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Sonal Vishnoi
Associate Professor, Department of Chemistry, P.G. Govt. Collage, Hisar, Haryana, India

Manju Bala
Guest Lecturers, Department of Applied Chemistry, Govt. Polytechnic Sonipat, Haryana, India

Anu Godara
Research Scholar, Department of Chemistry, JCDV, Memorial College, Sirsa, Haryana, India

Corresponding Author:
Sonal Vishnoi
Associate Professor, Department of Chemistry, P.G. Govt. Collage, Hisar, Haryana, India

Effect of chelators on phytoextraction of cadmium by Indian mustard from effluent contaminated soil

Sonal Vishnoi, Manju Bala and Anu Godara

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 Indian mustard crops. Second from the sewer water and sewage sludge polluted fields near the main disposal outlets behind main bus stand of Hisar to study uptake of Cadmium. 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 two crops *viz*., Indian mustard (three genotypes RH-819, Varuna and RH-9304) on Cd (@ 30, 60, 90, 120 and 150 mg kg^{-1}) spiked soil. The results concluded that availability of mean values of DTPA-extractable Cd increased significantly with increasing level of Cd. With the addition of chelating agents and FYM, the DTPA-extractable Cd decreased significantly as compared to where no chelates and FYM were applied and trend observed was control>FYM>NTA>EDTA>FYM+NTA>FYM+EDTA Indian mustard post harvested soils.

Keywords: Chelator, phytoextraction, cadmium uptake, heavy metal, toxicity, Indian mustard

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) [14]. In addition, mining, smelting, and the associated activities are one of important sources by which soils, plants, and surface waters are contaminated (Jung, 2008) [6].

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 hyper accumulator 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.

Materials and Methods

A pot experiment was conducted to investigate the Cd phytoextraction potential of three Indian mustard plant genotypes, namely, RH - 819, Varuna and Rh-9304, 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 deionized 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 deionized 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 ^{0}C 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

Phytoextracton of Cd by Indian mustard from Cd enriched soil as influenced by chelating agents

Toxicity symptom

Visual toxicity symptoms of Cd were also recorded from germination to harvesting of the crops. It was found that there was no adverse effect of applied Cd on germination of seeds. The effect of added dose of Cd was almost similar as in tolerance study. Overall growth of mustard crop was better in FYM amended soil over unamended soil. In FYM added soil, growth of Indian mustard at starting was slower but at advance stages its growth was better as compare to control. The application of chelating agents at 85 days of sowing, causes wilting of plants after a day of its application. These symptoms disappeared upto Cd_{90} level after 2-3 days, whereas at higher levels wilting of plant caused serve damage and growth of plants restricted.

Dry matter yield of shoot and root

Shoot

The mean dry matter yield of shoot was maximum, 44.81 g pot^{-1}, in Cd_{30} with FYM and the least, 8.21 g pot^{-1}, in Cd_{120} with EDTA treatment (Table 1). As compare to control, the application of FYM increased the dry matter yield of Indian mustard shoot by 4.88 per cent followed by FYM+NTA (2.37%). The highest decrease was reported with the application of EDTA i.e. 12.65 per cent as compared to control followed by application of NTA. The mean dry matter yield of shoot was 32.66, 31.88, 31.14, 30.05, 28.23 and 27.20 g pot^{-1} in FYM, FYM+NTA, control, FYM+EDTA, NTA and EDTA treated pots, respectively.

The interaction between chelating agents and Cd levels was found to be significant. The mean shoot dry matter yield of Indian mustard increased upto Cd_{30} treatments and thereafter, it decreased significantly. Moreover the mean dry matter yield differed significantly with the application of different chelating agents.

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

Treatments	Cd levels (mgKg^{-1} soil)					
	0	30	60	90	120	Mean
Shoot						
Control	38.74	39.91	35.46	26.29	15.28	31.14
EDTA	35.23	37.47	32.25	22.83	8.21	27.20
NTA	36.25	38.15	33.47	23.62	9.67	28.23
FYM	43.65	44.81	37.24	25.47	12.15	32.66
FYM+EDTA	39.53	41.23	36.15	23.15	10.19	30.05
FYM+NTA	43.72	44.2	36.82	23.85	10.81	31.88
Mean	39.52	40.96	35.23	24.20	11.05	30.19
CD(P=0.05) Chelating Agent-0.96; Cd Levels-0.87; CA x Cd-2.14;						
Root						
Control	5.39	5.67	4.59	2.95	2.74	4.27
EDTA	4.76	4.69	3.60	2.75	1.42	3.44
NTA	4.86	4.83	3.75	2.95	1.57	3.59
FYM	5.65	5.79	4.82	3.15	2.54	4.39
FYM+EDTA	5.26	5.18	4.24	2.45	1.98	3.82
FYM+NTA	5.05	5.96	4.63	2.75	2.21	4.12
Mean	5.16	5.35	4.27	2.83	2.08	3.94
CD(P=0.05) Chelating Agent-0.20; Cd Levels-0.18; CA x Cd-0.45;						

Root

The data in Table 1 further revealed that dry matter yield of root and shoot of Indian mustard was influenced variably depending upon the type of chelating agents. Addition of FYM increased the dry matter of root of Indian mustard from 4.27 to 4.39 g pot^{-1} grown on sewage sludge polluted soils. Application of other chelating agents significantly decreased the dry matter yield of root in comparison to control. The mean dry matter yield of root of Indian mustard was lowest (3.44 g pot^{-1}) in EDTA applied as compared to 3.59, 3.82, 4.12, 4.27 and 4.39 g pot^{-1} in NTA, FYM+EDTA, FYM+NTA, control and FYM, respectively. Application of chelating agents increased the dry matter of Indian mustard roots upto Cd_{30} level after that it started decreasing.

It revealed from table 1 that the application of FYM increased the mean dry matter yield of root by 2.81 per cent. While the application of EDTA, NTA, FYM+EDTA and FYM+NTA decreased the dry matter of Indian mustard roots by 19.43, 15.92, 10.53 and 3.5 per cent, respectively as compare to control. The mean dry matter of Indian mustard roots was 5.16, 5.35, 4.27, 2.83 and 2.08 g pot^{-1} at Cd_0, Cd_{30}, Cd_{60}, Cd_{90} and Cd_{120} level, respectively.

Kulli *et al.* (1999) [8] also found that application of 20 mmol kg^{-1} soil led to a reduction of dry matter harvest by 5 to 15 per cent in lettuce and ryegrass. No visual symptoms of toxicity were found. The NTA-70 treatment reduced growth by 15 to 25 per cent. In this treatment both lettuce and ryegrass showed some chlorosis, and the roots of lettuce plants were less developed than in the control treatment. The NTA 200 treatment reduced the harvested biomass by more than 90 per cent of the control plants for lettuce and by 97 per cent for ryegrass. The plants were strongly chlorotic and necrotic at the end of the experiment, and growth was severely impaired.

Uptake is a function of concentration and dry matter yield per pot. Increasing uptake of Cd with the application of CDTA as compared to other chelating agents was due to higher dry matter production and Cd accumulation in roots and shoots. FYM was found more beneficial in increasing the dry matter of roots. The increased phytoextractability of Cd by CDTA and other chelating agents might have been due to increased availability of Cd in the rhizosphere and higher dry matter yield of the *Zea mays* roots and shoots (Kulli *et al.*, 1999 and Tatiana, 2006) [8]. The beneficial effect of CDTA on uptake by plants was also observed by Cooper *et al.* (1999) [4].

The beneficial effect of sewage sludge and FYM and adverse effect of Cd on different crops have also been reported by several workers. The possible reason for beneficial effect of chelating agents on dry matter yield of shoot of *Zea mays* in Cd contaminated soil might have been due to increased availability of essential micronutrients. The beneficial effect of FYM on plant growth may be ascribed to the improvement in soil physico-chemical environment which might have increased the availability of other essential nutrients (Kos *et al*, 2003) [7].

Pereira *et al.* (2007) [9] reported that EDTA chelant applied to soil caused a decrease in shoot dry matter yield of maize plants. Such effect might be explained by EDTA increase on availability of heavy metals (HM) or due to EDTA toxicity (Cui *et al.*, 2004) [15].

Cadmium concentration

Shoot

Table 2 showed the effect of different treatments on Cd concentration of shoot and root of Indian mustard. It was observed that the Cd concentration increased with the increasing level of Cd in the soil. The Cd concentration increased significantly from 2.51 to 105.95 µg g^{-1} in shoot as the Cd level increased from 0-120 mg Kg^{-1} soil). The highest Cd concentration in Indian mustard plants was recorded (124.65 µg g^{-1}) with the addition of EDTA at Cd_{120}, whereas the lowest Cd concentration (1.10µg g^{-1}) was recorded with the addition of FYM at Cd_0 level.

The application of FYM decreased the mean Cd concentration 10.09 per cent in Indian mustard plants while the addition of EDTA, NTA, FYM+EDTA and FYM+NTA increased the mean Cd concentration 34.23, 12.17, 11.03 and 2.41 per cent, respectively as compared to control. However, the magnitude of increase in Cd concentration varied with the chelating agent. The mean Cd concentration of shoot was 67.38, 56.31, 45.13, 55.74 and 51.41µg g^{-1} with the application of EDTA, NTA, FYM+EDTA and FYM+NTA, respectively. The interaction between chelating agents and Cd levels was found to be significant.

Application of chemical chelators like CDTA, DTPA, NTA, Citric acid etc. increasing the metal bioavailability, uptake and translocation in the plants has been reported by Cooper *et al.* (1999) [4]; Kulli *et al.* (1999) [8] and Romkens *et al.* (2002) [11].

Kulli *et al.* (1999) [8] also reported that application of NTA @ 200 mg kg^{-1} soil increased the Cd concentration in lettuce and rye grass. Wallace *et al.* (1977) [13] observed that application of NTA increased the levels of Cd in the leaves of bush beans. NTA increased Cd uptake by 26 mg g^{-1} dry weight compared with control of 15 µg g^{-1} dry weight

Table 2: Cadmium concentration (µg g^{-1}) in root and shoot of selected Indian mustard genotype as influenced by different chelating agents in Cd contaminated soil.

Treatments	Cd levels (mgKg^{-1} soil)					
	0	30	60	90	120	Mean
Shoot						
Control	1.41	28.81	37.30	84.67	98.82	50.20
EDTA	3.89	38.78	66.64	102.92	124.65	67.38
NTA	3.27	34.35	50.72	87.53	105.72	56.31
FYM	1.10	20.63	34.74	73.61	95.58	45.13
FYM+EDTA	2.77	30.75	50.37	85.20	109.61	55.74
FYM+NTA	2.65	26.28	44.25	82.57	101.30	51.41
Mean	2.51	29.93	47.34	86.08	105.95	54.36
CD(P=0.05) Chelating Agent-0.63; Cd Levels-0.58; CA x Cd-1.42;						
Root						
Control	2.10	58.60	75.68	113.45	136.38	77.24
EDTA	6.75	68.75	137.39	146.80	150.48	102.03
NTA	6.21	64.39	104.54	135.71	140.60	90.29
FYM	1.93	42.23	70.84	103.59	128.36	69.39
FYM+EDTA	6.37	58.45	89.45	131.47	138.10	84.77
FYM+NTA	6.05	54.84	82.33	120.38	128.64	78.45
Mean	4.90	57.88	93.37	125.23	137.09	83.70
CD(P=0.05) Chelating Agent-0.72; Cd Levels-0.66; CA x Cd-1.61						

Root

Similar to shoot Cd concentration, the minimum content (69.39 µg g^{-1}) of Cd in root was also observed at Cd_0 added with FYM but when chelating agents were applied, it increase significantly. The mean Cd content in control was 77.24 µg g^{-1} in root but it was 102.03, 90.29, 84.77 and 78.45 µg g^{-1} with the addition of EDTA, NTA, FYM+EDTA and FYM+NTA, respectively. Application of EDTA increased the mean root Cd content by 32.09 per cent as compare to control. While application of FYM decreased the mean Cd concentration in Indian mustard root by 10.61 per cent.

The interaction effect between Cd levels and chelating agents was significant. It is also evident from Table 2 that the root Cd concentration was also significantly affected by the chelating agents and addition of Cd in the soil.

Reported that Indian mustard root Cd concentrations decreased after EDTA application, but shoot concentrations increased when soil Cd levels were more than 130 mg kg^{-1}. Moreover, the increases in soil Cd induced by EDTA did not increase plant total Cd uptake but appeared to stimulate the translocation of the metal from root to shoot when the plants appeared to be under Cd toxicity stress.

Reported that addition of EDTA increased the plant concentration of Cd by almost 10-fold in soils contaminated with $CdCl_2$, with a concentration of 1283 mg Cd kg^{-1} in the dried EDTA-treated plants over a concentration of 131 mg Cd kg^{-1} in plants without added chelate. However, EDTA increased the aqueous solubility of Cd by 36 times over the soil matrix without added chelator, and thereby, increased the possibility of leaching. Other chelators used in both experiments were ethylenebis (oxyethylenenitrilo) tetraacetic acid, *trans*-1, 2-diaminocyclohexane-*N*, *N*, N′, N′-tetraacetic acid, and DTPA increasing Cd in plants to 1240, 962, and 437 mg Cd kg^{-1}, respectively.

Cadmium uptake

Shoot

The data on Cd uptake by Indian mustard as affected by Cd levels and chelating agents are presented in Table 3. The results indicated that the mean Cd uptake increased significantly with the response to added Cd treatments. It increased significantly from 97.26 to 2076.60 µg pot^{-1} with the increasing levels of added Cd from 0 to 90 mg Kg^{-1} soil. Further increase in the level of added Cd resulted in significant decrease (44.38%) in comparison to the Cd_{90} treatment.

The interaction between CA x Cd was found to be significant. The results showed the effect of addition of chelating agents to Cd uptake by Indian mustard varied considerably. Application of EDTA, NTA, FYM+NTA, FYM+EDTA and FYM increased the Cd uptake by Indian mustard shoot from 54.62 in control to 137.04 (118.5%), 118.53, 115.86, 109.50 and 48.02 µg pot^{-1}, respectively in plants grown on sewage sludge polluted soils. Moreover the addition of Cd to the soil further increases the Cd uptake by shoot of Indian mustard. The highest Cd uptake was observed at Cd_{90} with the addition of EDTA, followed by NTA, FYM+EDTA, FYM+NTA and FYM.

Root

The Table 3 further showed that application of all the chelating agents significantly increased the Cd uptake in root in comparison to control. Application of EDTA at Cd_0 level increased the Cd uptake by Indian mustard root by 183.83 per cent in plants grown on sewage sludge polluted soils. The Cd uptake increased from 11.32 µg pot^{-1} in control (Cd_0) to 33.51, 32.13, 30.55, 30.18, and 10.90 µg g^{-1} due to application of FYM+EDTA, EDTA, FYM+NTA, NTA and FYM by the root of Indian mustard plant.

The mean Cd uptake by root of Indian mustard due to application of different chelating agents was highest (389.32 µg g^{-1}) at Cd_{60} level instead of Cd_{90} level as in case of shoot. Application of NTA increased the Cd uptake of Indian mustard root upto Cd_{90} level. Only the EDTA increased the mean root Cd uptake by 4.80 per cent as comparison to control. The interaction between CAxCd was found to be significant.

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

Treatments	Cd levels (mgKg^{-1} soil)					
	0	30	60	90	120	Mean
Shoot						
Control	54.62	1149.81	1322.66	2225.97	1509.97	1252.61
EDTA	137.04	1453.09	2149.14	2349.66	1023.38	1422.46
NTA	118.53	1310.45	1697.6	2067.46	1022.31	1243.27
FYM	48.02	924.43	1293.72	1874.85	1161.30	1060.46
FYM+EDTA	109.50	1267.82	1820.88	1972.38	1116.93	1257.50
FYM+NTA	115.86	1161.58	1629.29	1969.30	1095.05	1194.22
Mean	97.26	1211.20	1652.22	2076.60	1154.82	1238.42
CD(P=0.05) Chelating Agent-1.49; Cd Levels-1.36; CA x Cd-3.33;						
Root						
Control	11.32	332.26	347.37	334.68	373.68	279.86
EDTA	32.13	322.44	494.60	403.70	213.68	293.31
NTA	30.18	311.00	392.03	400.34	220.74	270.86
FYM	10.90	244.51	341.45	326.31	326.03	249.84
FYM+EDTA	33.51	302.77	379.27	322.10	273.44	262.22
FYM+NTA	30.55	326.85	381.19	331.05	284.29	270.79
Mean	24.77	306.64	389.32	353.03	281.98	271.15
CD(P=0.05) Chelating Agent-.34; Cd Levels-0.31; CA x Cd-0.77;						

These results clearly indicate that applications of chelating agents are helpful in increasing Cd uptake by Indian mustard. EDTA was found to be more effective in enhancing the Cd uptake by Indian mustard root as well as shoot of Indian mustard as than other chelating agents. The increased phytoextractability of Cd by EDTA and other chelating agents may be ascribed to increase availability of metals in soil and higher dry matter yield of the Indian mustard root and shoot (Blaylock *et al* 1997; Kos *et al* 2003) [2, 7].

Debra *et al.* (2007) [5] repoted that chelating agents increased plant uptake of Cd from the $CdCl_2$ soil but did not significantly increase *Brassica Spp.* uptake of Cd from the $CdCO_3$ contaminated soil. Addition of EDTA increased the plant concentration of Cd by almost 10-fold in soils contaminated with $CdCl_2$, with a concentration of 1283 mg Cd kg^{-1} in the dried EDTA-treated plants over a concentration of 131 mg Cd kg^{-1} in plants without added chelate. However, EDTA increased the aqueous solubility of Cd by 36 times over the soil matrix without added chelator.

Conclusions

Among these amendments the EDTA was most effective in total uptake and concentration of Cd point of view. The post-harvest soil samples of Indian mustard crops was analyzed for the mean DTPA-extractable Cd as influenced by application of chelating agents. The results concluded that availability of mean values of DTPA-extractable Cd increased significantly with increasing level of Cd. With the addition of chelating agents and FYM, the DTPA-extractable Cd decreased significantly as compared to where no chelates and FYM were applied and trend observed was control>FYM>NTA>EDTA>FYM+NTA>FYM+EDTA Indian mustard post harvested soils.

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