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Effect of chelators on phytoextraction of nickel by maize from effluent contaminated soil

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Abstract

The pot experiment was conducted by taking three bulk surface soil samples varying in textures. First sample was taken from the sand dune area of Balsamand Village of Hisar District to study the tolerance of heavy metals by different genotypes of maize crop. Second sample was taken from the Atlas Industry effluent polluted field from the Kabirpur Village of Sonipat District to study uptake of Nickle. The soil samples were air dried, grounded and passed through a 2 mm stainless steel sieve and was mixed thoroughly. The processed soil samples were used for laboratory and pot experiments. The pot experiment for heavy metal tolerance study was conducted, taking crops *viz*., maize (three genotypes J-1006, HM-4 and HKH-1183) on Ni (@ 30, 60, 90, 120 and 150 mg kg^{-1}) spiked soil. The post-harvest soil samples of crop was analysed for the mean DTPA-extractable Ni as influenced by application of chelating agents. The results revealed that availability of mean values of DTPA-extractable Ni increased significantly with increasing level of Ni. FYM were applied and trend observed was control>FYM>NTA>EDTA>FYM+NTA>FYM+EDTA in maize post harvested soils.

Keywords: Chelator, phytoextraction, nickel uptake, heavy metal, toxicity, maize

Introduction

In an ideal ecological system, there would be no pollution. However, with the establishment of permanent human settlements by great numbers of people, pollution became a problem and has remained one ever since. Cities of ancient times were often noxious places, fouled by human wastes and debris. In the middle Ages, unsanitary urban conditions favoured the outbreak of population-decimating epidemics. During the 19th century, water and air pollution and accumulation of solid wastes were largely problems of only a few large cities. But, over the course of recent decades, urbanization, industrial and agricultural activities have led to a continuous production of huge amount of heavy metals contaminated solid, liquid and as fine particles directly into atmosphere and ultimately deposited on the surface of land and water bodies which finally on reaching the agricultural fields get accumulated in soil at hazardous levels (Raskin *et al.*, 1997 and Al-Hawari and Mulligan, 2006) [12, 2]. In addition, mining, smelting, and the associated activities are one of important sources by which soils, plants, and surface waters are contaminated (Jung, 2008) [7].

In Haryana also large amount of sewage water and industrial effluent is produced every day which is used as a potential source of irrigation of fields. Long term application of effluent for irrigating crops may cause potentially toxic metal accumulation in soil to such an extent that they may cause toxic effect to plant growth. Soil contamination by heavy metals is of major concern because of their toxicity and threat to both human health and environment.

Hence, there is a need to develop suitable biological soil remediation technique to remove contaminants. In fact, traditional state-of-the-art technology for the remediation of metal polluted soils is the excavation and burial of the soil at a hazardous waste site. However, these approaches are expensive, disruptive, and are not economically viable. Recently, efforts have been made towards finding remediation strategies that are less expensive and less damaging to soil properties than current approaches. One such method is phytoextraction in which plants uptake heavy metal from the soil, followed by harvesting the above ground biomass. Harvested material is disposed in brick kilns (as bio-energy source) and byproduct in a landfill (kilns ash) or also treated to recover metals. Use of chelating agents to enhance heavy metal uptake is another new line in the technique of phytoremediation. To be successful on a specific site, the remediation technique must be selected according to heavy metals on the soil particles. Some scientists recommended the use of hyperaccumulator species, other prefer plants with a lower accumulation rate but high biomass.

Amongst the commercial crops grown in Haryana rabi season, Indian mustard has been reported to produce high biomass.

Material and Methods

A pot experiment was conducted to investigate the Ni phytoextraction potential of three maize plant genotypes, namely, J-1006, HM-4 and HKH-1183) on Ni (@ 30, 60, 90, 120 and 150 mg kg^{-1}), in a light soil from sand dune areas of Balsamand, Hisar. Five levels of Cd concentration ranging from 0-120 mg kg^{-1} soil were taken for the study. The toxicity symptoms were recorded; biomass production, Cd concentration and finally the Cd uptake were measured to screen the best Cd tolerant Indian mustard genotype. The plants were harvested at maturity.

The polythene lined earthen pots were filled with 5 Kg of thoroughly mixed, air dried bulk soil sample collected from sand dune area of Balsamand village, District Hisar. Basal dose of N, P, K, S, Mn, Fe and Zn @ 50, 50, 62, 20,10,10 and 5 mg Kg^{-1} soil, respectively was applied in solution form in each pot through urea, KH_2PO_4, $MnSO_4$. H_2O, Fe $SO_4.7H_2O$ and $ZnSO_4.7H_2O$, respectively in Indian mustard genotypes. To create desired level of Cd in their respective pots, appropriate volumes of $CdCl_2$ solutions were added. The treatments were imposed 15 days before sowing. The entire material in the pots was taken out. Treatment and nutrient solution mixed thoroughly and refilled. Each treatment was replicated three times. After addition of heavy metals and nutrient solution, the pots were wetted with deionised water to nearly 30 per cent moisture content, and kept for equilibration and drying 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. The contents of each pot were again taken out, mixed thoroughly and refilled to ensure uniformity.

Ten healthy seeds of each selected genotypes of Indian mustard were sown in pots. After germination, the seedlings were thinned to four plants per pot and grown to maturity. The pots were irrigated with deionised water as and when required. Second dose of nitrogen was applied @ 20 mg N Kg^{-1} soil at pod initiation stage as solution form. The metal toxicity symptoms were recorded during the growth period of crop. The growth parameters such as chlorophyll content (before application of second dose of fertilizer), plant height, and plant dry weight were also recorded at harvesting.

The Indian mustard genotypes were harvested and the leaves sheded by plants grown in different pots were collected pot wise. The leaves and above ground harvested plants were washed with 0.1N HCl, then with distilled water to remove dust etc. The washed plant material was put in paper bags, air dried and then oven dried at 65 ± 2^0C for constant weight. Thereafter, pot wise dry weight of plant materials were recorded and grounded in a stainless steel grinder, mixed and stored in polythene bags for chemical analysis. On the basis of highest heavy metal uptake a highly Cd tolerant genotype of Indian mustard was selected.

Results and Discussion

Phytoextraction of Ni by maize from Ni enriched soil as influenced by chelating agents

Toxicity symptom

Visual toxicity symptoms of Ni were recorded from germination to harvesting of crops. There was no adverse effect of applied Ni on germination of seeds in both unamended and FYM amended soil. But after few days of seed emergence, some chlorosis appeared on leaves, which get disappeared as growth progress. Overall growth of maize crop was better in Ni contaminated soil as compare to unamended soil at Ni_{100} levels. Moreover, it was also observed that growth of maize crop in Ni treated pot was comparatively better over Cd treated pot.

In FYM added soil, growth of maize at starting was slower as compare to other but on advance stage its growth was better as compare to other. When the chelating agents were applied 15 days before harvesting, the plant grown under chelating agents treated soil started showing some wilting symptoms after a days of application of chelating agents, which disappeared after 2-3 days. Such wilting symptoms were severe in EDTA treated soil and higher level of Ni**.**

Dry matter yield of shoot and root

Shoot

The data on shoot and root dry matter yield of maize crop as influenced by different chelating agents are presented in Table 1. The mean dry matter yield of shoot also increased significantly in the FYM alone and FYM in combination with EDTA and NTA amended soils over the control. The increase was 16.86, 10.22 and11.74 per cent in FYM (60.22 g pot^{-1}), EDTA+FYM (56.80 g pot^{-1}) and NTA+FYM (57.58 g pot^{-1}) amended pots over control, respectively. It might be due to fact that FYM contain essential nutrient and improve the soil health, which might have positive effect on growth of plant. While the application of EDTA and NTA significantly decreased 4.32 and 2.50 per cent mean dry matter yield of shoot over control, respectively. The interaction between CAxNi was found to be significant.

Application of Ni levels significantly decreased the mean dry matter yield of shoot over Ni_0 except Ni_{100} which increased the dry matter yield of shoot. Similarly Aziz *et al.* (2007) [4] also repoted that the application of Ni at 25 mg kg^{-1}soil gave the highest effect on increasing plant height, number. of branches as well as fresh and dry weight of roselle calyces. The mean dry matter yield of shoot was 71.60, 72.92, 57.88, 47.74 and 21.25 g pot^{-1} in Ni_0, Ni_{100}, Ni_{150}, Ni_{200} and Ni_{250} amended soils, respectively. The highest dry matter yield of shoot was recorded 81.35 g pot^{-1} due to FYM at Ni_{100} level and lowest 20.65 g pot^{-1} EDTA at Ni_{250} level.

Root

The result indicated that mean root dry matter yield increased significantly in FYM and NTA+FYM amended soils over control. The increase was 10.36 and 4.56 per cent. Data further showed that mean dry matter yield of root significantly decreased the mean dry matter yield of root over control with application of EDTA (5.06 g pot^{-1}), NTA (5.33 g pot^{-1}) and EDTA+FYM (5.65 g pot^{-1}). This could be ascribed to FYM providing the necessary essential nutrient to the plants whereas chelating agents immediately increased solubility of metal in soil and produce toxic effect after its application to the soil (Herencia *et al.*, 2008) [6].

The application of added Ni increased dry matter yield of root over Ni_0 level only upto Ni_{100}. After this level, the dry matter yield of root started decreasing significantly. Highest dry matter yield 7.84 g $pot^{-1}$of root was recorded in FYM added soil at Ni_{100} and lowest 3.05 g pot^{-1} was found in EDTA+FYM treated pots at $Ni_{250,}$ respectively.

The increase in dry matter yield of shoot due to FYM may be attributed to increase supply of nutrient through metal solubilization whereas decrease in dry matter yield of shoot due to EDTA and other chelating agents may be attributed to increase solubilization of Ni and subsequent increase concentration in root and shoot might have inhibited growth (Indoria and Poonia, 2006) [15].

High dose of Ni being toxic to plants, and reduced growth and impaired metabolism (Aery and Sarkar, 1991) [1] and interfered with photosynthetic and respiratory activities (Clijsters and Van-Assche, 1985) [5].

Robinson *et al.* (2000) showed that application of NTA @ 0.5 g kg^{-1} caused necrosis and abscission of most of the leaves by 1 week after treatment. The plant slowly recovered and had significantly lower biomass production at end of experiment relative to control.

The beneficial effect of Ni contaminated and FYM and adverse effect of Ni on different crops have also been reported by several workers (Narwal *et al.*, 1983 and Singh *et al.*, 1991) [10, 14].

Table 1: Dry matter yield (g pot^{-1}) of root and shoot of selected maize genotype as influenced by different chelating agents in Ni contaminated soil

Treatments	**Ni levels (mgKg^{-1} soil)**					
	0	**100**	**150**	**200**	**250**	**Mean**
Shoot						
Control	65.95	68.65	54.15	47.55	21.37	51.53
EDTA	64.73	65.94	49.78	45.38	20.65	49.30
NTA	65.21	67.70	51.39	45.73	21.16	50.24
FYM	79.24	81.35	68.72	49.87	21.90	60.22
FYM+EDTA	76.24	76.15	62.51	48.25	20.86	56.80
FYM+NTA	78.23	77.70	60.75	49.64	21.58	57.58
Mean	71.60	72.92	57.88	47.74	21.25	54.28
CD(P=0.05) Chelating Agent-1.18; Ni Levels-1.08; CA x Ni-2.64						
Root						
Control	6.83	6.95	6.05	5.17	3.43	5.69
EDTA	6.15	6.15	5.15	4.70	3.16	5.06
NTA	6.47	6.54	5.47	4.86	3.31	5.33
FYM	7.58	7.84	6.32	5.77	3.89	6.28
FYM+EDTA	6.74	7.10	6.14	5.22	3.05	5.65
FYM+NTA	6.96	7.45	6.20	5.51	3.65	5.95
Mean	6.79	7.01	5.89	5.21	3.42	5.66
CD(P=0.05) Chelating Agent-0.27; Ni Levels-0.24; CA x Ni-NS						

Nickel concentration in shoot and root

Shoot

The perusal of data in Table 2 on shoot and root Ni concentration of maize crop as influenced by different chelating agents revealed that similar to Indian mustard the mean Ni concentration of maize shoot also increased significantly the mean Ni concentration of shoot over control except the application of FYM which decreased mean Ni concentration by 8.40 per cent. The increase was 39.05, 31.55, 26.08 and 17.74 per cent with the application of EDTA, NTA, EDTA+FYM and NTA+FYM, respectively.

Data further showed that application of added Ni also significantly increased the mean Ni concentration of shoot over control. The mean Ni concentration of shoot was 16.66, 32.29, 42.19, 49.04 and 55.82 µg g^{-1} in Ni$_{0}$, Ni$_{100}$, Ni$_{150}$, Ni$_{200}$ and Ni$_{250}$ amended soils, respectively. Highest Ni concentration was reported due to EDTA (61.35 µg g^{-1}) at Ni$_{250}$ level and lowest Ni concentration in shoot was due to the FYM (10.87 µg g^{-1}) at Ni$_{0}$ level. The interaction between chelating agent and Ni level was significant. The shoot Ni concentration significantly and progressively increased with the increasing additions of Ni. Moreover, the highest mean Ni concentration was reported due to EDTA followed by NTA, EDTA+FYM and NTA+FYM whereas FYM decreased Ni concentration in maize shoot.

Root

The result indicated as like shoot the mean Ni concentration of root increased significantly the mean Ni concentration of root over control except the application of FYM which decreased mean Ni concentration by 6.82 per cent. The increase was 57.81, 51.00, 41.11 and 33.38 per cent with the addition of EDTA, NTA, EDTA+FYM and NTA+FYM. Application of chelating agents significantly increased the mean root Ni concentration over control. Highest mean Ni concentration was reported due to EDTA (95.40 µg g^{-1}) at Ni$_{250}$ level and lowest Ni concentration in shoot was due to the FYM (17.52 µg g^{-1}) at Ni$_{0}$ level.

Table 2: Nickel concentrations (µg g^{-1}) in root and shoot of selected maize genotype as influenced by different chelating agents in Ni contaminated soil

Treatments	**Ni levels (mgKg^{-1} soil)**					
	0	**100**	**150**	**200**	**250**	**Mean**
Shoot						
Control	12.14	22.17	35.38	43.73	53.12	33.31
EDTA	22.64	40.97	50.37	56.27	61.35	46.32
NTA	19.35	37.20	47.40	54.77	60.46	43.82
FYM	10.87	20.56	28.66	40.79	51.67	30.51
FYM+EDTA	18.50	38.65	47.50	50.51	54.86	42.00
FYM+NTA	16.45	34.19	43.84	48.17	53.45	39.22
Mean	16.66	32.29	42.19	49.04	55.82	39.20
CD(P=0.05) Chelating Agent-0.42; Ni Levels-0.38; CA x Ni-0.94						
Root						
Control	22.67	35.20	48.29	56.12	61.00	44.66
EDTA	37.22	58.30	75.15	86.35	95.40	70.48
NTA	34.93	54.37	70.21	83.47	94.23	67.44
FYM	17.52	31.92	45.47	52.41	60.72	41.61
FYM+EDTA	32.11	56.85	71.40	75.29	79.45	63.02
FYM+NTA	28.10	52.71	66.36	72.36	78.33	59.57
Mean	28.76	48.23	62.81	71.00	78.19	57.80
CD(P=0.05) Chelating Agent-0.53; Ni Levels-0.48; CA x Ni-1.19						

The interaction between CAxNi was significant. The mean Ni concentration of root was 28.76 – 78.19 µg g^{-1} as the Ni levels increased from 0–250 mg kg^{-1} soil. It was also revealed from the data that all chelates significantly increased the Ni concentration in root except FYM. The root Ni concentration also significantly and progressively increased with increasing additions of Ni.

Nickel uptake

Shoot

The Table 3 showed that the mean Ni uptake of shoot was significantly increased with the application of chelating agents. Highest mean Ni uptake by shoot was reported due the effect of EDTA+FYM (2180.87 µg pot^{-1}) followed by EDTA, NTA+FYM, NTA and FYM. The increase was 46.30, 40.81, 36.20, 34.17 and 2.90 per cent over control with application of EDTA+FYM, EDTA, NTA+FYM, NTA and FYM, respectively.

Data further showed that interaction between CAxNi was significant. The impact of added levels of Ni is further evident from the data that mean Ni uptake significantly increased from Ni_0 to Ni_{150} treatment but thereafter, it started decreasing. Moreover the application of EDTA, NTA and FYM alone increased Ni uptake upto Ni 200 mg Kg^{-1} soil level. Highest Ni uptake by shoot was recorded due to effect of EDTA+FYM at Ni_{150} (2969.23 µg pot^{-1}) and lowest was (800.63 µg pot^{-1}) in control (Ni_0) soil.

Table 3: Nickel uptake (µg pot^{-1}) of root and shoot of selected maize genotype as influenced by different chelating agents in Ni contaminated soil

Treatments	Ni levels (mgKg^{-1} soil)					
	0	100	150	200	250	Mean
Shoot						
Control	800.63	1521.97	1915.83	2079.36	1135.17	1490.59
EDTA	1465.49	2701.56	2507.42	2553.53	1266.88	2098.98
NTA	1261.81	2518.44	2435.89	2504.63	1279.34	2000.02
FYM	861.34	1672.56	1969.52	2034.2	1131.57	1533.84
FYM+EDTA	1410.44	2943.20	2969.23	2437.11	1144.38	2180.87
FYM+NTA	1286.88	2656.56	2663.28	2391.16	1153.45	2030.27
Mean	1181.10	2335.72	2410.20	2333.33	1185.13	1889.09
CD(P=0.05) Chelating Agent-0.90; Ni Levels-0.82; CA x Ni-2.00						
Root						
Control	154.84	244.64	292.15	290.14	209.23	238.20
EDTA	228.90	358.55	387.02	405.85	301.46	336.37
NTA	226.00	355.58	384.05	405.66	311.90	336.64
FYM	132.80	250.26	287.37	302.41	236.20	241.81
FYM+EDTA	216.42	403.64	438.40	393.01	242.32	338.76
FYM+NTA	195.58	392.69	411.43	398.70	285.90	336.86
Mean	192.42	334.23	366.74	365.96	264.50	304.77
CD(P=0.05) Chelating Agent-0.34; Ni Levels-0.31; CA x Ni-0.75						

Root

The results indicate that mean Ni uptake by root was influenced variably depending upon type of chelating agents applied. The mean Ni uptake of roots was increased with the application of EDTA+FYM, EDTA, NTA+FYM, NTA and FYM 42.21, 41.41, 41.21, 41.32 and 10.51 per cent over the control, respectively (Table 3). Highest mean Ni uptake by shoot was reported due to the effect of EDTA+FYM. The interaction between CAxNi was found to be significant. The mean Ni uptake by root increased significantly upto Ni_{150} treatments and thereafter, it decreased. Highest Ni uptake by root was recorded due to the effect of EDTA+FYM at Ni_{150} (438.40 µg pot^{-1}) and lowest was (132.80 µg pot^{-1}) in control Ni_0+FYM soil. The mean uptake by maize roots was 192.42, 334.23, 366.74, 365.74 and 264.30 g pot^{-1} in Ni_0, Ni_{100}, Ni_{150}, Ni_{200} and Ni_{250} amended soils, respectively. The low removal of metals from the sludge by various chelators may be due to overloading of metals (Nair *et al.*, 2008) [9].

In the presence of heavy metals, increasing EDTA concentrations led to decrease metal phytotoxicity. The highest total amount of metals in shoot was obtained at 250 µM EDTA, a close to equimolar concentration to metal with lowest phyto availability present in solution and the proper management of EDTA concentration can reduce metal phytotoxicity, maintain free uptake of some metals and, at same time, increase uptake of metals with low phyto availability (Allica *et al.*, 2007) [3].

Paulose *et al.* (2007) [11] reported that EDTA could successfully predict phytoavailability of Zn and Ni in amended soil, whereas it failed in case of Cu. By and large, application of $CaCO_3$, either alone or in combination with FYM had a positive effect on the retention of Zn, Cu and Ni in soil. Application of $CaCO_3$ alone or in combination with FYM was equally effective in reducing Zn content in lettuce, whereas sole application of $CaCO_3$ significantly reduced Ni content.

Malarkodi *et al.* (2008) [8] reported that *Ricinus communis* accumulated more Ni than *Tagetes erecta* and it was enhanced with farmyard manure application over control and poultry manure application. Roots of both crops contained higher Ni concentration than aerial parts. The Ni accumulation ratio was higher in *Ricinus communis* than *Tagetes erecta* but both recorded the Ni accumulation ratio of more than one (4.40 and 1.64 respectively). Even though *Ricinus communis* was found to be effective in removing Ni from the soil, it would take much longer period (about 125 years) to remediate the soil contaminated with 165 mg Ni kg^1 soil. The time requirement was reduced when farmyard manure was added as soil amendment.

Conclusions

The result concluded that amount of Ni desorbed from two soils was the highest in the first extraction followed by second, third and fourth successive extraction. The order of effectiveness of chelating agent towards the extraction of Ni was EDTA followed by NTA. Highest Ni desorbed from Ni treated soil subsequently by Ni+ FYM. The results revealed that availability of mean values of DTPA-extractable Ni increased significantly with increasing level of Ni. Further J 1006 of maize can be grown successfully in soils having low to medium levels of pollution due to industrial wests, sewer water and sewage sludge.

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