Comparative study of the efficiency of metal uptake Enhancers with EDTA Phytoremediation of Pb using Brassica.juncea (Indian mustard)

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Abstract

The effects of application of natural metal uptake enhancers like garlic and lime were investigated. Garlic being more effective than lime. It induces the metal tolerance in the plant cells and thereby facilitates metal sequestration. Plants were grown in garden soil (VNSGU, University campus, about 250 grams.), in plastic cups. The seeds were sown and spiked with 5, 50,500ppm of lead stock solution. Metal uptake enhancers were added one week before harvest and the plants were grown for a total period of 21 days. Comparison was done with synthetic chelator-EDTA which was the most effective followed by garlic, lime and citric acid. Metal was analyzed using ICP.

Key words: EDTA, lead, Garlic, Brassica juncea, metal uptake, sulphur metabolism, chelation

1.0 INTRODUCTION

Phytoremediation is based on the fact that a living plant can be considered as a solar driven pump, which can extract and concentrate toxic elements from the contaminated soil. (Raskin et al 1997.

Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, an d their remediation in soils and sediments are among th e most technically difficult (Cunningham et al., 1997). Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludges to land, and industrial manufacturing (Blaylock and Huang, 2000; Cunningham et al., 1997; Raskin et al., 1994). Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000). According to Raskin et al. (1994), the term heavy metal is arbitrary and imprecise. In this dissertation, 'heavy metal' will refer to any element that has metallic properties and atomic number greater than 20 (Raskin et al., 1994). Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Ow, 1996; NRC, 1997).Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibitin g symptoms of toxicity (Baker and Brooks, 1989; Baker et al., 1991; Reeves and Brooks, 1983). Chaney (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metalpolluted sites. However , hyperaccumulators were later believed to have limited potential in this area because oftheir small size and slow growth, which limit the speed of metal removal (Comis, 1996; Cunningham et al., 1995; Ebbs et al., 1997). By definition, a hyperaccumulator must accumulate at least 1000 µgAg-1 of Co, Cu, Cr, Pb, or Ni, or 10,000 µgAg-1 (i.e. 1%) of Mn or Zn in the dry matter (Reeves and Baker, 2000; Wantanabe, 1997).

2.0 METHODOLOGY

Soil preparation and plant material:
Soil (topsoil) used for study was obtained near the Department of Bioscience, VNSGU, Surat. Soil was collected, air dried, gently ground to pass through a 2mm sieve. This soil was filled in plastic glass. (250gm soil was filled in each plastic glass). For acclimatization of plant initially the seeds were germinated in normal soil and 3 days after germination the soil was spiked with metal.

Some amount of water was added to the soil to make it wet. Approximately 10-15 seeds were sown. Before sowing; seeds were selected each of approximate same size and colour; they were washed with distilled water and sown in to the soil. Small amount of wet soil was added to the top of the seeds for enhancing the germination process because they require moisture for germination.

B.juncea required 16hrs photoperiod, temperatures of 25/20degrees (day/night), and 75% humidity. The plastics glasses were kept in one line along the window pane to meet these physical requirements. Petri plates were kept below the pots to avoid leaching of metals; following seed emergence, the seedlings were spiked with lead solution.

SET I

In the first set plants were tested for phytoremediating lead from soil using EDTA as a chelator. Three concentrations of lead solutions were taken: 5ppm, 50ppm, 500ppm for the experiment two sets of plants was used.

SET II

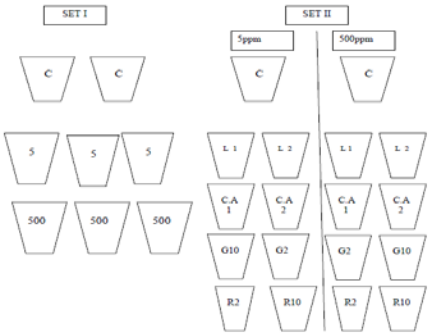
In the second set the plants were tested for determining the efficiency of other metal uptake enhancers-lime, citric acid and garlic as compared to EDTA.

In this set only 2 concentrations ie.5ppm (least) and 500ppm (max).Here there was control each for the 2 concentrations but devoid of any metal uptake enhancer. There were duplicates of each metal uptake enhancer. So there were 16pots+2 pots of control.

In the second set, the seeds were sown and after 3 days germinated plants were spiked with 2 concentration of lead: lowest (5ppm) and highest(500ppm).After about 2 weeks from the day of sowing or one week before harvesting; the plants were spiked with metal uptake enhancers respectively except the controls.

Duplicates of the garlic sets were performed wherein one set was harvested after 2 weeks (G2, G10) and other after 3 weeks (R2, R10).hence G2, G10 were spiked with garlic 1 week after sowing the seeds. And harvested the next week
Rest all were harvested after 3 weeks.

Figure 1: Schematic diagram of the pot planting method



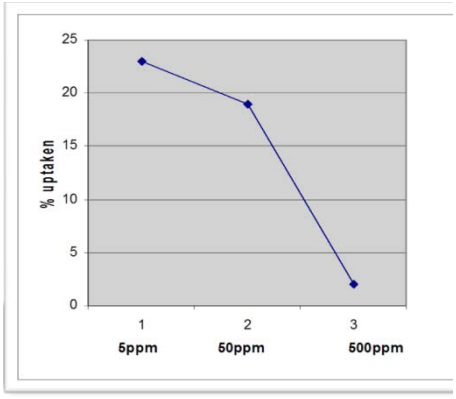
Harvesting and plant analysis: After harvesting the plants were washed with tap water followed by distilled water to ensure all the soil was removed from the plant surface and didn't interfere with analysis. They were dried on Petri plates, later oven-dried at 110 degrees for 24hrs. And weighed.

Dried material was ground using mortar and pestle. Some definite amount was taken for microwave digestion. Teflon vessels were used. About 0.5gm was taken in the vessel, 3ml of nitric acid was added to it closed tightly and microwave digested for 4min.The digested sample was filtered through what Mann paper no.42 and filtrate collected in 25ml standard flask. Finally the volume was made using ultra pure distilled water. Inductively coupled plasma spectrophotometer was used to analyze the samples for lead.

3.0 OBSERVATIONS

Table 1: Set I results

Metal uptake enhancers						
5ppm:1250µ.gms=1.25mgs of Pb spiked						
	µ.gm	Avg	Ppm	Avg	%	Avg
1	143.6	143.6	1.04	1.04	11	11
2	283.2	283.2	1.9	1.9	22.5	22.5
50ppm:12500µ.gms=12.5mgs of Pb spiked						
1	1099	1099	6	6	8.8	8.8
2	1871	1871	14	14	15	15
500ppm:125000µ.gms=125mgs of Pb spiked						
1	6892	6892	47	47	5.5	5.5
2	2979	2979	18.7	18.7	2.4	2.4



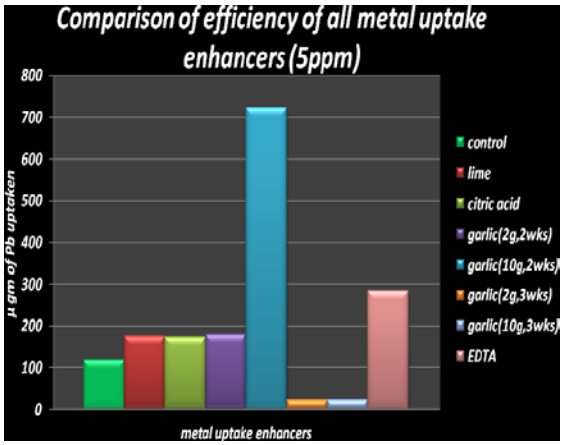
Graph1: Set I graphical representation of set I plants treated with 5, 50, 500 ppm of lead solution.

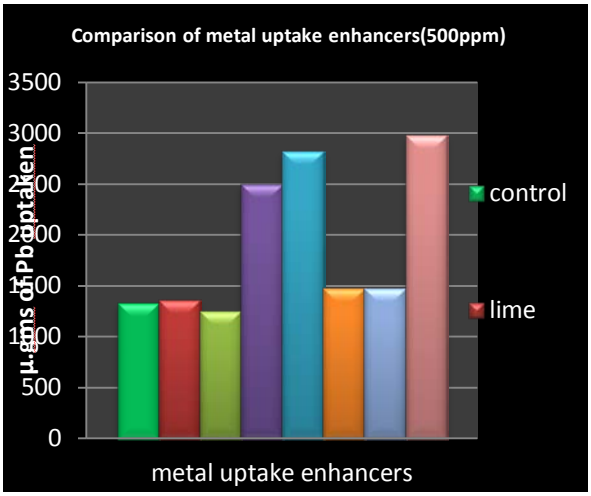
Table 2: Comparison of efficiency of metal uptake enhancers at 5 ppm and 500 ppm(**Set II)**

5ppm:1250µ.gms=1.25mgs of Pb spiked				
Sample	Conc.	Wks	µ.gm	%
Control	-	3	117	9.3
Lime 1	960ppm	3	185	14
Lime 2	960ppm	3	166	
C.A 1	960ppm	3	194	13.9
C.A 2	960ppm	3	153	
Garlic	2g	2	179	14.3
	10g	2	722.8	57.7
Garlic	2g	3	21.4	1.7
	10g	3	22.6	1.8
EDTA	2.68mM	3	283.2	22.5

500ppm:125000µ.gms=125mgs of Pb spiked				
Sample	Conc.	Wks	µ.gm	%
Control	-	3	1335	1
Lime 1	960ppm	3	1438	1.2
Lime 2	960ppm	3	1278	1
C.A 1	960ppm	3	1727	1.4
C.A 2	960ppm	3	770	0.6
Garlic	2g	2	2500	2
	10g	2	2823	2.3
Garlic	2g	3	1475	1.2
	10g	3	1479	1.2
EDTA	2.68mM	3	2979	2.4

Graph 2: Comparison of efficiency of metal uptake enhancers at 5 ppm and 500 ppm





4.0 RESULTS DISCUSSION

(I) Physical characteristics were studied

Visual differences in color, root growth, shoot length of all lead treated plants were retarded compared to controls.

Leaf diameter was largest in case of plants exposed to high conc. (500ppm) of lead. This could be attributed to high metal concentrations triggering the leaf growth. Others showed similar leaf area as in control. Shoot growth was inhibited in plants (stunting) with lead as compared with control.

Dark green leaves, stunted foiliage, an increase in amounts of shoots are the effects of lead on plants (Jack.E.Fergusson)

Roots of all lead treated plants were purplish, in contrast to the dirty white roots of untreated plants.

Stunting, chlorosis, blackening of roots are major toxic symptoms due to lead. The stunting and anthocyanin pigmentation (purple color of roots) in leaves of lead-treated plants can be ascribed to a deficiency of an element like phosphorous

Lead had been shown to form insoluble complexes with phosphorous (Johnson and Proctor,1997; Johnson, McNeilly and Putwain,1997) .Similar anthocyanin pigmentation and inhibited growth have been recently noted in Indian mustard treated with 500g/l lead (Daniels-Davis,1996)

II) Metal uptake in plants

Very high efficiency of uptake seen in plants treated with 5ppm of lead which indicates brassica.juncea are sturdy at low levels of lead (5ppm) & have the ability to uptake large amounts of lead as compared to 50ppm,500ppm.

In case of 50ppm there was moderate uptake activity but declined considerably at higher concentration of 500ppm.This shows that 50ppm is the threshold limit for brassica.juncea to uptake lead. Refer figure ##

For further study purpose, harvesting was conducted at intervals of 2 and 3 weeks. while comparing the results it was concluded that the plants uptake better in 3 weeks period at low lead concentration .but in case of 500ppm the lead toxicity increases to a greater extent which can cause degeneration of plant roots in the 3 weeks. Hence shows marginal uptake of lead.

But it's also concluded that at higher concentrations 2 week period shows better results.

In case of plant treated with 5ppm, garlic (10g, 2weeks) showed extra-ordinary results. Low toxicity and effective sulphur metabolism induces tolerance to this set of plant The possible reason which accounts for the high metal uptake is the role of sulphur. Sulphur added by the garlic plays an important role in formation of phytochelatins which are cysteine rich moieties.Phytochelatins bind with Pb and the PC-Pb complex so formed brings about its detoxification thereby rendering the plant more tolerance. Hence increased tolerance induces more metal uptake in brassica.juncea .the exact nature of this mechanism is not investigated and has future implications in research.

EDTA is established as the best chelator for Pb uptake but due to its potential risks to contaminate groundwater and further food chain, other natural substitute were tested of which citric acid and lime were equally efficient but not very effective.

Garlic (2g) of 2 weeks-showed moderate results like citric acid and lime.

On the whole when all the metal uptake enhancers were compared with EDTA; showed lesser efficiency than EDTA except for garlic (10g, 2weeks).This confirms that EDTA is a very effective synthetic chelator.

When all of them were compared with control, except Garlic (2g, 10, 3weeks) all showed better efficiency. The drastic reduction in metal uptake is attributed to high toxic effects by the third week which causes degeneration of roots within the soil and they go back to the soil unavailable for analysis.

In case of plants treated with 500ppm, it must be noted however that brassica.juncea showed the ability to accumulate Pb, its sensitivity to higher levels (500ppm) of lead as evidenced by its reduced biomass may limit its potential in phytoextraction effort.

In this case, Garlic (10g, 2weeks) showed equally good results

But EDTA served to be the best. Low conc. of lead (5ppm) posed lesser toxicity problems and hence roots were enough to uptake high amounts of Pb whereas in higher concentration (500ppm) roots degenerated and become weak which led to lesser uptake of lead. Hence both concentrations of garlic harvested at 3 weeks showed poor activity due to the degeneration of roots exposed to Pb for elongated periods.

Lime and citric acid enhanced the metal uptake ability to a lesser extent (Lime was better than control.

In conclusion; synergistic effect of optimum Pb concentration and garlic concentration (10g, 2weeks) enabled sulphur metabolism (PC synthesis detoxification) possible easily and hence the extra ordinary results.

5.0 CONCLUSIONS

Brassica.juncea (Indian mustard) exhibited Pb uptake and is a hyperaccumulator of lead.

Garlic (2weeks, 10g) showed the best results in the experiment conducted.

As compared efficient synthetic chelators like EDTA, garlic proved to be an efficient natural substitute for phytoremediating lead.

6.0 RECOMMENDATIONS

Mechanism of enhanced metal uptake due to garlic has been attributed to increased sulphur metabolism and phytochelatin activity in the present study. Biochemical pathway responsible for the sequestration of Pb in brassica.juncea needs to be worked upon and has got further scope for research.

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