Comparison of Phytoremediation of Cadmium and Nickel from Contaminated Soil by *Vetiveria Zizanioides L.*

Isha Gunwal*, Lata Singh ** and Payal Mago**

*, ** (Department of Botany, Sri Aurobindo College, University of Delhi, Delhi)
** (N.R.E.C College, Khurja, C.C.S University, Meerut)

Abstract- Industrial waste is one of the most important sources of contamination in the surface environment. The impact of heavy metals in soils, plants, animals and humans is due to the unabated expansion of toxic effects. The toxic wastes generated are treated by physicochemical processes in which "Bioremediation" is the microbial clean up approach. Plant based remediation (Phytoremediation) is an emerging biotechnological tool, for cleaning metal polluted or contaminated soil. The present study deals with phytoremediation of metal polluted soil collected from Delhi and surrounding areas utilizing Vetiver grass, which is one of the best "Hyperaccumulators" for the phytoremediation of metal polluted sites. *Vetiveria zizanioides (Linn) Nash)* were grown in three types of experimental plots containing sterilized soil with a known quantity of Cd and Ni salts as present in polluted soils (i) Control without any kind of AM spores, (ii) AM inoculum prepared from spores extracted from non Cd and Ni polluted soils,(iii) AM inoculum prepared from spores extracted from Cd and Ni polluted soils. Plants were observed for their growth and harvested after 3 months. The soil and plant samples were analyzed for cadmium (Cd) and nickel (Ni) using Atomic Absorption Spectrophotometry (AAS) at regular intervals i.e. every 15 days till harvesting of experimental plant. The results indicated that roots and shoots taken from plot II showed maximum uptake of Cadmium then Nickel in comparison to roots and shoots taken from plot I and plot III. Pytoremediation is found to be cost effective and highly efficient in remediating the heavy metal polluted sites.

Index Terms- Phytoremediation, Arbuscular Mycorrhizae (AM), Vetiver, Atomic Absorption Spectrophotometry (AAS), Hyperaccumulator, Nickel

I. Introduction

Industrial waste is one of the most important sources of contamination in the surface environment. Numerous studies of environmental contamination due to industrial wastage activities have been undertaken to further the understanding of the impacts of heavy metals and metalloids in soils, plants, animals and humans. The unabated expansion of chemical industries and technologies indeed left a trial of anxiety and global concern. The toxic metals may be absorbed by plants grown in contaminated soil which then accumulate in animals eating those plants perhaps reaching to chronic toxic levels. Most heavy metals become quite insoluble in soil at pH 6 or more. Cadmium, being highly soluble than other heavy metals, is a frequent contaminant. Other metals such as Ni, Cu, Mo and Zn are also soluble but to a lesser extent [19] .Soil pollution by heavy metals such as mercury, cadmium, chromium and lead are of great concern to public health [16]. The source of heavy metal in plant is the environment in which they grow and their growth medium (soil) from which heavy metals are then taken up by roots or foliage of plants [22]. Plants growing in polluted environment can accumulate heavy metals at high concentration causing serious risk to human health when consumed [2, 4, 30].

Cadmium is Natural. Cadmium (elemental symbol Cd and CAS registry number 7440-43-9) is a member, along with zinc and mercury, of Group 12 (CAS IIB) of the Periodic Table of the Elements. It is generally characterized as a soft, ductile, silver-white or bluish-white metal, and is listed as 64th in relative abundance amongst the naturally occurring elements. Cadmium is often described as a "heavy metal", but the term has no meaning in fact since it possesses an average density, atomic number and atomic weight compared to other metals. The density of cadmium is 8.64 g/cm^3 at room temperature compared to 1.85 g/cm^3 for beryllium, the lightest of the metallic elements, to 19.3 g/cm^3 for gold or tungsten, amongst the heaviest of the metallic elements. Nickel is a transition element with atomic number 28 and atomic weight 58.69. In nature, Ni is mostly present in the form of nickelous ion, Ni2+. The hydrated form as Ni (H2O)6 is the most common form of Ni found in the soil solution. Phytoremediation is the use of certain plants to clean up soil, sediment, water and polyaromatic hydrocarbons (PAHs). Phytoremediation is an aesthetically pleasing mechanism that can reduce remedial costs, restore habitat and clean up contamination in place rather than entombing it in place or transporting the problem to another site. Phytoremediation can be used to clean up contamination in several ways such as Phytovolatilization, Microorganism stimulation, Phytostabilization, Phytoaccumulation or extraction and Phytodegradation by plants[12,15] (Cunningham et al., 1997; Flathman and Lanza, 1998).The primary motivation behind the development of phytoremediative technologies is the potential for low cost remediation [14 (Ensley, 2000). [8] Chaney, (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metalpolluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal [10, 11,13] (Cunningham et al., 1995; Comis, 1996; Ebbs et al., 1997). By definition, a hyperaccumlator must accumulate at least 100 mg g 1 (0.01% dry wt [24,31] (Reeves and Baker, 2000; Wantanabe, 1997). Vetiver grass (*Vetiveria zizanioides L*. Nash) has been one among the well documented grasses to have strong resistance to the execrable environment and to be able to survive in high concentrations of heavy metals [32] (Xia et al., 1999). Although certain plants like *Brassica juncea* (cv. Vardan), *Vetiveria zizanioides*, *Viola sp.* posses a higher potential for removing Cd from moderately contaminated soil and could be used as a hyper accumulator for contaminated soil remediation. Recently vetiver grass, due to its ecofriendly nature, found a new use for phytoremediation of contaminated sites. Vetiver grass is both a xerophyte and a hydrophyte and, once established, is not affected by droughts or floods [17](Greenfield, 1988). It is highly tolerant to droughts and water logging, frost, heat, extreme soil pH, solidity, salinity, Al and Mn toxicity [27]*(Truong and Claridge, 1996).* It is also highly tolerant to a range of trace elements such as As, Cd, Cu, Cr, and Ni [27] (Truong and Claridge, 1996). It is suitable for the stabilization and rehabilitation and reclaiming of acid sulfate and trace metals contaminated soils, i.e. phytoremediation The present study hypothesises that *Vetiver* sp., a plant which shows high mycorrhizal colonization, is a hyper accumulator, with a high biomass, has the potential that mycorrhizal *Vetiver* could enhance Cd and Ni uptake due to more access to soil beyond rhizospere with the help of mycorrhizal hypae.

II. MATERIALS AND METHODS

Plants of *Vetiveria zizanioides* were grown under natural conditions in experimental plots in Department of Botany, Sri Aurobindo College, University of Delhi. The soil samples of all the experimental plots were used for analysis of cadmium and Nickel in soil as well as plant parts at regular intervals.

Experimental setup

The experiment was conducted in the Botanical Garden, Sri Aurobindo College, University of Delhi in 3 micro-plots of 10 m2 area each. The top soil (up to 30 cm depth) was fumigated twice with 0.1% formaldehyde at an interval of 15 days. Then the soil was allowed to dry and the fumigant was dissipated. Vetiver was grown in three types of experimental plots containing sterilized soil with a known quantity of Cd and Ni salts as present in polluted soils (i) Control without any kind of AM spores, (ii) AM inoculum prepared from spores extracted from non Cd and Ni polluted soils,(iii) AM inoculum prepared from spores extracted from Cd and Ni polluted soils. Clums rate was kept uniform for all treatments and when clums were 15 days old, thinning was done to maintain spacing of 10 cm between the plants and 20 cm within the rows. The plants were allowed to grow and no fertilizer or pesticide was added to the soil during the course of the experiment. Weeding was done mechanically at regular intervals and plots were irrigated with tap water.

I –Cadmium and Nickel contaminated soil + Vetiver plants without AM fungal spores

II – Cadmium and Nickel contaminated soil + AM fungi spores from non contaminated soil + Vetiver plant

III – Cadmium and Nickel contaminated soil +AM fungi spores from contaminated soil+ Vetiver plant

Description of sampling points

Three different points (A, B and C) 10 km around Delhi were sampled for soil and plant while the fourth sampling point is located in garden of Sri Aurobindo college, Delhi. The samples from this point served as control and the point was designated as D. Soil was collected from different localities in and around Delhi. These sites are (a) Shastri park area- dumping ground for burnt and half burnt electronic waste and disposal ground for nickel-cadmium batteries, (b) Dump yard of Okhla sewage treatment plant where treated sewage is converted into manure, (c) Sanganer soil from Rajasthan where effluents from dying industries are dumped into the soil, (d) Yamuna soil from the three different regions i. e. near okhla barrage, Wazirabad barrage and ITO barrage. These parts of Yamuna are its flood plains and receive water from Yamuna during rainy season. The water of Yamuna is highly polluted as it receives waste domestic sewage as well industrial effluents of Delhi yet there is large scale cultivation of vegetables and horticulture plants at its bank, (e) Uncontaminated garden soil from Sri Aurobindo college**.** The soils collected from above sites contain moderate level of Ni so Nickel was supplied in the form of soluble nickel salt dissolved in double distilled water (DDW), was mixed thoroughly in the soil and the soil was homozinised and kept in polythene bags and labeled to avoid a mix-up of the different soil samples and later analyzed for their Ni contamination. Plant samples were collected carefully using hand trowel to dig the soil around the plant and the plants were pulled out carefully, ensuring that no part of the root was lost. The different plant samples were kept in different polythene bags and properly labeled. Soil samples were collected from the same point where the plant samples were uprooted. Clums of *Vetiveria zizanioides*(Linn.) procured from Central Soil Salinity And Research Institute, Karnal, Haryana and planted in prepared plots (4x4inch) of 15 kg soil capacity containing soil collected from the different locations. Four plots were used for cultivating the plants. The prepared plots were placed in field conditions to expose the growing plants to natural environment. Ten plants per plot were maintained. Plants and soil was analyzed at 15 days interval for investigating the uptake potential, spore density and % root colonization. Every year plants of Vetiver grass uptake Ni from the soil and again the healthy clums of Vetiver grass were planted in the same soil. The culms of *Vetiveria zizanioides* (Linn.) grass with root (10 cm) and shoot (20 cm) were selected for the study. This experiment was conducted for three consecutive growth period with three treatments to *Vetiveria zizanioides*.

Collection of plant and soil samples

Pilot study has been carried out in the laboratory, Department of Botany, Sri Aurobindo College, University of Delhi, New Delhi-Clums of Vetiver were procured from Central Soil Salinity And Research Institute, Karnal, Haryana and planted in prepared plots (4x4inch) of 15 kg soil capacity containing sandy soil from the different locations in and around New Delhi, India (28.38N, 77.11E, 228 m altitude) during the winter season. The climate of Delhi is semi-arid and subtropical with the mean annual rainfall of about 650 mm. Three plots for each treatment were used for cultivating the plants. Cadmium and Nickel were supplied in the form of Cadmium nitrate and Nickel nitrate dissolved in double distilled water (DDW), was mixed thoroughly in the soil. The prepared plots were placed in field conditions (in Sri Aurobindo College) to expose the growing plants to natural environment. Ten plants per plot were maintained. Ninety-day-old plants were analyzed at 15 days interval for investigating the uptake potential. The culms of vetiver grass with root (10 cm) and shoot (20 cm) were selected for the study. This experiment was conducted over a 12-week growth period with three treatments to *Vetiveria zizanioides.*

Estimation of Cadmium and Nickel in soil and plant parts
Soil and plant collection and Preparation-

The soil (loamy sand) used in this study was collected from the experimental plots at regular intervals. The soil was sieved (4 mm) and steam sterilized (100_C for 1 hour for 3 consecutive days) to eliminate naturally occurring AMF propagules. Root samples along with surrounding soil were taken in polythene bags and stored at 4*c until they were processed further. The root samples were preserved in Formalin Alcohol Acetone (FAA) in the ratio of 90:5:5 (v/v/v) before clearing and staining.

Sample preparation:

Each plant sample was separated into leaves, roots, and stems and then dried at 50ºC for 8 hours using an oven. The dried plant samples were milled using a laboratory blender and kept for digestion. Unwanted materials such as stones, leaves and debris were removed from the soil samples by handpicking. The soil sample was further broken down into finer particles using a laboratory mortar and pestle. The soil samples were dried for 8 hours at 80ºC using an oven.

Sample digestion:

Soil and plant samples were digested before analysis to reduce organic matter interference and allow for the conversion of the metal into a form that can be analyzed by the Atomic Absorption Spectrophotometer (AAS). (Tandon, 1993)

Plant sample digestion:

Plant samples were digested following the Mixed Acid Procedure of Allen *et al [3]*

Reagents-

1. Perchloric Acid, 60%
2. Nitric Acid, concentrated.
3. Sulphuric Acid, concentrated.

Procedure-

1. Weigh 0.20-0.50g of air-dried or oven-dried milled plant sample into a 50 ml Kjeldahl flask using a digital weighing balance.
2. Add 1 ml of 60% Perchloric acid, 5 ml nitric acid and 0.5 ml Sulphuric acid to the weighed milled plant sample.
3. Swirl gently and digest slowly at moderate heat on a laboratory hot plate, increasing the heat later, until the white fume evolving from the Kjeldahl flask turned brown.
4. Digest for 10-15 minutes after the appearance of white fumes.
5. The digest was allowed to cool (the cold digest is usually colourless or occasionally pink).
6. Dilute to about 10 ml and boil for a few minutes and then filtered through a Whatman's filter paper (No. 44 paper) into 50 ml volumetric flask, leaving a whitish residue.
7. The filtrate was then made up to 25 ml using distilled water and kept for further analysis.
8. The residual acid is now about 1% (v/v).

Soil sample digestion:

The same procedure for the digestion of plant sample was used according to the method of Allen *et al. (1974)*

III. RESULT AND OBSERVATION

The results of the chemical analysis of cadmium (Cd) and Nickel in soil and plant samples collected are presented in **Table 1, 2 & Figure 1, 2**. The concentration of cadmium is higher in soil samples obtained from the experimental plot I when compared with those obtained from the experimental plots II & III. The concentrations of the heavy metals in the plant tissue were higher in the experimental plots II & III than the experimental plot I. Irrespective of the concentration of the metals and the presence or absence of AM spores the bioaccumulation of the selected metal was higher in the roots than shoots. It was 140% and 101% respectively in cadmium exposed vetiver plant. These findings suggested that the soil type could cause the difference in cadmium accumulation in vetiver plant. [5] Alloway (1997), Baker and Senft (1997) reported that plant species as well as cultivars differ widely in their uptake ability and accumulation of heavy metals.

The average highest cadmium concentration in shoots of vetiver grass was 5.11ppm. It was lower than the toxic threshold level [28] (Truong, 1999).The average highest Cd concentration in roots of vetiver grass was 11.83 ppm. Comparing the distribution of Cd concentration in the parts of vetiver grass, Cd was found to accumulate more in roots than in shoots (the accumulation rate in shoot/root is from 1.85% to 13.32%). This finding is similar to the results of [28] Truong (1999) and Roongtanakiat et al. (2002). They found that a small amount of Cd was translocated to the shoot.

The average highest Nickel concentration in shoots of vetiver grass was 3.99 ppm. It was lower than the toxic threshold level [28] (Truong, 1999).The average highest Ni concentration in roots of vetiver grass was 10.84 ppm. Comparing the distribution of Ni concentration in the parts of vetiver grass, Ni was found to accumulate more in roots than in shoots (the accumulation rate in shoot/root is from 1.05% to 10.32%). This finding is similar to the results of [28] Truong (1999) and Roongtanakiat et al. (2002). They found that a small amount of Ni was translocated to the shoot.

Table 1 – Mean Concentration of Cadmium mg/kg or ppm in soil samples and plant tissues of *Vetiveria zizanioides* at regular intervals

Time Interval	Experimental Plot	Cd Concentration (mg/kg or ppm)		
		Soil	Root	Shoot
15 Days	I	30.6	8.4	1.83
	II	29.5	9.3	2.74
	III	28.4	6.74	1.59
30 Days	I	27.3	10.14	2.19
	II	26.2	10.63	3.71
	III	25.1	7.74	1.74
45 Days	I	24.1	11.33	2.79
	II	23.1	11.85	4.71
	III	22.0	9.03	2.44
60 Days	I	20.9	12.13	3.21
	II	19.8	13.12	4.91
	III	18.7	10.04	3.14
75 Days	I	17.6	14.83	4.39
	II	16.5	15.82	5.11
	III	15.4	10.74	3.84
90 Days	I	17.6	14.83	4.39
	II	16.5	15.82	5.11
	III	15.4	10.74	3.9

Table 2 – Mean Concentration of Nickel mg/kg or ppm in soil samples and plant tissues of *Vetiveria zizanioides* at regular intervals

Time Interval	Experimental Plot	Ni Concentration (mg/kg or ppm)		
		Soil	Root	Shoot
15 DAS	I	12.5	1.88	0.83
	II	22.4	9.12	1.63
	III	28.4	6.74	1.59
30 DAS	I	10.3	2.63	1.01
	II	20.2	10.14	1.89
	III	25.1	7.74	1.74
45 DAS	I	9.1	2.85	1.71
	II	18.1	10.33	2.59
	III	22	9.03	2.44
60 DAS	I	7.9	3.12	2.41
	II	17.8	11.13	3.29

	III	18.7	10.04	3.14
75 DAS	I	6.6	3.82	3.11
	II	16.5	11.83	3.99
	III	15.4	10.74	3.84
90 DAS	I	7	3.82	3.11
	II	16.5	11.83	3.99
	III	14.5	10.74	3.84

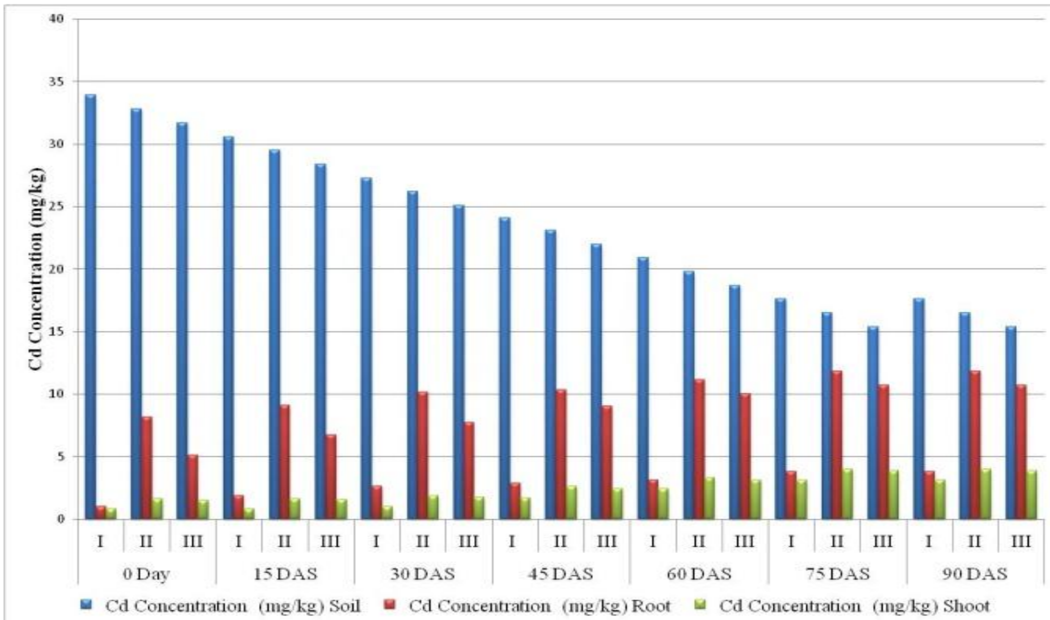

Fig. 1- Mean Concentration of Cadmium mg/kg or ppm in soil samples and plant tissues of *Vetiveria zizanioides* at regular intervals.

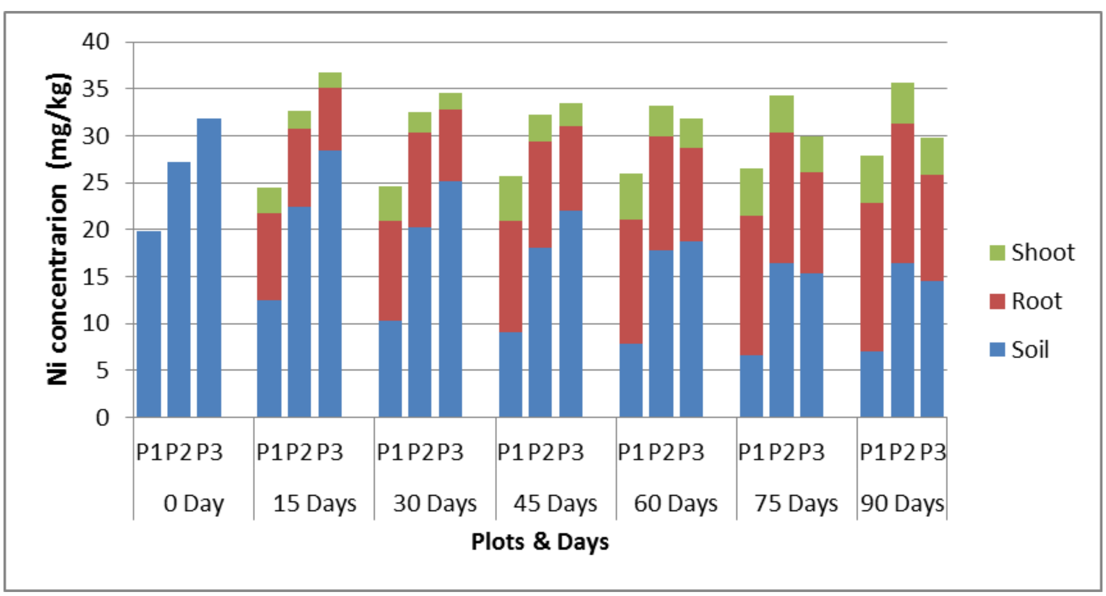

Fig. 2- Mean Concentration of Nickel mg/kg or ppm in soil samples and plant tissues of *Vetiveria zizanioides* at regular intervals.

Plants of *V. zizanioides* were harvested at regular intervals (15 days) from experimental plots. Roots and shoots of vetiver were taken at regular interval (15 days) and analyzed for Cd and Ni uptake every year on 15 DAS (Initial), 45 DAS, 90 DAS (maturity).It was observed that shoot started uptake on 45 DAS and uptake was slow and much less than roots in all the 3 plots studied because shoot showed stunted growth, chlorosis of shoot occurred, no. of fronds decreased and Stability in uptake was observed on 75 DAS and 90 DAS in all the 3 plots studied because the plant attain maturity and started dying

- At the end of cropping it was observed that there is depletion in cadmium from soil in each plot and roots had accumulated more cadmium in comparison to shoots because of early chlorosis, stunted growth, decrease in no of fronds of shoots were observed due to high concentration of cadmium in soil in each plot. Stability in uptake was observed on 75 DAS and 90 DAS in all the 3 plots studied because the plants attain maturity and started dyeing**.(Table 1 & Fig. 1)**
- At the end of cropping it was observed that there is depletion in Nickel from soil in each plot and roots had accumulated more Nickel in comparison to shoots because of early chlorosis, stunted growth, decrease in no of fronds of shoots were observed due to high concentration of cadmium in soil in each plot. Stability in uptake was observed on 75 DAS and 90 DAS in all the 3 plots studied because the plants attain maturity and started dyeing**.(Table 2 & Fig. 2)**
- It was observed that roots and shoots taken from plot II showed maximum Cadmium uptake in comparison to Nickel uptake in all years in comparison to roots and shoots taken from plot I and plot III.
- From the above result it is concluded that roots have maximum uptake potential than shoots. **(Table 1,2 & Fig. 1, 2)**

IV. DISCUSSION

The concentration of Cd in the soil was higher in soil samples from the industrial site compared with the non-industrial site. There is no doubt that heavy metals are present in soil naturally and non-degradable, and can be accumulated in the plant tissues [3,17] as shown by the concentrations of heavy metals obtained in soils from non-industrial site, but their concentrations can be increased by industrial activities[20].Vetiver is native to India, its environmental application for soil and water conservation is traditionally practiced for longer time. Systematic efforts to develop the vetiver grass technology for mitigation of soil erosion and water conservation were first initiated in India; however it was not practiced seriously. Several countries on the other hand, taking cues from the Indian initiative extensively implemented environmental applications of this grass [18]*(Lavania, 2004)*. Currently two main methods for treating contaminated water namely, 'engineering' and 'biological' are being used. The biological method consists of land irrigation, wetland and hydroponics system [9]*(Chomchalow, 2003).*

As vetiver has been found to be highly tolerant to extreme soil condition including heavy metal contamination, the present study was conducted to check the reduction level of Cd contaminants of soil on the plant's ability to tolerate toxic levels of Cd and on the ability to accumulate these heavy metals in roots and shoots. [29]*Truong and Baker (1998)* have proved the similar results. It might be concluded that heavy metals in soil even at the higher level to plant growth have no negative effect on vetiver root growth but showed negative effect on the shoots and the growth of shoots sustained after some days so as the growth of plant. Our study did not in coordination with the mentioned results. [25]*(Roongtanakiat and Chairoj, 2001)* .

For successful phytoremediation, the first requisite is high production of plant biomass. Since metal removal is a function of metal concentration in the harvestable biomass, the plant should be able to produce enormous biomass so that it can grow successfully at the contaminated site. Cr uptake is an important parameter in understanding the cellular responses of high HM concentration in plants and is one of the requisites contributing to the success of phytoremediation. The ambient metal concentration in the soil was the major factor influencing the metal-uptake efficiency as the metal uptake was observed to increase with increase in treatment doses *(Ghosh & Rhyne 1999; Begonia et al. 2005).* Considering the definition of hyperaccumulator given by *Foy (1984), [6,7,24]Baker & Brooks (1989), Baker et al. (2000) and Reeves & Baker (2000),* Indian mustard behaves as a potential Cr hyperaccumulator. The present study is not in accordance with the above mentioned results because plant chosen in the present study has a higher biomass of roots as compared to arial parts. Pods are also used commercially for essential oil extraction (Khus-Khus) which is used in perfumery and beverage industry. Also the roots of the plant are extensively used for making Chiks (a type of curtain sprayed with water ocassionaly in summer months and keep the area cool and gives sweet fragrance at the same time. Due to higher biomass of its roots the plant is also used for keeping a check on soil erosion. So here due to higher biomass of the roots as compares to shoots the more cadmium accumulation is seen in roots as compared to shoots) On the other hand, Cd accumulation was enhanced in various plant parts with time. In general, when metal concentration was increased, the amount of metal accumulation in plants increased. Similar results were obtained for Lemna minor *(Jain et al. 1990*) and water hyacinth [33]*(Zhu et al. 1999).* In conclusion, our test plants displayed enhanced tolerance towards a range of Cd concentration besides accumulating high Cd in its various parts, thus serving as a suitable contender for phytoremediation. It is suggested that the remediation potential of Vetiver can be tapped to the utmost by periodically harvesting the plant from the site being remediated, avoiding chances of the plant attaining lethal concentration of metal that could lead to oversaturation and hence the damage. The harvested biomass could then be incinerated and disposed off or the accumulated metal could also be recovered for commercial uses and thus recycled and reused.

The results of the present investigation showed that the cd uptake by roots was more in comparision to the shoot and the concentration of cadmium in soil decreased slowly initially but pick up more later and steadily in three consecutive year of cropping and slightly reached below the permissible level and continuously present study showed is not in accordance with the following studies.

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AUTHORS

First author- Isha Gunwal, M.sc, M.phil, PhD, isha_gunwal83@yahoo.co.in
Second author- Lata Singh, M.sc, M.phil, PhD, drsamindersinghrana@gmail.com
Third author- Payal Mago, Associate Professor, Sri Aurobindo College, University of Delhi, Payal500@hotmail.com

Correspondence author- Isha Gunwal, isha_gunwal83@yahoo.co.in, contact no- 9560922869