grass

	Root	Shoot	Leaves
Control	4.3 ± 1.6	3.1 ± 1.2	2.3 ± 1.6
Pb+0 EDTA	374.9 ± 19.9*	166.33 ± 6.4*	45.1 ± 4.0*
Pb+3 mmol kg^{-1} EDTA	793.0 ± 249.0*	304.0 ± 116.2*	80.0 ± 30.3*
Pb+5 mmol kg^{-1} EDTA	1034.0 ± 215.0*	490.6 ± 210.0*	81.0 ± 36.0*
Pb+10 mmol kg^{-1} EDTA	1054.0 ± 232.6*	510.8 ± 216.2*	86.0 ± 29.7*

*Significant at 5% level of significance concentration in vetiver roots as compared to vetiver treated with lead only. It was also found that lead is most accumulated in roots while shoots and leaves show much less lead accumulation. Huang *et al*. (1997) has previously shown that lead phytoextraction can be enhanced with the addition of chelating agents to the soil and that of several chelating agents tested, EDTA was shown to be most effective Pb^{+2} ion chelator. Vulava and Seaman (2000) also determined that EDTA was superior to other extracting agents for increasing the mobility of lead in highly weathered soils. Lai and Chen (2004) found that EDTA increased lead uptake in vetiver roots but did not significantly increase Cd or Zn uptake. However, the influence of EDTA on lead uptake in vetiver was not as great (upto 100 fold) as demonstrated in other species (Huang *et al*., 1997; Suresh and Ravishankar, 2004).

Chelation of heavy metal ions has long been recognized as an important factor in metal uptake by plants. Results from this study demonstrate that EDTA can induce Pb accumulation in vetiver grass. Most of the increased lead uptake after the chelate treatments could be explained as an effect of enhancing Pb solubility (Lai and Chen, 2004). It is well known that metal solubility and bioavailability can be increased by application of synthetic chelates *viz.* EDTA, HEDTA, NTA and citric acid to soil (Hunag *et al*., 1997; Ebbas and Kochian 1998; Kavser *et al*., 2000). The effects of chelates on metal uptake may also be related to different uptake mechanism of metal concerned. For example, Zn transport into cytosol is *via* a protein mediated transport system with a fairly high affinity for Zn (Laset *et al.,* 2000) which can be contrasted to passive uptake of Pb by plants (Huang and Cunningham,1996). The application of EDTA solubilized large quantities of lead from soil which then diffuses down its concentration gradient into plant root and can be taken up by mass flow. Recent studies have shown that lead accumulation in plant parts is correlated with formation of Pb-EDTA complex and this complex is major form of lead absorbed and translocated by plant (Epstein *et al*., 1999; Barona *et al.,* 2001).After being taken up by the roots, the localization of lead is greater in roots than in other parts of the plants. Lead moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of lead between the root and shoot. This may in part account for the reports of higher lead accumulation in roots as compared to shoots (Verma and Dubey, 2003). It has been suggested that metal chelate complex may enter the root at breaks in the root endodermis and casparian strip and be rapidly transported to shoots (Shen *et al*., 2002).

The results of the present investigation are significant for several reasons. It clearly demonstrates that growth and performance of vetiver were not affected by exposure to lead solution. Accumulation of lead in roots of vetiver makes them useful for phytostabilization. It may be due to its larger biomass apart form strongest metal uptake ability. Furthermore, it could yield better covering and revegitating benefits (Xia, 2004). The fact that vetiver can live for about 50 years. (NRC, 1995) make this species an efficient, enduring low cost and long term remedial option for phytoremediation.

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