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Effect of chelating agents on phytoextraction of Ni from contaminated Soil by *Zea mays*

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Abstract: The effects of application of CDTA, (CA), DTPA, NTA and FYM on the growth of *Zea mays* and its Ni uptake and accumulation were investigated 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 (11.35 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 (Ni_{90}). Dry matter yield of shoots of *Zea mays* increased over control due to application of CDTA and FYM. The highest value of dry matter yield of shoot (86.05 g pot$^{-1)}$ was observed in case of CDTA with sewage sludge amended soil at 80 days after sowing. Whereas reverse trend was observed in NTA, CA and DTPA treated soils. Chelating agents enhanced the Ni uptake by both roots and shoots, higher values of Ni uptake by roots (3415.44 µg pot^{-1}) and shoots (10104.98 µg pot^{-1}) Was observed in NTA and CDTA treated soil after 80 days of sowing in amended as compared to sewage sludge unamended soil. Application of CDTA followed by NTA was found more effective in enhancing the Ni uptake by *Zea mays* roots and shoots than any other chelating agents at both the growth stages. The chelating agents are found useful in enhancing phytoextractability of Ni by *Zea mays*. Hence, marginally Ni contaminated soil may be remediated by adding chelating agents.

Keywords: Chelating agents, Nickel, Phytoextraction, *Zea mays*

INTRODUCTION

Mining, manufacturing and other anthropogenic activities have contributed to extensive soil contamination over the past century, metals being the main group of inorganic contaminants. The untreated effluents, mixed sewer waters are rich sources of almost all the essential and toxic elements and their continuous use may lead to their accumulation in soil to such an extent that may prove toxic to plant and beneficial microorganisms. Though, the industrial effluent mixed sewer waters and sewage sludge improve soil fertility by enhancing the status of essential plant nutrients, yet there is a need to monitor their impact on of soil health due to addition of toxic metals. Soil contamination by Ni originates from silver refineries, electroplating, zinc-based casting and storage battery, mining, mineral and organic fertilizers, pesticides, and urban disposals (Ali *et al.* 2013, Wuana and Okieimen 2011). At very low concentrations, Ni is an essential element for some higher plants, which acts as a cofactor for the urease enzyme (Gheibi *et al.,v* 2009). It is also well known for its detrimental nature to plants and the environment when present in excess concentrations. The chronic toxicity of Ni to human health has been also recognized, such as gastrointestinal irritation, allergy, and cancer of lungs, nose, and bone (Ali *et al*., 2013).Contamination of soil with heavy metals may be avoided by chemical, physical or biological techniques (McEldowney *et al.*, 1993). Chemical and physical treatments are often destructive to the soil as they irreversibly alter important soil properties. Physicochemical technologies of soil remediation render the land useless as a medium for plant growth as they remove all the biological activities in the process of decontamination causing adverse effect on biodiversities. In view of the above, there is a need to develop suitable technique for soil remediation by enhancing phytoextraction of Ni from contaminated soils which is termed as phytoremediation. Phytoremediation has emerged as aneconomical, eco-friendly, and aesthetically acceptable technology in the recent years amongst the various strategies adopted for removal of the metal from the ontaminated sites (Ghosh and Singh 2005, Dickinson *et al.*, 2009, Sainger *et al.*, 2011, Bauddh and Singh 2012a, 2012b, Olivares *et al.* 2013, Kumar *et al.*, 2014). 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., hyperaccumulations), and (2) chelateenhanced phytoextraction based on the application of chelating agents to the soil to enhance metal uptake by plants (Garbisu and Alkorta, 2001; Zhou and Song,

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2004; Alkorta *et al.* 2004).

Among different types of phytoremediation, phytoextraction is the most efficient option in large-scale remediation projects, in which hyperaccumulator plants are used to extract and translocate heavy metals from the contaminated soil to their harvestable above-ground parts (Bert *et al.* 2009; Ali *et al.* 2013,). 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. However, use of sewage sludge continuously may increase heavy metals in soil but organic matter, present in the sludge can complex/immobilize Ni and decrease their bioavailability (McBride *et al.,* 1998). The main objective of the current work was to assess phytoextraction of heavy metal by *Zea mays* from contaminated soil. To this point, chelator-induced phytoextraction study was carried out with DTPA, CDTA, NTA and citric acid (CA) to determine the capacity of *Zea mays* to phytoextract Ni from contaminated soil.

MATERIALS AND METHODS

Pot culture experiment: The bulk surface sample (0-15 cm) of a sandy loam soil was collected from the experimental area of the dry land Agriculture, CCS Haryana Agricultural University, Hisar, Haryana. It was air dried, ground to pass through a 2 mm sieve and mixed thoroughly. The processed soil sample was used for the analysis of physico-chemical properties like Organic carbon (Walkley and black, 1934), CEC (Hesse, 1971) and available N (Subbiah and Asija, 1956), P (Olsen, 1954) and K were determined by flame photometer.

The bulk sewage sludge sample was collected from the sewer treatment plant, industrial estate, Okhala, New Delhi farmyard manure (FYM) was taken from the manure pit of dairy farm, CCS Haryana Agricultural University, Hisar It was air dried, ground to pass through a 2 mm sieve and mixed thoroughly before use. The processed sewage sludge sample was used for determination of different physico-chemical properties like total N by Nessler's reagent method (Lindner, 1944), P (Koenig and Johnson, 1942) and K were determined by Flame photometer. Heavy metals Pb, Cd, Cr and Ni were determined by atomic absorption spectrophotometer(GBC-932 plus). The physico-chemical properties of soil, sewage sludge and FYM used are given in Table (1). Two soil treatments, viz., Ni spiked @ 90 mg Ni kg^{-1} soil and Ni spiked (at 300 mg Ni kg^{-1} sludge) sewage sludge added at 3% on dry weight basis, six chelating agents, viz. Control Control (Ni_{90}), Nitrilotriacetic acid (NTA), Diethylenetriamine-pentaaceticacid (DTPA), Cyclohexanediaminotetraacetic acid (CDTA), Citric acid (CA) and FYM were used. The Ni treated soil was filled separately in 72 polyethylene lined earthen pots (25 inch diameter) each at 5 kg soil per pot. 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 their 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 at 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 at 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.

Analysis of plant samples: The leaves and harvested plants were washed with 0.1N HCl followed by distilled water and finally with double distilled water. The washed plant samples was transferred to paper bags, air dried and finally oven dried at 65±2 ^{0}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 Ni in root and shoot sample, wet digestion was carried out in a diacid mixture of nitric acid and perchloric acid in 4:1 ratio. Nical in the digest was determined by using atomic absorption spectrophotometer (GBC-932 plus).

Statistical analysis: All treatments were replecated six times in the experiments the mean were calculated by the microsoft office Excel 2003. Stastistcal analysis were done using factorial completely randomized design.

RESULTS AND DISCUSSION

Toxicity symptoms: The present study observed that there was no adverse effect of applied Ni on the germination in both sewage sludge unamended and amended soils. Overall growth of *Zea mays* plants was better in sewage sludge amended than unamended soil. Application of chelating agents after 40 days of sowing caused temporary wilting of *Zea mays* leaves recovered within 2 to 3 days after the application of chelating agents. Such wilting symptoms were more prevalent in plants grown on when NTA was applied.

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	-
Nutrients (%)				
(a) Nitrogen		0.09	1.29	1.18
(b) Phosphorus		0.01	0.41	0.70
(c) Potassium		0.10	0.63	2.50
Total metals (mg kg^{-1})				
	Cr	0.12	7.2	
	Pb	0.98	64.2	
	Cd	3.22	7.2	
	Ni	11.37	64.2	
	Zn	29.72	215	
	Mn	145.90	360	
	Cu	22.10	263	

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

	50 DAS		80 DAS		
Treatment	Without SS	With SS	Without SS	With SS	Mean
Root					
Ni_{90}	7.35	8.12	9.30	9.64	8.60
Ni_{90}+CDTA	6.60	7.50	8.00	9.00	7.78
Ni_{90}+CA	6.03	6.90	7.42	7.80	7.04
Ni_{90}+DTPA	6.20	7.00	7.63	7.95	7.20
Ni_{90}+NTA	5.77	6.70	6.90	7.20	6.64
Ni_{90}+FYM	7.67	8.35	10.21	11.35	9.40
Mean	6.60	7.43	8.24	8.82	
CD(P=0.05) Soil=0.09 Time=0.09 Chelating Agent=0.16 SxT=0.13 SxCA=0.23 TxCA=0.23 SxTxCA=0.32					
Shoot					
Ni_{90}	52.90	56.73	75.93	80.15	66.43
Ni_{90}+CDTA	54.80	61.50	78.40	86.05	70.19
Ni_{90}+CA	51.61	55.00	68.35	75.15	62.53
Ni_{90}+DTPA	52.54	55.70	73.33	79.06	65.16
Ni_{90}+NTA	49.89	54.57	59.70	74.90	59.77
Ni_{90}+FYM	53.60	58.41	76.32	84.40	68.18
Mean	52.56	56.99	72.00	79.95	
CD(P=0.05) Soil=0.19 Time=0.19 Chelating Agent=0.33 SxT=0.27 SxCA=0.47 TxCA=0.47 SxTxCA=0.67					

Dry matter yield of roots and shoots: The dry matter yield of roots and shoots of *Zea mays* as influenced by different treatments in Ni contaminated soil is presented in (Table-2). The mean dry matter of roots ranged from 6.64 to 9.40 g pot^{-1}. The maximum mean dry matter of roots was observed in Ni_{90}+ FYM and the least in Ni_{90}+NTA treated soil. The mean dry matter of roots in sewage sludge unamended and amended soils at first stage was 6.60 and 7.43 g pot^{-1}, respectively. Similar trend was also observed at second stage of growth. The interaction between soil, time and chelating agent was significant. Application of CDTA, CA, DTPA and NTA chelates caused 9.5%, 18.1%, 16.27% and 22.79% significant reduction at probability level 0.05 in the dry matter of roots as compared to control. FYM addition increased the dry matter of *Zea*

Table 3. Effect of chelating agents and sewage sludge on Ni uptake (µg pot^{-1}) by roots and shoots of *Zea mays* crop in Ni contaminated soil.

	50 DAS		**80DAS**		
Treatment	**Without SS**	**With SS**	**Without SS**	**With SS**	**Mean**
			Root		
Ni_{90}	574.11	694.31	1695.07	1864.92	1207.10
Ni_{90}+CDTA	926.70	1092.06	2803.96	3261.31	2021.00
Ni_{90}+CA	761.30	894.85	1730.39	1922.08	1327.15
Ni_{90}+DTPA	845.10	977.50	2535.39	2730.92	1772.23
Ni_{90}+NTA	833.74	1007.61	3214.41	3415.44	2117.80
Ni_{90}+FYM	770.05	887.99	2035.12	2321.91	1503.77
Mean	785.17	925.72	2335.72	2586.10	
CD(P=0.05) Soil=20.90 Time=20.90 Chelating Agent=36.20 SxT=29.56 SxCA=51.20 TxCA=51.20 SxTxCA=72.41					
			Shoot		
Ni_{90}	2221.83	2445.08	5695.42	6259.78	4155.53
Ni_{90}+CDTA	2718.18	3579.79	8792.63	10104.98	6298.90
Ni_{90}+CA	2415.25	2824.38	6233.54	7389.46	4715.66
Ni_{90}+DTPA	2512.22	2922.57	8100.93	8344.79	5472.63
Ni_{90}+NTA	3838.94	4305.41	7618.66	9921.40	6421.11
Ni_{90}+FYM	2385.26	2943.88	6912.24	8148.84	5097.56
Mean	2681.95	3170.19	7225.57	8363.21	
CD(P=0.05) Soil=23.74 Time=23.74 Chelating Agent=41.12 SxT=33.58 SxCA=58.16 TxCA=58.16 SxTxCA=82.24					

mays roots by 4.35 at first and 9.78% at second stage of growth in sewage sludge unamended soil whereas the corresponding values are 2.83% and 17.74% in sewage sludge amended soil over control (Ni_{90}). The minimum dry matter of roots was observed in NTA treated pots. Significant increase in the dry matter of root was also observed due to sewage sludge application. Application of CDTA, DTPA, Citric acid and NTA reduced the root growth but addition of FYM caused significant increase in root dry matter at both growth stages.

In case of shoots, the mean dry matter yield ranged from 59.77 to 70.19 g pot^{-1}. Maximum mean dry matter yield of shoots was observed in Ni_{90}+ CDTA and the least in Ni_{90}+NTA treated pots. The dry matter yield of shoots increased due to application of CDTA and FYM but it decreased in citric acid, DTPA, and NTA treated pots as compared to control where none of the chelating agents was applied. The mean dry matter yield of shoot obtained at first stage was 52.56 and 56.99 g pot^{-1} in sewage sludge unamended and amended soils, respectively. Similar trend was visible at second stage of growth. The dry matter yield of *Zea mays* shoots was influenced significantly by different treatment combinations i.e. soil, time and chelating agents which is evident from the fact that about 3.59 and 3.25 per cent increase in shoot yield was observed at first and second growth satge, respectively in CDTA treated sewage sludge unamended and amended . However, there was significantly at probability level 0.05 lower as compared to control where none of the chelating agents was applied.

Positive effect of FYM on root growth might have been due to the complexation of Ni resulting in decreasing the toxic effect of Ni on emerging roots (Maiti, 2001); (Indoria, 2004). It also clear from data that among the chelating agents, NTA was most effective in decreasing the dry matter of roots probably due to increased availability of Ni in the rhizosphere (Kulli *et al*, 1999, Robinson *et al*, 2000, Mishra, 2004). Addition of CDTA improved shoots dry matter yield significantly over others. Similar results were also observed by (Ramprakash *et al.*, 2013) who reported that dry matter yield of roots and shoots of *zea mays* increase due to application of FYM and CDTA whereas reverse trend was observed in NTA, Citric acid and DTPA treated soils. (Lombi *et al*, 2001) who observed that compared with the control, the application of EDTA at the flowering stage did not significantly affect the shoot and root biomass of maize in the first cropping. However, the biomass of maize at the second and third harvest was significantly lower than that in the first. (Greman *et al.,* 2003) reported that single and weekly addition of 10 m mol kg^{-1} EDTA resulted in rapid senescence of the plant shoots and lowered the yield of cabbage biomass compared with that of control. Plant growth was strongly inhibited by the highest single dose of EDTA. Ethytenediamine dissuccinate (EDDS) was less phytotoxic than EDTA but the results were statistically non significant. This could be explained either by inherently lower phytotoxicity of EDDS or as a result of EDDS rapid biodegradation in the soil. (Tandy *et al*., 2006) reported that application of EDDS (10 mmol kg^{-1} soil) showed adverse effect on plants. Two days after the addition of EDDS to the soil, shoots started to show sign of toxicity and by 3 days they were necrotic resulting in reduced dry weight of sunflower shoots.

Nickel uptake by roots and shoots: Application chelating agents significantly increased the Ni uptake by roots in comparison to control (Ni_{90}) at both the growth stages (Table 3). The mean Ni uptake by roots varies from 1207.10 to 2117.80 µg pot^{-1}. The maximum mean Ni uptake by shoots was observed in plants grown on Ni_{90}+NTA treated soil and the least in Ni_{90}. Application of CDTA increased the mean Ni uptake by 67.43% as compared to Ni_{90}. The mean uptake of Ni by roots was 785.17 and 925.72 µg pot^{-1} in sewage sludge unamended and amended soils at first stage of growth while the corresponding values were 2335.72 and 2586.10 µg pot^{-1} at second stage of growth respectively. Application of all the chelating agents significantly increased Ni uptake in roots as compared to control (Ni_{90}) at both the growth stages. Application of CDTA increased the Ni uptake by *Zea mays* roots at first stage by 61.42 and 57.29% in sewage sludge unamended and amended soils, respectively as compared to where chelating agents were not applied. Significantly higher uptake of Ni was observed in roots of *Zea mays* from amended soil as compare to sewage sludge unamended soil. NTA was found to be more effective in enhancing the Ni uptake by maize roots than other chelating agents at both the growth stages.

The mean Ni uptake by shoots varied from 4155.53 to 6421.11 µg pot^{-1} (Table-3). The maximum mean Ni uptake by shoots was observed in plants grown on Ni_{90}+NTA treated soil and the least in Ni_{90}. The mean Ni content of shoots, was 2681.95 and 3170.19 µg pot^{-1} in sewage sludge unamended and amended soil at first stage of growth, respectively. Application of CDTA increased uptake of Ni by *Zea mays* shoots by 22.34 and 46.41% in sewage sludge unamended and amended soils at first stage of growth but it eas relatively higher (54.38 and 61.43%) at second stage of growth, respectively as compared control (Ni_{90}). The results further reveal that addition of sewage sludge increased the Ni uptake at both the growth stages.

It is clear from the data that uptake of Ni increased due to NTA than any other chelating agent. Similar results were also observed by (Ramprakash *et al.,* 2013) who reported that application of NTA was found more effective in enhancing the chromium uptake by *Brassica juncea* root and shoots than other chelating agents at both the growth stages, by Meers *et al.* (2005) who reported that EDDS was more effective in increasing the metal uptake followed by EDTA, NTA and citric acid, by Tandy *et al*, (2006) who reported that uptake of metals during chelate assisted phytoextraction is related to the solublized metal concentration. Addition of chelating agents such as NTA, DTPA, CDTA, Citric acid etc have also been reported to increase Cd and Ni contents in the soil solution phase (Gray *et al.* 1999; Hong and Pintauro, 1996). Hence, higher extractability of Cd and Ni due to chelating agents may lead to their increased availability to plants Indian mustard as reported by Mishra (2004) and Tatiana (2006).

Conclusion

The maximum mean dry matter of roots was observed in Ni_{90}+ FYM and the least in Ni_{90}+NTA treated soil. Application of all the chelates caused significant reduction in the dry matter of roots of *Z.mays* as compared to control. FYM addition increased the dry matter of the roots by 4.35 at first and 9.78% at second stage of growth in sewage sludge unamended soil whereas the corresponding values are 2.83% and 17.74% in sewage sludge amended soil over control (Ni_{90}). The minimum dry matter of roots was observed in NTA treated pots. Significant increase in the dry matter of root was also observed due to sewage sludge application. Application of CDTA, Citric acid, DTPA and NTA reduced the root growth but addition of FYM caused significant increase in root dry matter at both growth stages.

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