

COMPARATIVE UPTAKE AND PHYTOEXTRACTION STUDY OF SOIL INDUCED CHROMIUM BY ACCUMULATOR AND HIGH BIOMASS WEED SPECIES

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Abstract. Plant species have been recently used for heavy metal accumulation and most of the studies have been done on hyperaccumulator tolerant species. Metal hyperaccumulator plants though useful to phytoextract metal contaminant from soil, have many shortcomings such as low biomass, edible nature and difficult to harvest. This study is part of a series of studies that attempt to evaluate the phytoextraction potential of commonly found high biomass weed species that are harmless, non-edible in nature. We have investigated and compared five weed species (*Ipomoea carnea*, *Dhatura innoxia*, *Phragmites karka*, *Cassia tora* and *Lantana camara*), with two accumulator plants (*Brassica juncea* and *Brassica campestris*), in a pot study to assess Cr uptake in the range of 5 to 200 mg kg⁻¹ soil. The results indicated that *P. karka* showed much greater tolerance to metals than other plants, though the uptake was low. It was more effective at translocating Cr from soil to plant shoot. The order of Cr extraction was *I. carnea* > *D. innoxia* > *C. tora* > *P. karka* > *B. juncea* > *L. camara* > *B. campestris*. Among the studied plants *I. carnea* showed maximum chromium extraction and biomass growth, but the difference of shoot by root chromium concentration was least. Other than *Lantana camara*, all the tested weeds were better for chromium extraction than the accumulator *Brassica species*. To save the *Brassica species* infested by army moth, pesticide application was required, whereas weeds required no care.

Keywords: *phytoremediation, weeds, bioconcentration factor, transportation index*

Introduction

India is one of the largest producers of leather and nearly 80 % of the tanneries are engaged in chrome tanning process [19]. Leather tanning, electroplating and stainless steel industries contribute to most of the chromium contamination, by disposal of wastewater directly to the streams and/or by over land disposal of sludge or solid waste [21, 24]. Non-biodegradability of chromium is responsible for its persistence in the environment; once mixed in soil, it undergoes transformation into various mobile forms before ending into environmental sink. The dominant forms of chromium in waste contaminants are dichromate (Cr₂O₇²⁻) and/or chromate (CrO₄⁻). Chromate, (CrO₄²⁻), is the predominant form at pH > 6. It exists in pH-dependent equilibrium with other forms of Cr(VI), such as HCrO₄⁻ and dichromate (Cr₂O₇²⁻); these oxyanions are actively transported to cells by the sulfate transport system. Adsorption of Cr (VI) is considerably less; it is soluble at neutral to alkaline pH than at more acidic pH values [5, 6].

In view of the seriousness of Cr pollution, considerable efforts have been made to develop suitable methods for the remediation of chromium-contaminated soil. Phytoextraction; a part of phytoremediation technology, is considered for remediation of inorganic and organic contaminated sites because of its cost effectiveness, aesthetic advantages, and long-term applicability. It is well suited for large sites where other

methods may prove impractical. For a country like India phytoremediation is best suited as it requires low investment, and relies on plants natural capability to take up metal ions from soil. After accumulation of contaminants, plants can be harvested and the biomass can be used as a source of energy along with recovery of metal from ash. This will complete a biogeochemical cycle and heavy metals can be isolated.

Role of Chromium in Plants

Chromium is not considered to be essential for plant growth and development; some studies have indicated that at low concentrations ($1\mu\text{M}$), Cr stimulates plant growth [8]. Chromium (Cr) exists predominantly in III and VI oxidation states. The hexavalent chromium Cr(VI) compounds are comparatively more toxic than Cr(III) due to their high solubility in water [12], rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids [7]. Chromium is toxic for agronomic plants at about 0.5 to 5.0 mg l^{-1} in nutrient solution and 5 to 100 mg kg^{-1} of available Cr in soil [11]. The species found to accumulate Cr are largely exotic; research into the mechanisms of Cr hyperaccumulation is scarce. The plant species *Leptospermum scoparium* (Myrtaceae) is an accumulator of Cr, it showed up to $20,000\text{ mgCr kg}^{-1}$ in the foliage ash when grown on serpentine soils. Few Cr hyperaccumulator species have been identified to date, [3]. *Brassica juncea* has been found to be an excellent accumulator plant for Cr in soils, other metals accumulated by it are Cd, Ni, Zn and Cu [13, 22]. Hence it was considered as reference plant in our study, along with this *Brassica campestris* was also selected, as it belongs to the same family.

Looking at the environmental conditions of the polluted areas, hardy tolerant weed species were selected for phytoextraction study. Plants that can grow in both dry lands and marshy conditions were considered. These are *Ipomoea carnea*, *Datura innoxia*, *Cassia tora* and *Lantana camara* (all are local weeds). Another example of a plant that has shown much promise in the treatment of metal pollutant is *Phragmites karka*, it has the ability to take up chromium from contaminated water [10, 22]. Though studies on *Phragmites karka* were hydroponic, soil studies on chromium accumulation are scarce. Thus the study focuses on the ability of high biomass weed species to extract and decontaminate Cr spiked soil and discounts on the plants ability to accumulate large amounts of Cr in the tissues. All these plants naturally grow in the tropical climates and large parts of the Indian subcontinent. Cr (VI) was added to soil as Potassium dichromate solution at various concentrations, and chromium uptake and extraction were compared between accumulator and weed species.

Materials and methods

As the pH of the soil is 8 ± 0.2 , and Cr (VI) is more soluble than Cr (III), easily transported inside plant and is the predominant form at $\text{pH} > 6$; Cr (VI) was chosen as the study species. Topsoil from botanical garden was air-dried, sieved to ($< 2\text{ mm}$), and thoroughly mixed. Soil pH was measured in double distilled water using a solid: liquid ratio of 1: 2.5 after equilibrium for 2.5 hrs. The Organic Carbon is $5.5 \pm 0.3\text{ g kg}^{-1}$, CaCO_3 $70 \pm 6\text{ g kg}^{-1}$, Clay 590 g kg^{-1} , semectite is the dominant mineral of the studied soil [20].

Soil Treatments and Sowing

Pot culture experiments were conducted using soil treated (spiked) with Potassium dichromate solution. The final concentration of Cr added in soil was 5, 10, 20, 50, 100 and 200 mg kg⁻¹ respectively, and for comparison an unamended (control) was taken. Chromium solution was uniformly mixed with air-dried soil, kept for two weeks to stabilize and filled in pots (8 kg). Twenty seeds were sown in the soil to germinate; out of them only six uniform plants were allowed to grow in each pot, at a uniform distance. Pots were placed in net house shaded with transparent polythene sheet, to protect from rainwater leaching. Plants were grown under natural light and ambient temp in order to keep all plants under conditions as similar as possible. Fertilizers or soil amendments were not added to enhance growth or metal uptake.

Plant Growth and Harvesting

For growth studies individual plants were grown under similar conditions and at set time intervals 10 plants for each concentration and interval (i.e. 15, 30, 60 and 90 days) were harvested from the six replicate pots, without damaging the roots. Maximum recoverable portion of roots were procured and plants were rinsed in distilled water to remove dust and soil mineral particles. The plants were separated into leaves, stem and roots and oven dried at 85^o C for 36 hours and weighed. Shoot and root length (cm) and dry biomass (g) of different plant parts (leaves, stem, and roots) were taken for each treatment and interval. All the calculations of Cr uptake and extraction were done on dry weight basis.

Analysis of Plant Mass

Dried samples were homogenized using a wily mill before analysis. The samples were digested in acidic mixture of HNO₃: HClO₄ (APHA-1992), and chromium analysis was done in triplicate by Atomic Absorption Spectrophotometer (GVC 902).

Transportation index (T_i) gives the leaf/root chromium concentration and depicts the ability of the plant to translocate the metal species from roots to leaves at different concentrations. It was calculated, as:

$$T_i = \frac{\text{Cr content of the leaves mg kg}^{-1}}{\text{Cr content of root mg kg}^{-1}} \times 100$$

Metal uptake is also depicted as Bioconcentration Factor (BCF). It provides an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate [27]. It was calculated as follows:

$$\text{Bioconcentration Factor} = \frac{\text{Average chromium conc. in the plant tissue (mg kg}^{-1}\text{)}}{\text{Chromium added in soil (mg kg}^{-1}\text{)}}$$

It has limited application if one wishes to compare uptake of a plant species under different treatments. Since change in BCF is related to the individual plant biomass and soil elemental concentration, the efficiency of BCF is a better understood when compared between different harvests, plant species or elements.

Data analysis

Statistical significance of the observed differences between samples was determined by Student's *t*-test and ANOVA test. Differences were considered to be significant at $p \leq 0.05$ and highly significant at $p \leq 0.005$, level of significance.

Results and Discussion

Chromium toxicity was evident in form of reduction of shoot and root length and total biomass of the plants. The growth of the plants was highly affected with increase in chromium concentration. Among all the tested plants *Phragmites karka* was the only plant to grow above 20 mg Cr kg⁻¹ soil. Plants other than *P.karka* grew till maturity (90 days) only up to 20 mg kg⁻¹. At 50 mg kg⁻¹ Cr plants were able to germinate and grow up to 10 days, this clearly shows that conc. of 50 mg Cr kg⁻¹ soil is toxic to plants and this is in agreement with range mentioned earlier as the toxic range. Significant reduction ($p < 0.05$) in shoot length of plants was observed in comparison to control [18]. in their study concluded that Cr(VI) seems to act principally on plant roots, resulting in intense growth inhibition; this was evident in form of reduction of mass. As shown in Table 1, in comparison to control all the seven tested species exhibited sensitivity to Cr and all the plants showed highly significant ($p < 0.005$) reduction in dry biomass. *Brassica campestris* and *Brassica juncea* were the most sensitive showing 58% and 48% reduction in total dry mass respectively, *Datura* was least affected and showed a biomass reduction of only 21% at 20 mg Cr kg⁻¹ soil. *Phragmites karka* was tolerant to 200 mg Cr kg⁻¹ soil, but the biomass showed 93 % reduction in comparison to control. The correlation of increase in biomass with respect to time was positive, whereas with increase in chromium conc. it was negative. Beyond 90 days only *I. carnea* and *L. camara* continued to grow, whereas other plants did not add biomass. The pattern of addition of biomass is important for phytoextraction studies because it is needed to estimate the best time to harvest the biomass, in this case harvesting after 90 days was best, as no mass was added beyond this period.

Table 1. Average Dry biomass (g) grown in chromium treated soils (n = 6) on 90th day.

Total Cr added in Soil (mg kg ⁻¹)	<i>Brassica campestris</i>	<i>Brassica juncea</i>	<i>Datura innoxia</i>	<i>Ipomoea carnea</i>	<i>Phragmites karka</i>	<i>Cassia tora</i>	<i>Lantana camara</i>
Control	3.28	3.31	12.32	19.59	11.46	12.45	5.43
5	1.86**	2.89*	8.57**	15.01**	7.66**	7.90**	2.27**
10	1.47**	2.16**	7.24**	11.33**	5.93**	7.30**	1.93**
20	1.36**	1.17**	6.49**	10.50**	7.64**	7.21**	1.76**
50	NG	NG	NG	NG	1.51**	NG	1.09**
100	NG	NG	NG	NG	1.06**	NG	NG
200	NG	NG	NG	NG	0.78**	NG	NG

Significantly different * ($p \leq 0.05$) & ** ($p \leq 0.005$) in comparison to control plant.

NG = No Growth observed

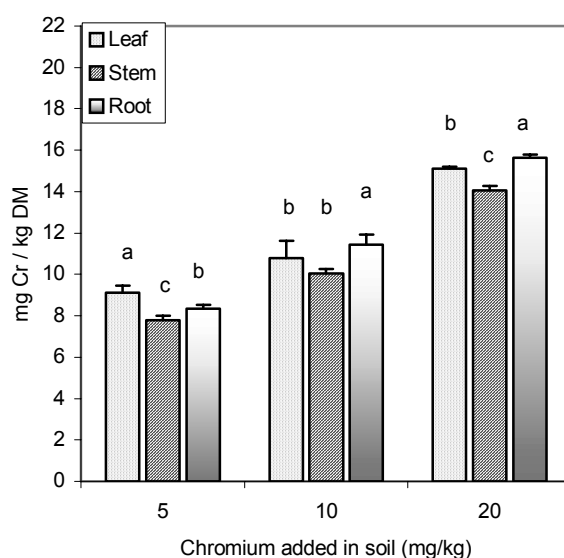
n = number of plants

Chromium uptake by plant tissues

Chromium uptake by plants is mainly non-specific, probably as a result of plant uptake of essential nutrients and water. Plants can absorb both Cr(VI) and Cr(III);

though Cr was added as hexavalent form it is expected that both the forms are simultaneously present in soil. At the end of 90 days, maximum Cr accumulation was in roots followed by leaves and stem in all the species, except *P.karka*. Statistically significant ($p < 0.05$) difference in accumulation of Cr in leaves, stem and roots has been shown in Figure 1 (a to g). At the earlier stage (15 days) concentration was maximum in leaves, but later roots accumulated most of the Cr followed by leaves and stem, this confirms the earlier results that metals get accumulated in leaves due to transpiration pull. Chromium (VI) is more easily transported inside the plant, as it has been reported to occur by an active mechanism [17].

Figure 1a. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *B. campestris*. Different letters indicate significant difference between parts ($p < 0.05$).



The initial symptoms of Cr toxicity appeared as severe wilting and chlorosis in *Datura* and *Brassica* species, as confirmed by [25]. They proposed that chlorosis appeared in the upper leaves of these plants, as an indirect effect of Cr, probably due to the retardation of Fe and Zn translocation. The primary toxic effect seemed to be membrane damage due to the high oxidative potential of Cr (VI), this was observed as necrosis in the lower leaves of *D.innoxia*. Two plants *I.carnea* and *P.karka* did not show any deformation except reduction in biomass. Most researchers [9, 23], using non-hyperaccumulator plants have reported that Cr is mainly accumulated in the roots, and relative lower in the leaves, this was observed in all the plant species except *P.karka*.

Maximum accumulation of Cr in plants was observed between 31 to 60 days, but the extraction was highest between 61 to 90 days. This shows that the increase in biomass was at a higher rate than accumulation of chromium between 31 to 60 days, which got reduced at a later stage. The amount of chromium increased with both increase in time and soil Cr concentration, this depicts the accumulation of chromium was linear and showed a positive correlation. Only in case of *P.karka* the correlation was negative with increase in time. The average chromium uptake was significant ($p < 0.05$) with increase in metal concentration.

Figure 1b. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *B. juncea*. Different letters indicate significant difference between parts ($p < 0.05$).

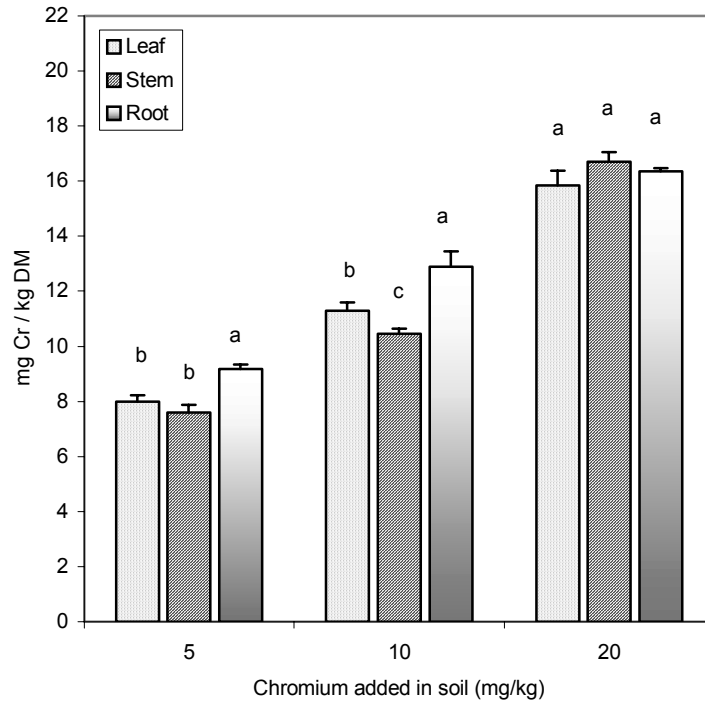


Figure 1c. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *D. innoxia*. Different letters indicate significant difference between parts ($p < 0.05$).

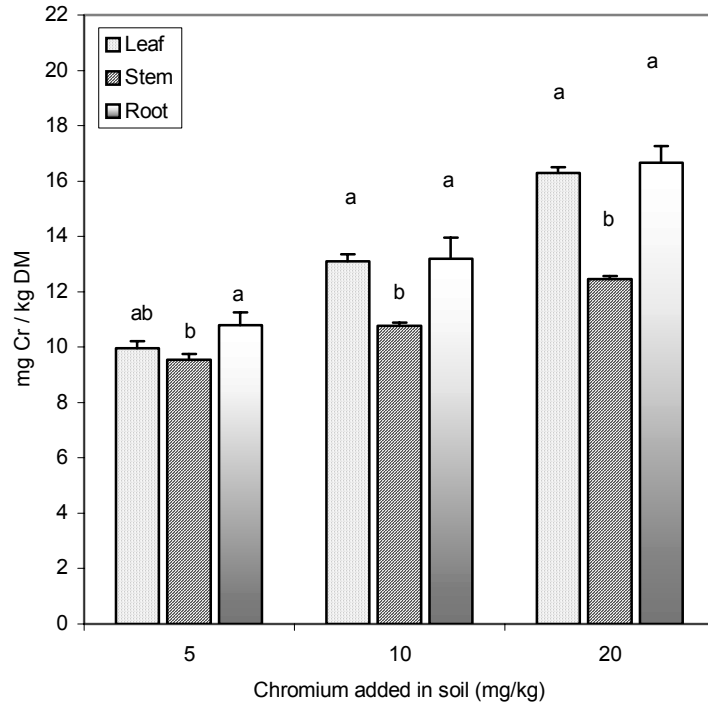


Figure 1d. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *I. carnea*. Different letters indicate significant difference between parts ($p < 0.05$).

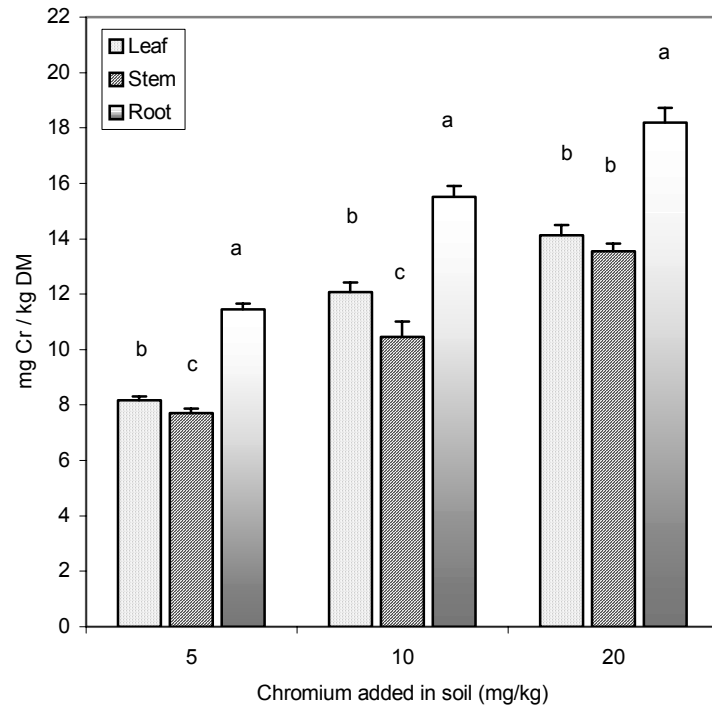


Figure 1e. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *P. karka*. Different letters indicate significant difference between parts ($p < 0.05$).

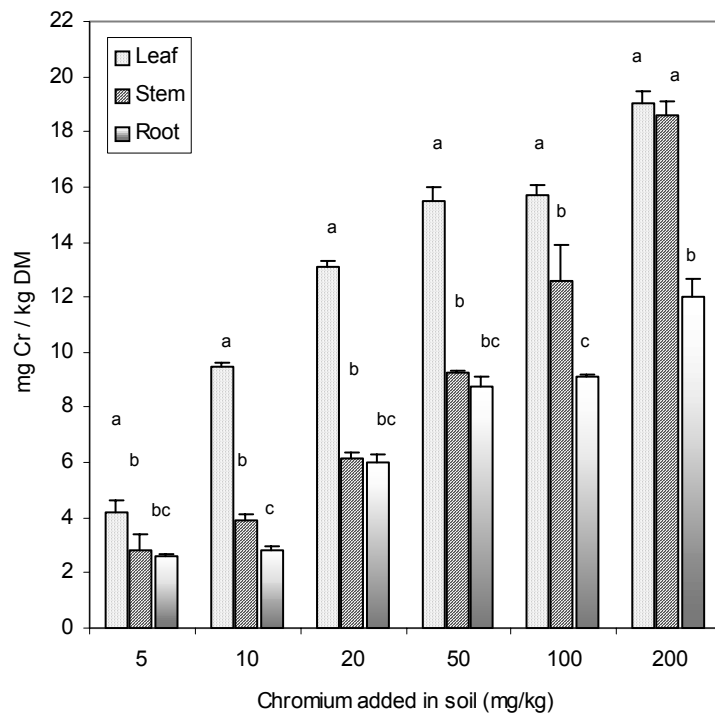


Figure 1f. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *Cassia tora*. Different letters indicate significant difference between parts ($p < 0.05$).

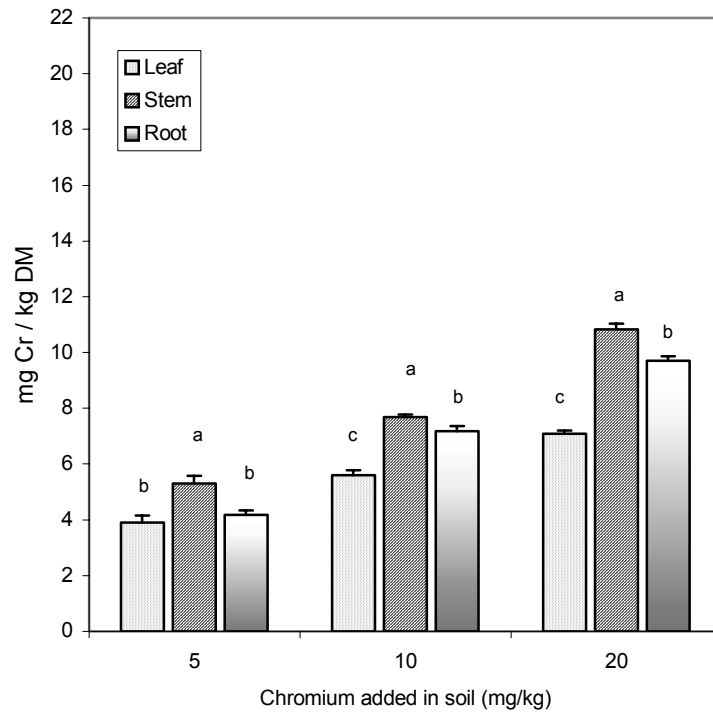
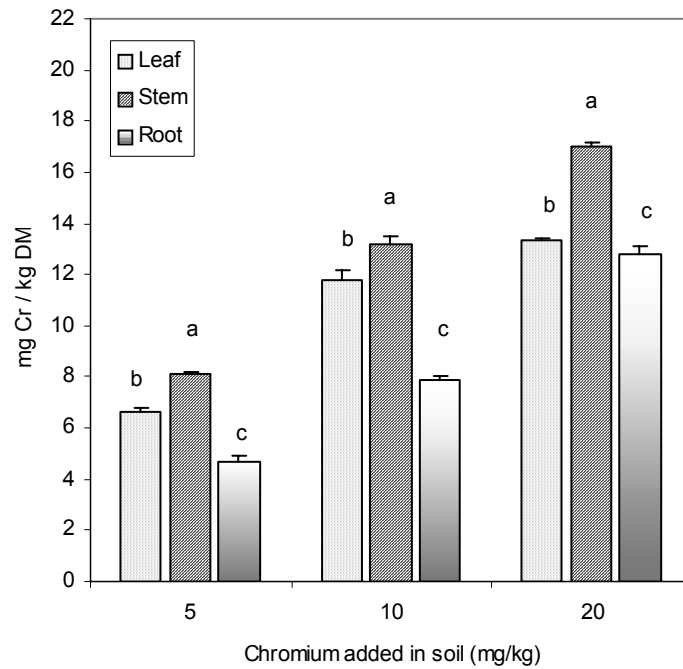


Figure 1g. Chromium concentration (mg kg^{-1} of dry matter) in leaves, stem, and roots of *Lantana camara*. Different letters indicate significant difference between parts ($p < 0.05$).



Transportation index (T_i) - Plants must have the ability to translocate Cr from the root to the shoot, or to compartmentalize it, in order to continue absorption of Cr from the substrate. Better translocation is advantageous to phytoextraction; (i) it can reduce Cr concentration and thus reduce toxicity potential to the root, and (ii) translocation to the shoot is one of the mechanisms of resistance to high Cr concentration. *P.karka* showed maximum translocation but this was due to lower Cr accumulation in roots. Transportation index (T_i) was least and accumulation was lower in *I.carnea* but it had maximum shoot extraction. All the plants showed a general trend of fall in T_i with increase in chromium concentration, thus showing inhibition of its translocation in plant body.

Bioconcentration factor (BCF) of Cr increased with increase in time, this means Cr accumulated up to maturity, as shown in Table 2. BCF is a useful comparison of metal accumulation power of different plants at same background metal concentration. It is bound to get reduced with increase in metal conc. in soil, but the trend in change of BCF at various intervals shows that the plant is extracting metal or not. In our study the value increased with increase in time.

Table 2. Bioconcentration Factor (BCF) of the studied plant species at different intervals.

Plant Species	Total Cr added in Soil (mg kg^{-1})	15 Days	30 Days	60 Days	90 Days
<i>Brassica campestris</i>	5	0.30	0.54	1.4	1.75
	10	0.33	0.41	0.87	1.07
	20	0.30	0.32	0.57	0.75
<i>Brassica juncea</i>	5	0.83	1.13	1.44	1.71
	10	0.53	1.03	1.11	1.20
	20	0.41	0.62	0.62	0.72
<i>Dhatura innoxia</i>	5	0.72	1.12	1.51	1.58
	10	0.88	1.01	1.15	1.12
	20	0.49	0.63	0.69	0.81
<i>Ipomoea carnea</i>	5	0.79	1.17	1.42	2
	10	0.56	0.72	1.18	1.21
	20	0.38	0.54	0.68	0.75
<i>Phragmites karka</i>	5	0.48	0.65	0.77	0.66
	10	0.58	0.78	0.85	0.58
	20	0.41	0.72	0.63	0.416
	50	0.22	0.30	0.28	0.248
	100	0.12	0.16	0.15	0.136
	200	0.06	0.08	0.09	0.089
	5	0.37	0.67	0.77	1.145

Table 2. continued from previous page

Plant Species	Total Cr added in Soil (mg kg ⁻¹)	15 Days	30 Days	60 Days	90 Days
<i>Cassia tora</i>	10	0.35	0.68	0.67	0.83
	20	0.34	0.72	0.55	0.501
	5	0.28	0.41	0.45	1.37
<i>Lantana camara</i>	10	0.30	0.53	0.67	1.163
	20	0.31	0.54	0.51	0.726

Extraction of Chromium

Chromium extraction is amount of metal accumulated by the whole plant mass or plant part. At 20 mg Cr kg⁻¹ soil and maturity, maximum extraction of Cr (151 μg plant⁻¹) was observed in *I.carnea* followed by *D.innoxia* (97 μg plant⁻¹) at maturity. The order of Cr extraction at above Cr conc. was *I.carnea* > *D.innoxia* > *C.tora* > *P.karka* > *B.juncea* > *L.camara* > *B. campestris*. Table 3, shows the Cr average extraction by a single plant at different treatments and intervals. *I.carnea* extracted more than five (5.2) times that of *B.juncea*, this was mainly because the biomass was more than five (5.8) times its dry biomass. *B.campestris*, *D. innoxia*, *P. karka* and *I. carnea* extracted maximum amount of Cr at 20 mg Cr kg⁻¹ soil. *P.karka* was able to grow above this conc. but the Cr extraction potential showed a sharp drop.

Table 3. Chromium extraction (μg plant⁻¹) by whole plant, at given Cr treatments (mg Cr kg⁻¹) soils at different intervals.

Plant Species	Total Cr added in Soil (mg kg ⁻¹)	15 Days	30 Days	60 Days	90 Days
<i>Brassica campestris</i>	5	0.3	0.8	6.9	16.2
	10	0.5	0.9	7.3	15.5
	20	0.9	1.3	8.8	20.6
<i>Brassica juncea</i>	5	1.2	2.2	11.9	23.0
	10	2.6	3.8	16.4	30.4
	20	2.4	3.1	14.8	28.1
<i>Dhatura innoxia</i>	5	0.3	3.3	22.1	85.4
	10	0.5	3.7	25.7	87.9
	20	0.4	2.1	17.3	97.5
<i>Ipomoea carnea</i>	5	0.9	4.0	30.9	128.9
	10	1.2	6.0	40.7	136.2
	20	1.0	5.0	9.0	151.6
	5	0.6	4.0	11.5	25.3

Table 3. continued from previous page

	10	1.2	7.4	18.2	33.2
	20	1.8	8.4	18.5	63.6
<i>Phragmites karka</i>	50	3.9	8.8	12.2	18.9
	100	3.6	9.0	11.8	14.5
	200	3.6	5.8	12.0	14.1
	5	0.3	2.4	11.3	15.24
<i>Cassia tora</i>	10	0.5	3.1	13.2	18.41
	20	0.8	3.6	14.0	22.28
	5	0.1	1.6	6.8	15.59
<i>Lantana camara</i>	10	0.3	2.9	8.1	22.53
	20	0.4	3.6	10.4	25.56

In *B. juncea* and *P.karka* the extraction decreased with increase in Cr concentration. Relative percentage extraction in case of *B.campestris*, *B.juncea*, *D.innoxia* and *P.karka* was more by the foliage and in *I.carnea* the extraction was more by stem. The distribution of Cr between root and shoot in accumulator plants, however, indicated that the leaves also contained a much higher Cr concentration than that of non-hyperaccumulator plants, suggesting better translocation of Cr from root to shoot for hyperaccumulator plants.

Conclusion

This study provides a promising start for biomass-based phytoextraction as it includes high biomass producing species, growing these species is practically easier than accumulators. The accumulator *Brassica species* infested by army moth, and pesticide was applied to save the plants, but for better results the both the *Brassica species* had to be replanted. Whereas weeds required no care to grow, this is a practical problem that will require care in field applications. Next research should focus on identifying the proper usage of the biomass produced, as it can be used as a source of energy. The results indicate that plant species differ significantly in Cr uptake capacity and distribution within the plant. *I.carnea* was much more effective than *B.juncea* for phytoextraction of Cr contaminated soils. This was due to two factors, (i) It was able to tolerate/ accumulate equivalent levels of Cr in soil. (ii) Though shoot/root ratio of Cr was much lower in *I.carnea*, its shoot biomass was much higher; a great proportion of total metal in plant is therefore sequestered in harvestable tissue. In the soil having more than 20 mg Cr kg⁻¹ soil, only *P.karka* showed the potential for phytoextraction.

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