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Effect of Chelators on phytoextraction of Cadmium by Indian mustard from industrial effluent contaminated soil

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ABSTRACT

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. Results of the study showed that symptoms of chlorosis, yellowing and burning of lower leaves and poor growth were more prominent with the increasing level of Cd and finally drastic reduction in dry matter yield was recorded at Cd_{60} and Cd_{90} levels. Moreover at Cd_{120} level, only genotype RH-819 was survive with acute chlorosis, burning of leaves and producing 1.16 g pot^{-1} dry matter yield even though having highest Cd concentration 68.67 µg g^{-1}. Overall, growth of genotype RH-819 was better as compared to other two genotypes. The mean dry matter yield was highest (18.32 g pot^{-1}) in RH-819 followed by Varuna (14.75 g pot^{-1}) and RH-9304 (12.68 g pot^{-1}). The shoot Cd concentration significantly and progressively increased with the increasing additions of Cd. Similar to the dry matter yield, the mean Cd concentration was highest (30.94 µg g^{-1}) in shoot of RH 819. It was 4.84 per cent higher than Varuna and 15.96 per cent higher than RH-9304. The results further indicated that the mean Cd uptake by shoot was significantly more in RH-819 as compared to Varuna and RH-9304. In RH-819 it was 47.08 per cent higher than Varuna and 76.24 per cent than RH 9304. In all the three genotypes, the Cd uptake by shoot increased significantly up to Cd_{30} treatment and thereafter it decreased significantly in comparison to this level. On the basis of highest Cd uptake and biomass production RH-819 genotype was found best accumulator among three genotypes of Indian mustard.

Key words: **Phytoextraction, Nickel uptake, Heavy metal, Indian mustard, Toxicity symptom, Chlorophyll content.**

INTRODUCTION

Phytoextraction is a green technology for sustainable remediation of surface soils contaminated with toxic heavy metals. Indian mustard is a high biomass producing crop having heavy metal tolerance characteristics. This biomass produced from contaminated soils would be an important source of energy in developing country and its use is still increasing in the domestic sector and small-scale industries such as brick kiln etc in India. Moreover, it also reduces the risk of entering in the food chain when used as biomass energy and also reduces the re-entry of heavy metals in the agricultural field by using the byproduct in land fill and in making bricks also.

In case of heavy metals, increasing attention is being directed towards more diffuse forms of soil contamination. Large areas of Indian soils have received significant amount of Cadmium (Cd) as an impurity of phosphatic fertilizers (Alloway, 1995). Other sources of Cd contamination is sewage sludge, industries involved in protective plating on steel and production of various alloys, pigments, stabilizers for plastic, Ni-Cd dry batteries and miscellaneous items including photovoltaic cells, TV screens and control rods for nuclear reactors. Its contamination also comes from mining, ore dressing and smelting of cadmium sulphide ore, which may contain up to 5 per cent Cd. Cd has no essential biological function and is highly toxic to plants and animals. It is recognized as one of the most hazardous element and not essential for plant growth (Kabata and Pendias, 1992).

MATERIAL 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,

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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 was applied in solution form in each pot through urea, KH_2PO_4 and $ZnSO_4.7H_2O$, respectively. To create desired level of Cd in their respective pots, appropriate volumes of $CdCl_2$ solutions were added for each Indian mustard. 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 three different 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 in solution form in Indian mustard genotypes. 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 for each crop 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 to 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 for further heavy metal uptake study.

RESULTS AND DISCUSSION

Tolerance of Cd by Indian mustard genotypes from Cd enriched soil

Toxicity symptom

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Visual toxicity symptoms due to Cd were recorded from germination to harvesting of the crops. There was no adverse effect of different levels of applied Cd on the germination of seeds of all the three genotypes of Indian mustard, however the growth and development of plant was adversely affected by increasing levels of the Cd (Photo plate-1). The leaves were narrow and small with stunted growth, poor branching, and poor seed development of the plant. At Cd 120 and 150 mg kg^{-1} soils, plants leaves started wilting after 2-3 weeks and ultimately the plants died in case of all the three genotypes (i.e. RH-819, Varuna and RH-9304), except RH-819 which survived in case of Cd 120 mg kg^{-1} soil with very poor growth and biomass (1.16 g pot^{-1}) yield (Table 4).

Table 1 Effect of Cd application on Chlorophyll content (a, b and total) of Indian mustard genotypes.

Cd levels (mg/Kg soil)	Chlorophyll content (Mg/g)								
	Chlorophyll content 'a'			Chlorophyll content 'b'			Total Chlorophyll content		
	RH 819	Varuna	RH-9304	RH 819	Varuna	RH-9304	RH 819	Varuna	RH-9304
0	12.14	10.21	9.43	3.73	2.96	2.87	15.87	13.17	12.3
30	10.67	8.55	7.84	3.21	1.98	1.63	13.88	10.53	9.47
60	9.26	7.76	7.15	2.17	1.69	1.48	11.43	9.45	8.63
90	8.12	7.32	7.06	2.07	1.48	1.36	10.19	8.8	8.42
120	4.65	-	-	1.11	-	-	5.76	-	-
Mean	10.05	8.46	7.87	2.8	2.03	1.83	12.84	10.49	9.71
CD (P=0.05)	Genotype -0.21	Cd Levels- 0.24	G x Cd- 0.41	Genotype -0.05	Cd Levels- .06	G x Cd- 0.10	Genotype -0.36	Cd Levels- 0.42	G x Cd- 0.72

According to Williams and Mills, 2005 number of heavy metals, including copper (Cu), zinc (Zn), manganese, and iron, are essential micronutrients for a wide variety of physical processes. These micronutrients can serve structural roles in proteins, act as enzyme cofactors, and function in cellular redox reactions. Where as cadmium (Cd), mercury, silver, and lead (Pb), are generally considered nonessential to plants and are potentially highly toxic because of their reactivity with sulfur and nitrogen in amino acid side chains and effect the growth and development of plant (Clemens, 2001). Cd in higher concentration has been shown to disturb chlorophyll synthesis (Stobart *et al.*, 1985) and many metabolic processes like nucleic acid and protein synthesis (Bingham *et al.*, 1993).

Chlorophyll content

The perusal of data in Table 1 revealed that chlorophyll content 'a' in Indian mustard significantly affected by Cd application in soil. The mean chlorophyll content 'a' in Indian mustard plant was 10.59, 9.02, 8.06 and 7.50 mg g^{-1} with application of 0, 30, 60 and 90 mg Cd kg^{-1} soil respectively. The mean chlorophyll content 'a' decreased from 10.59 – 7.50mg g^{-1} with increase

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in Cd concentration from 0-90 mg Cd kg^{-1} soil. However, the magnitude of chlorophyll content 'a' varied with genotypes. In all studied genotypes the highest values of chlorophyll 'a' content were recorded at Cd_0 having 12.14 mg g^{-1} in RH-819 and minimum (7.06 mg g^{-1}) in RH-9304 at Cd_{90}. The decrease in mean chlorophyll 'a' value was 15.82 per cent in Varuna and 21.7 per cent in RH-9304 as compare to RH- 819.

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Genotype Varuna　　Genotype RH-9304　　Genotype RH-819

Photo plate-1: Effect of different Cd levels on germination of Indian Mustard genotypes

It is evident from Table 1 that the chlorophyll content 'b' in Indian mustard plant was also affected by soil Cd levels. The mean chlorophyll content 'b' decreased from 3.19 to 1.64 mg g^{-1} with increase in Cd concentration from 0 to 90 mg Cd kg^{-1} soil. The chlorophyll content 'b' varied with the genotypes and decreased from 3.73 to 2.07, 2.96 to 1.48 and 2.87 to 1.36 mg g^{-1} in RH-819, Varuna and RH-9304 Indian mustard genotypes, respectively with increase in Cd concentration from 0-90 mg Cd kg^{-1} soil.

Similarly, the significant reduction in total chlorophyll was also observed with the increase of Cd concentration table 1. This decrease was 18.30 per cent in Varuna and 24.37 per cent in RH- 9304 as compare to RH- 819 in case of total chlorophyll content. The mean of total chlorophyll content of genotypes RH-819 was 12.84 mg g^{-1} followed by genotype Varuna (10.49 mg g^{-1}) and RH-9304 (9.71 mg g^{-1}). The highest chlorophyll content was recorded at Cd_0 in RH-819, whereas the lowest chlorophyll content was recorded at Cd_{90} in RH-9304. The mean total chlorophyll content decreased from 13.78 – 9.14 mg g^{-1} with increase in Cd concentration from 0-90 mg Cd kg^{-1} soil.

The interaction between Genotype X Cd was found to be significant. The total chlorophyll content of genotypes RH 819 significantly decreased from 15.87 mg g^{-1} in Cd_0 to 10.19 mg g^{-1} in Cd_{90} treatment. Similar trend was also observed in Varuna and RH-9304. The total chlorophyll content of RH-819 differed significantly from Varuna and RH-9304 at each level of added Cd.

These results are consistent with observations of Wei *et al.* (2006) who found that longer exposure to Cd concentration reduced chlorophyll contents. This may be due to interference in Fe^{2+} ions translocation to leaves by Cd^{2+} ions thus inhibiting chlorophyll-haeme

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biosynthesis. Whereas Muthuchelian *et al.* (1988) reported that decrease in chlorophyll content seems to be mainly due to decrease in chlorophyll biosynthesis which is probably caused by binding of Cd to the –SH groups of active sites of all the enzymes involved in chlorophyll biosynthesis. Reduction in chlorophyll content by Cd application have also been reported by Baszynski *et al.* (1980), Goldberg *et al.* (1980), Wong *et al.* (1984), and Klobus and Buczek (1985). The increasing level of Cd in the soil was adversely effect the chlorophyll content 'a' and 'b' in all the three genotypes of Indian mustard. Therefore, the biomass of plants also decreased as the chlorophyll is the main factor for synthesis of carbohydrates in plants.

Nagajyoti *et al.* (2008) observed that at 25 per cent industrial effluent concentration, there was growth in the root length, an increase in shoot length, germination percentage, chlorophyll a, chlorophyll b, total chlorophyll content in *Arachis hypogaea* L. Chlorophyll content have increased upto the 20th day and then decreased from 25th day onwards. Moreover, Shi *et al.* (2008) reported that the concentration of Cd in soil significantly and negatively correlated with chlorophyll content, photosynthetic rate, transpiration rate and biomass production.

Haider *et al.* (2007) concluded that Cd is synergistic in their effect on plant growth, physiological traits and nutrient uptake. The Cd has been proved to be an aggressive, oxidative damage inducing agent and an effective competitor for essential metal cofactors participating in the chlorophyll biosynthesis (Sanita and Gabbrielli, 1999, Cho and Seo 2004, and Surjenru *et al.*, 2007). Although roots were the main sites of metal accumulation, metals induced oxidative damage mainly in the leaves (Hegedus *et al.*, 2005).

Dry matter yield

To study the tolerance of Cd by Indian mustard genotypes from Cd enriched soil, dry matter yield of plant shoot were taken into account. Dry matter yield of Indian mustard influenced by different levels of Cd (Table 2). The mean dry matter yield of shoots ranged from 5.28 to 25.52 g pot^{-1}. It was the maximum in Cd_0 and the minimum in Cd_{90} treatment. The mean dry matter yield of shoot was highest (18.32 g pot^{-1}) in RH-819, while 14.75 and 12.68 g pot^{-1} in Varuna and RH-9304, respectively. The genotypes showed variability in their mean dry matter yield. The data further showed that the interaction between Cd levels and genotypes was significant. The shoot dry matter yield of RH-819 significantly decreased from 29.34 g pot^{-1} in Cd_0 to 8.21 g pot^{-1} in Cd_{90} treatments. Compared to $Cd_{0,}$ the minimum decrease in the shoot dry matter yield with the application of Cd_{90} was 72.01 per cent in RH-819 followed by RH-9304 (78.28%) and Varuna (88.31%).

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Quartacci *et al.* (2007) reported that out of nine species tested in multiple metal-polluted soil (As, Cd, Cu, Pb and Zn), *Brassica carinata* was the species that accumulated the highest amounts of metals in shoots without suffering a significant biomass reduction.

Table 2 Dry matter yield (g/pot) of different genotypes of Indian mustard as affected by Cd levels

Genotypes	Cd levels (mg/Kg soil)				
	0	**30**	**60**	**90**	**Mean**
RH 819	29.34	20.45	15.28	8.21	18.32
Varuna	26.18	18.24	11.5	3.06	14.75
RH-9304	21.05	14.34	10.76	4.57	12.68
Mean	25.52	17.68	12.51	5.28	15.25

CD (P=0.05) Genotype-0.94; Cd Levels-1.08 G x Cd-1.87

Cadmium concentration

A perusal of data in Table 3 revealed that application of increasing Cd levels significantly increased the Cd content in shoots of Indian mustard. The mean value of Cd content in shoots was 0.013, 30.01, 36.75, and 49.41 µg g^{-1} in control, Cd_{30}, Cd_{60} and Cd_{90} treated pots, respectively. The shoot Cd concentration significantly and progressively increased with the increasing additions of Cd and the interaction between Genotype X Cd was found to be significant.

Similar to the dry matter yield, the mean Cd concentration was highest (30.94 µg g^{-1}) in shoot of RH-819, which was followed by Varuna (29.54 µg g^{-1}) and RH-9304 (26.68 µg g^{-1}). In shoot of RH-819 it was 4.84 per cent higher than Varuna and 15.96 per cent higher than RH-9304. The shoot Cd concentration of RH-819 significantly increased from 0.02 µg g^{-1} in Cd_0 to 52.65 µg g^{-1} Cd_{90} treatment.

Table 3 Cd concentration (µg/ g) in different genotypes of Indian mustard as affected by Cd levels

Genotypes	Cd levels (mgKg-1 soil)				
	0	**30**	**60**	**90**	**Mean**
RH 819	0.02	32.66	38.44	52.65	30.94
Varuna	0.01	30.54	38.23	49.25	29.51
RH-9304	0.01	26.82	33.58	46.32	26.68
Mean	0.013	30.01	36.75	49.41	29.04

CD (P=0.05) Genotype-0.29; Cd Levels-0.33 G x Cd-.58

Cadmium uptake

The amount of metals uptake by plants arises from the metal concentration in dry plant tissue and the total biomass of the plant. The product of these estimates the total amount of metal uptake from the contaminated soil. The data on Cd uptake by shoot of Indian mustard as affected by different levels of Cd are presented in table 4. The

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results indicated that the mean Cd uptake by shoot was significantly more in RH-819 as compared to Varuna and RH-9304. In RH-819 it was 47.08 per cent higher than Varuna and 76.24 per cent than RH-9304. In all three genotypes, the Cd uptake by shoot increased significantly upto Cd_{30} treatment and thereafter, it decreased significantly in comparison to this level.

Table 4 Cd uptake (µg /pot) by different genotypes of Indian mustard as affected by Cd levels

Genotypes	Cd levels (mg/Kg soil)				
	0	**30**	**60**	**90**	**Mean**
RH 819	**0.59**	**667.9**	**587.36**	**432.26**	**422.03**
Varuna	**0.26**	**557.05**	**439.65**	**150.71**	**286.92**
RH-9304	**0.21**	**384.6**	**361.32**	**211.68**	**239.45**
Mean	**0.35**	**536.52**	**462.78**	**264.88**	**316.13**

CD (P=0.05) Genotype-0.40; Cd Levels-0.46; G x Cd-0.80

The maximum Cd uptake by shoot 667.90 µg pot^{-1} was recorded in RH-819 at Cd_{30} treatment. The interaction between Genotype X Cd was found to be significant. The Cd uptake by shoot of RH-819 significantly increased from 0.59 µg pot^{-1} in Cd_0 to 667.90 µg pot^{-1} in Cd_{30} treatment but thereafter, it decreased to 432.26µg pot^{-1} at Cd_{90} treatment. Similar trend was also observed in Varuna and RH-9304. The Cd uptake by shoot of RH-819 differed significantly from Varuna and RH-9304 at each level of added Cd. The reduction in cadmium uptake at higher Cd treatments may be due to drastic reduction in biomass (Marchiol *et al.*, 2006) of all the three genotypes.

CONCLUSIONS

The results of this study showed that out of three genotypes of Indian mustard tested, only RH-819 genotype survived upto Cd 120 mg kg^{-1} soil level. The significant reduction in total chlorophyll and dry matter yield of shoot was observed with the increasing level of Cd concentration. The mean dry matter yield of shoot, Cd concentration and its uptake was highest in RH 819, followed by Varuna and RH-9304. The Cd uptake by shoot of RH-819 differed significantly from Varuna and RH-9304 at each level of added Cd. Hence, genotype RH-819 of Indian mustard was found the best Cd tolerant genotype and was selected for uptake study.

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