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Effect of chelating agent on phytoextraction of lead from contaminated soil by *Zea mays*

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Abstract

Phytoextraction of heavy metals enhanced by using chelating agents was proposed as an effective approach to remove heavy metals from contaminated soil by use of hyper accumulator plants. The effects of application of chelating agents *viz*., Cyclohexane diamino tetraacetic acid (CDTA), Citric acid (CA), Diethylene triamine pentaacetic acid (DTPA), Nitrilo triacetic acid (NTA) @ 10 mmol kg^{-1} soil and FYM (at 3%) on the growth of *Zea mays* and its Pb uptake were investigated in sewage sludge un-amended and amended (at 3%) soil using the pot-culture experiments. Application of chelating agents decreased the dry matter yield of roots of *Zea mays* while, higher values of dry matter yield (8.86 g pot^{-1}) was observed in case of FYM sewage sludge amended soil at 80 days after sowing. FYM addition was found beneficial as compared to control (Pb_{180}). Dry matter yield of shoots of *Zea mays* increased over control due to application of CDTA and FYM. The highest dry matter yield of shoot (63.29 g pot^{-1}) was observed in CDTA with sewage sludge amended soil at 80 days after sowing. Whereas reverse trend was observed in NTA, CA and DTPA treated soils. Addition of sewage sludge at 3% on dry weight basis was found beneficial in improving the plant growth. Chelating agents enhanced the Pb uptake by both roots and shoots. The higher values of Pb uptake by roots (2.89 mg pot^{-1}) and shoots (13.21 mg pot^{-1}) was observed in CDTA treated soil after 80 days of sowing in sewage sludge amended soil as compared to un-amended soil. Application of CDTA was found more effective in enhancing the Pb uptake by *Zea mays* roots and shoots than any other chelating agents at both the growth stages. Concentration of Pb in root was higher than that of shoots. Maximum Pb concentration was observed in NTA treated soil in both roots and shoots compare with other chelating agents. The chelating agents were found useful in enhancing phytoextractability of Pb by *Zea mays*. Hence, marginally Pb contaminated soil may be remediated by adding chelating agents.

Keywords:Chelating agents, Lead, Maize, Phytoextraction

Abbreviations: CA: Citric acid, **CDTA:** Cyclohexane diamino tetra acetic acid, **DAS:** Days after sowing, **DTPA:** Diethylene triamine pentaacetic acid, **FYM:** Farm yard manure, **NTA:** Nitrilo triacetic acid, **Pb :** Lead, **SS:** Sewage Sludge

Introduction

Large areas of land are contaminated with heavy metals due to increased industrilization, urbanization and various anthropogenic activities such as, use of treated and untreated waste water, sewage sludge, organic manure and compost, excessive use of fertilizer and pesticides. The heavy metals accumulated on agricultural lands are not subjected to degradation processes and therefor remain almost identifinitely in the environment, although the bioavailability of these chemicals can change considerably depending on their interactions with the various soil constituents. Excessive accumulation of heavy metals in agricultural soils has led to elevate heavy metal uptake by crops, and thus affect food quality and safety (Wang *et al.,* 2001), which may cause a potential hazard to human health by way of food chain. In fact, heavy metals have a significant toxicity for humans, animals, microorganisms and plants (Wagner, 1994; Hernandezochoa *et al.,* 2005; Fotakis and Timbrell, 2006). Therefore, the cleanup of heavy metal contaminated soils is emergent and imperative. Phytoremediation was considered as an alternative cleanup method by using plants such as trees, ornamentals and grasses to remove, destroy or sequester hazardous contaminants from environmental media, such as soil, water and air (Chen and Cutright, 2001; Prasad, 2003). It is an emerging technique which is cost-effective, environmentally friendly and technically applicable in situ, making it preferable to other chemical or mechanical techniques (Prasad, 2003). At present, there are two strategies of phytoextraction, (1) continuous phytoextraction which depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle *(e.g.,* hyper accumulations) and (2) chelate enhanced phyto-

-extraction based on the application of chelating agents to the soil to enhance metal uptake by plants (Garbisu and Alkorta, 2001; Alkorta *et al.,* 2004). Hyper accumulators or hyper accumulating plants are capable of accumulating large amount of trace elements including nickel (Ni), arsenic (As), zinc (Zn), cadmium (Cd) and lead (Pb) in their above ground tissues without any toxic symptoms (Sun *et al.,* 2007). However, the use of those species for phytoremediation on a commercial scale is limited due to its low biomass production and slow growth rate. Amongst the commercial crops grown in this region *Zea mays* has been reported to produce high biomass and accumulate significant amount of heavy metals in their tissues when induced through the addition of chelating agents (Blaylock *et al.,* 1997). Use of sewage sludge and FYM as source of organic matter is a common practice. Addition of chelating agents was reported to increase Pb contents in the solution phase (Gray *et al.,* 1999). Hence, higher extractability of Pb due to chelating agents may lead to their excessive accumulation in plants. The main objective of the present work was to assess the effect of DTPA, CDTA, NTA and citric acid (CA) on phytoextraction of Pb by *Zea mays* from contaminated soil.

Materials and Methods

***Site characteristics*:** Screen house experiment was conducted in CCS Haryana Agricultural University, Hisar, Haryana (latitude 29º-10' N, longitude 75º-46' E and an altitude of 215.2 m). The climate of the area is semi-arid type with hot summers and cool and dry winters. The temperature generally fluctuated between 1.9ºC (January) and 46ºC (May) during the year.

***Experimental details*:** Surface sandy loam soil (0-15cm) was collected from the experimental field of the dry land Agriculture, CCS Haryana Agricultural University, Hisar, Haryana, air dried and ground to pass through a 2 mm sieve and mixed thoroughly. The processed soil sample was used for laboratory and screen house studies. The bulk sewage sludge sample was collected from the Sewer Treatment Plant, Industrial Estate, Okhala, New Delhi. The bulk sample of well decomposed farmyard manure (FYM) was taken from the manure pit of Dairy Farm, CCS Haryana Agricultural University, Hisar. The physico-chemical properties of soil, sewage sludge and FYM used are given in table 1. Two soil treatments, *viz*., Pb spiked at 180 mg Pb kg^{-1} soil and Pb spiked (at 6000 mg Pb kg^{-1} sludge) sewage sludge added at 3% on dry weight basis, six chelating agents, *viz.* Control (Pb_{180}), Nitrilo triacetic acid (NTA), Diethylene triamine pentaacetic acid (DTPA), Cyclohexane diamine tetraacetic acid (CDTA), Citric acid (CA) and FYM were used. The Pb treated soil was filled with 5 kg soil per pot separately in 72 polyethylene lined earthen pots (25 inch diameter). A basal dose of N, P, K, Mn, Fe, Zn and Cu at 50, 50, 60, 10, 10, 5 and 5 mg kg^{-1} soil was applied in solution form in each pot through urea, DAP, MOP, $MnSO_4$, FeSO4, ZnSO4 and $CuSO_4$ analytical grade salts. After addition of nutrient solutions, pots were wetted with deionised water to field capacity moisture content, and kept for equilibration for one week and dried to workable moisture content. The contents of each pot were then taken out, mixed thoroughly, refilled and incubated for ten days at near field capacity moisture content and process of mixing was repeated again. Soil samples were drawn from each pot before sowing. Ten healthy seeds of the *Zea mays* cultivar were sown in each pot. After emergence of seedlings, only four plants per pot were allowed to grow. Second dose of nitrogen was applied @ 25 mg N kg^{-1} soil in solution form 30 days after sowing (DAS). The pots were irrigated with deionised water as and when required. Chelating agents were applied @ 10 mmol kg^{-1} soil (1 mmol daily for 10 days in 10 split doses) 40 DAS. Ten days after application of chelating agents half of the Maize crop (*i.e.* 3 replication out of 6) were harvested 50 DAS and remaining 3 replications of each treatment where harvested after 80 DAS.

***Plant and soil analysis*:** The leaves and harvested plants were washed with 0.1 N HCl followed by distilled water and finally with double distilled water. The washed plant samples were transferred to paper bags, air dried and finally oven dried at 65±2 °C to constant weight. Thereafter, the dry weight of shoots and roots were recorded. The samples were ground in a stainless steel grinder. In order to determine Pb in root and shoot sample, wet digestion was carried out in a diacid mixture of nitric acid and perchloric acid in 4:1 ratio. Lead in the digest was determined by using atomic absorption spectrophotometer (GBC-932 plus). After harvesting of *Zea mays*, soil in each pot was wetted with about one litre of distilled water, covered with plastic sheet and allowed to equilibrate for three days. Then representative soil samples (covering full depth of soil in pot) were drawn from each pot separately with the help of a stainless steel tube augar, air dried, ground, sieved and kept in polyethylene bags for chemical analysis. Stastistcal analysis were done using factorial completely randomized design.

Table 1. Physico-chemical characteristics of the experimental soil, sewage sludge and FYM

Properties		Content		
		Soil	Sewage sludge	FYM
Mechanical composition				
(a) Sand (%)		69.70	-	-
(b) Silt (%)		16.50	-	-
(c) Clay (%)		13.80	-	-
Textural class	Sandy loam		-	-
pH (1:2)		8.10	7.20	-
$EC_{1:2}$ (dS m^{-1})		0.50	2.10	-
Organic carbon (%)		0.32	12.20	27.80
CEC [Cmol(P^+) kg^{-1}]		11.80	-	-
$CaCO_3$ (%)		0.40	0.25	-
Total nutrients (%)				
(a) Nitrogen		0.09	1.29	1.18
(b) Phosphorus		0.01	0.41	0.70
(c) Potassium		0.10	0.73	2.50
Total metals (mg kg^{-1})				
(a) Cr		0.12	7.2	
(b) Pb		0.98	64.2	

Table 2. Effect of chelating agents and sewage sludge on dry matter yield (g pot^{-1}) of roots and shoots of *Zea mays* in Pb contaminated soil

Treatment	50 DAS		80 DAS		
	Without SS	With SS	Without SS	With SS	Mean
Root					
Pb_{180}	5.89	6.14	7.63	7.96	6.90
Pb_{180}+CDTA	4.91	5.20	6.51	7.21	5.96
Pb_{180}+CA	3.96	4.39	5.00	6.14	4.87
Pb_{180}+DTPA	4.21	5.00	5.91	6.53	5.41
Pb_{180}+NTA	3.11	4.11	4.96	5.64	4.45
Pb_{180}+FYM	6.06	7.23	7.81	8.86	7.49
Mean	4.69	5.34	6.30	7.05	
CD ($P<0.05$)	S=0.14 T=0.14 CA=NS SxT=0.19 SxCA=NS TxCA=0.34 SxTxCA=NS				
Shoot					
Pb_{180}	43.16	46.89	58.61	60.16	52.20
Pb_{180}+CDTA	45.69	49.99	61.30	63.29	55.07
Pb_{180}+CA	40.14	42.12	55.11	58.14	48.88
Pb_{180}+DTPA	42.31	45.84	56.69	59.19	51.01
Pb_{180}+NTA	37.14	40.13	49.11	52.61	44.75
Pb_{180}+FYM	44.16	47.65	59.14	62.15	53.27
Mean	42.10	45.44	56.66	59.26	
CD ($P<0.05$)	S=0.14 T=0.14 CA=NS SxT=0.19 SxCA=NS TxCA=0.34 SxTxCA=NS				

Results and Discussion

***Dry matter yield of root and shoot*:** The addition of chelating agent inhibited the plants growth and the dry biomass yields of root and shoot with different degrees as compared to control. The maximum mean dry matter yield of roots was observed in soil treated with FYM (7.49 g pot^{-1}) followed by CDTA (5.96 g pot^{-1}). However the addition of chelating agents CDAT, CA, DTPA and NTA decreased the root dry matter yield as compared to control (Table 2). The dry matter yield of shoots of *Zea mays* was also influenced variably by different chelating agents at both the growth stages. The higher mean dry matter yield of shoots (55.07 g pot^{-1}) was observed in soil treated with CDTA followed by soil treated with FYM

Table 3. Effect of chelating agents and sewage sludge on Pb concentration (ìg g^{-1}) in roots and shoots of *Zea mays* in Pb contaminated soil

Treatment	50 DAS		80 DAS		
	Without SS	With SS	Without SS	With SS	Mean
Root					
Pb_{180}	98.16	105.61	200.14	212.61	154.13
Pb_{180}+CDTA	170.34	178.63	390.61	400.63	285.05
Pb_{180}+CA	140.12	148.91	264.91	271.63	206.39
Pb_{180}+DTPA	155.17	161.31	365.14	374.10	263.93
Pb_{180}+NTA	180.91	187.63	495.81	509.61	343.49
Pb_{180}+FYM	121.31	127.63	225.14	236.14	177.55
Mean	144.33	151.62	323.62	334.12	
CD ($P<0.05$)	S=1.75 T=1.75 CA=NS				
	SxT=2.48 SxCA=4.29 TxCA=NS SxTxCA=NS				
Shoot					
Pb_{180}	79.12	95.63	154.16	162.61	122.88
Pb_{180}+CDTA	101.63	119.14	199.13	208.73	157.16
Pb_{180}+CA	90.61	99.61	175.18	182.16	136.89
Pb_{180}+DTPA	99.18	105.54	185.14	193.63	145.87
Pb_{180}+NTA	119.63	125.63	231.61	238.11	178.84
Pb_{180}+FYM	88.61	95.64	171.63	179.63	133.88
Mean	96.46	106.86	186.14	194.14	
CD ($P<0.05$)	S=1.19 T=1.19 CA=2.07				
	SxT=1.69 SxCA=2.93 TxCA=2.93 SxTxCA=4.14				

Table 4. Effect of chelating agents and sewage sludge on Pb uptake (ìg pot^{-1}) by roots and shoots of *Zea mays* in Pb contaminated soil

Treatment	50 DAS		80 DAS		
	Without SS	With SS	Without SS	With SS	Mean
Root					
Pb_{180}	577.19	648.42	1527.04	1692.46	1111.28
Pb_{180}+CDTA	837.61	928.89	2542.84	2888.19	1799.38
Pb_{180}+CA	555.05	653.49	1324.64	1668.00	1050.29
Pb_{180}+DTPA	653.59	806.64	2158.37	2442.96	1515.39
Pb_{180}+NTA	562.78	771.07	2460.52	2876.38	1667.69
Pb_{180}+FYM	735.23	922.96	1759.30	2092.21	1377.42
Mean	653.57	788.58	1962.12	2276.70	
CD ($P<0.05$)	S=37.41 T=37.41 CA=64.79				
	SxT=52.90 SxCA=91.63 TxCA=NS SxTxCA=NS				
Shoot					
Pb_{180}	3415.06	4488.86	9035.69	9786.20	6681.45
Pb_{180}+CDTA	4647.16	5963.79	12209.08	13212.07	9008.02
Pb_{180}+CA	3638.16	4204.55	9654.40	10593.72	7022.71
Pb_{180}+DTPA	4205.25	4851.77	10501.04	11464.32	7755.59
Pb_{180}+NTA	4444.84	5042.80	11376.42	12527.15	8347.80
Pb_{180}+FYM	3914.38	4558.33	10152.42	11164.91	7447.51
Mean	4044.14	4851.68	10488.17	11458.06	
CD ($P<0.05$)	S=234.95 T=234.95 CA=NS				
	SxT=332.27 SxCA=575.50 TxCA=NS SxTxCA=NS				

S=Soil, T= Time, CA= Chelating agent, SS= Sewage sludge

(53.27 g pot^{-1}) as compared to control. The minimum dry matter yield of both root and shoot was observed in NTA treated pots. Quartacci *et al.* (2006) demonstrated that the dry weight of *B. Junce*a shoot showed a significant (33%) reduction in NTA amendment soil at 5 mmol kg^{-1} in comparison to control. Application of chelating agents influenced dry matter yield of roots differentially depending upon the nature of the chelating agents. The addition of chelating agents such as CDTA, Citric acid, DTPA and NTA was reported to decrease the root dry weight probably due to increased availability of Pb in the rhizosphere (Mishra, 2004). The beneficial effect of FYM on root growth might have been due to the inactivation of Pb, thereby avoiding its direct injury to the newly emerging roots (Mishra, 2004). Among the chelating agents, NTA was found most effective in decreasing the dry weight of roots. Similar results were also observed by Kulli *et al*, (1999) and Mishra (2004). The mean value of root dry matter yield was increased when amended with sewage sludge (5.34 g pot^{-1} after 50 DAS and 7.05 g pot^{-1} after 80 DAS) over un-amended sewage sludge soil (4.69 g pot^{-1} after 50 DAS and 6.30 g pot^{-1} after 80 DAS). The same trend was observed in shoot dry matter yield (Table 2). Ramprakash *et al.* (2012) also reported the similar results that among the chelating agents, NTA was most effective in decreasing the dry matter yield of root and shoot of *Zea mays* crop in Cr contaminated soil due to improved availability of Cr in the rhizosphere, Narwal *et al.* (1983) reported that despite adequate nutrient supply to all pots, application of sewage sludge significantly increased the dry matter yield of fodder rape. This is due to release of mineral N during the process of mineralization in sludge amended soil (Soni *et al.,* 1994).

***Lead content in Zea mays*:** Application of all the chelating agents significantly increased the Pb content in roots and shoots in comparison to control (Pb_{180}) at both the growth stages (Table 3). Application of NTA increased the Pb concentration by 84.30 and 77.67% in root and 51.14 and 31.37% in shoot of sewage sludge un-amended and amended soils at first stage of growth, respectively. Increased accumulation of Pb in plants due to application of chelating agents has also been reported by Kulli *et al.*, 1999 and Lesage *et al.*, 2005. The mean Pb content in roots was 144.33 and 151.62 µg g^{-1} and in shoots 96.46 and 106.86 µg g^{-1} in sewage sludge un-amended and amended soils at first and 323.62 and 334.12 µg g^{-1} in root and 186.14 and 194.14 µg g^{-1} in shoot at second stage of growth, respectively. Lead content was significantly higher in root than shoot of plants at both the growth stages which was consistent with the report by Xiong et al. (2004 a, b). Removal of toxic lead from the rhizosphere is necessary in order to avoid its accumulation in the above ground edible parts of plant. Marginally contaminated soils may be remediated by increasing phytoextractability of metals by adding chelating agents in soils because they form soluble complexes with metallic ions (Kulli *et al*, 1999 and Tatiana *et al*, 2006).

***Lead uptake by roots and shoots*:** Application of CDTA increased the Pb uptake by *Zea mays* roots by 45.11 and 43.25% and in shoot 36.08 and 32.08 % in sewage sludge un-amended and amended soils at first stage of growth and 66.52 and 70.64% in root and 35.11 and 35.00%, in shoot at second stage of growth, respectively as compared to control (Pb_{180}). Significantly higher values of Pb uptake were observed in roots with sludge amended as compare to un-amended soil (Table 4). The effectiveness of chelator enhanced Pb uptake in the plants was in sequence of CDTA>NTA>DTPA>FYM>CA. Increased uptake of Pb with the application of CDTA as compared to other chelating agents was due to increased availability of Pb in rhizosphere, higher dry matter yield production and Pb concentration in roots and shoots of *Zea mays* (Kulli *et al.,* 1999; Mishra, 2004 and Tatiana, 2006).

Conclusion

It was concluded that chelating agents decreased the dry matter yield of root of *Zea mays* while FYM addition was found beneficial as compared to control (Pb_{180}). Dry matter yield of shoot of *Zea mays* increased due to application of CDTA and FYM whereas reverse trend was observed in NTA, CA and DTPA treated soils. Pb concentration was found higher in root as compared to shoot. NTA was found more effective in enhancing Pb accumulation in maize root and shoot than other chelating agents. Application of CDTA was found more effective in enhancing Pb uptake by *Zea mays* root and shoot than any other chelating agents at both the growth stages.

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