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Tolerance of Three Ornamental Plant Species to Chromium Contamination in Soil and their Potential for Phytoextraction and Phytostabilization of the Toxic Metal

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Abstract

Chromium is a reactive and toxic heavy metal that enters the soil through various anthropogenic activities and moves through food chain affecting adversely the higher trophic levels including humans. While engineering techniques to remediate metal contaminated sites are costly and energy intensive, phytoremediation with suitable plant species is a low cost, easy and eco-friendly technique, which uses solar energy in the process. Using suitable non-edible plants makes the process of remediation safe and sustainable. The present study was therefore, carried out to study growth, Cr tolerance and phytoremediation potential of three ornamental plant species *Sansevieria trifasciata*, *Canna indica* (L) and *Nephrolepis exaltata* (L) for removal of chromium from soil. Pot culture experiments were conducted in greenhouse using soils artificially spiked with chromium (Cr_{250}, Cr_{500}, Cr_{750} mg/kg soil). Tolerance to different concentrations of Cr varied with the plant species as reflected by the trends and magnitude of change in aboveground and belowground biomass. Leaf chlorophyll and carotenoid were quite tolerant at Cr_{250} for all the species, and up to Cr_{500} for *Sansevieria*. The antioxidant enzyme superoxide dismutase (SOD) showed elevated activity in aboveground parts at Cr_{250}, while catalase (CAT) activity declined in response to the metal. All the three species showed significant accumulation of Cr, and more so in the belowground parts. Total Cr phytoextraction was the highest in *N. exaltata*, followed by *C. indica* and *S. trifasciata*. In all the three species, bioconcentration factor (BCF) was >1, indicating the suitability of these species for phytoremediation of chromium contaminated soils.

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Introduction

Various anthropogenic activities as well as geological processes lead to chromium contamination of soils and being non-biodegradable, the metal persists in the soil system for years, affecting soil quality and plant life. Use of chromium in several industries like electroplating, manufacturing alloy products, nuclear reactor vessels, leather tanning, textile and dye synthesis ultimately leads to its discharge in the wastewaters and sludge that impacts both aquatic and terrestrial ecosystems.[1] Out of the two forms of chromium, Cr (III) occurs naturally in soil and is used by organisms as a micro-nutrient for their growth and development,[2] whereas Cr(VI) is a potent toxin that is produced by various anthropogenic activities and also, by natural oxidation of Cr(III). Hexavalent chromium is extremely reactive and hazardous in nature. It has been found that 75-100 ppm concentrations generally do not adversely affect plant growth, but above this concentration it is toxic and inhibitoryc Concentration of Cr in contaminated soils, particularly in industrial and mined areas often exceeds 1000ppm. Therefore, it becomes particularly important to decontaminate soils that have concentrations of Cr exceeding 100ppm. Various conventional methods used for removal of contaminants at large scale are not only expensive but also affect soil constitution and its fertility.[4] While using a method for soil decontamination, it is important to see that it does not lead to pollution of other environmental components such as air or water. Phytoremediation has emerged as one such method that is less expensive as well as eco-friendlyinnature. Phytoextraction and phytostabilization are the two important strategies that are useful for the phytoremediation of metal contaminated soils.

Phytoextraction has been widely studied because metal removal from polluted soils in such systems is high and economic. However, when the metals present in the soil get accumulated in plants, there are chances of their getting transferred through the food chain. It is, therefore proposed to use such plants for phytoremediation, which do not contribute directly towards food production and hence can be used in an environmentally safe manner. A few studies using ornamentals such as *Tagetes erecta* and *Helianthus annus* that have shown promise or phytoremediation of heavy metals like zinc and cadmium.[5,6] Considering the enormous diversity of plants and their wide range of metal tolerance and accumulation capacity, it is worth while to explore newer species that might prove useful in phytoremediation.

The present study is aimed at exploring Cr tolerance and phytoremediation potential of some fast-growing herbaceous ornamental plants (*Sansevieria trifasciata* var. *hahnii*, *Canna indica* (L.) and *Nephrolepis exaltata* (L.), which possess good biomass with a robust root system. Based on a preliminary screening study using a variety of plant species for extraction of multi metals, these plants were selected.[7] These plants showed metal uptake capacity and were easy to grow, and their perennial nature provides environmentally sound long-term phytoremediation. Besides, they add to the aesthetics and also have very little chances of transfer of the metals through food chain.

Considering the fact that tolerance of the species to high concentrations of the metal and good biomass would be vital in this context, plant growth, aboveground and belowground biomass, biochemical parameters like chlorophyll, carotenoids and oxidative enzymes like superoxide dismutase (SOD) and catalase (CAT) were studied in response to a range of Cr (VI) concentrations and bioremediation potential was assessed by determining their bioaccumulation and bio-concentration factors.

Methodology

Pot-Culture Experiments

The plant species selected for the study, namely, *Sansevieria trifasciata* var. *hahnii*, commonly known as bird's nest snake plant (family *Asparagaceae*), *Canna indica* (L.) commonly known as Indian shot (*Cannaceae*) and *Nephrolepis exaltata* (L.) known as Boston fern or sword fern (*Nephrolepidaceae*) are grown in the tropics and sub-tropics as ornamental species. These plants have good biomass and are not used as a food resource in these regions.

Plants of all the three species were obtained from a local nursery in New Delhi, India and young plants approximately of same age, height and biomass were selected for the experiments. Pot culture experiments were carried out in greenhouse within the university campus under controlled conditions

($27^0C \pm 3^0C$). Different concentrations of Cr (250, 500, 750 mg Cr^{kg-1}soil) were artificially created by spiking normal garden soil with calculated amounts of $K_2Cr_2O_7$, the pre-treatment soil without any Cr addition with pH 6.8 and organic carbon 0.28% served as control. A total of 72 pots (3 species x 4 treatments x 2 sampling days x 3 replicates) were taken. Each pot was filled with 5kg soil of the desired Cr concentration. The desired concentrations of Cr were added after accounting for Cr already present in the original pre-treated soil (11.6 mg Cr^{kg-1} soil) as determined by using atomic absorption spectrophotometer explained ahead. Each pot was watered equally as and when required using tap water. Calculated quantity of Cr was added to fixed amount of soil in pots and saucers were placed under each pot to collect the drained water, which was then poured back to pots so that any loss of Cr leached out of the pot was restored.

Growth and Tolerance Studies

Tolerance of the plant species to different concentrations of Cr was studied in terms of their aboveground and belowground biomass, leaf chlorophyll, carotenoid content and anti-oxidative enzyme activities. Plants were harvested twice (30d, 60d after transplantation), washed in deionized water and separated into aboveground and belowground parts. Harvesting of the plants was done on 30 and 60 days after sowing, when the plants showed good foliage growth. In a preliminary screening experiment these plant species were selected and their harvesting time were decided on the basis of the results obtained on metal uptake. Plant parts were separated into aboveground and belowground parts,the separated parts were dried in oven at 75°C for 48h.The samples were then weighed on electronic balance to obtain dry weight of plant parts independently and was expressed as g per plant.

Estimation of total chlorophyll contents in leaves was done following protocol given by Arnon[8] and carotenoids by following Lichtenthaler.[9] 0.5 gram of fresh leaves samples were cut into small pieces and macerated with 80% acetone for a few minutes .The homogenate was then centrifuged at 10,000g. Supernatant was then separated and analysed for the chlorophyll concentration by reading the absorbance of the supernatant on UV-vis spectrophotometer at 470, 645 and 663nm, using the formulae:

Chl a (mgg^{-1} fw) = [12.7 (A_{663}) -2.69 (A_{645})] x V/ 1000 x W

Chl b (mgg^{-1} fw) = [22.9 (A_{645})- 4.68 (A_{663})] x V/ 1000 x W

Total Chlorophyll (mgg^{-1} fw) = [20.2 (A_{645})– 8.02 (A_{663})] x V/ 1000 x W

Carotenoids (mgg^{-1} fw) = [1000 A_{470} -1.82 x Chl a -85.02 Chl b] x V/ 198x1000xW

V-volume of the sample (ml)

W-Fresh weight of the sample (g)

Antioxidative enzymes, Superoxide dismutase (SOD) and catalase (CAT) for both aboveground and belowground parts were assayed considering their role in metal tolerance. Superoxide dismutase activity was determined at 4°C following Nishikimi[10] as modified by Kakkar[11] and SOD activity is expressed as U g^{-1} FW where one unit of SOD activity refers to the amount of enzyme required for 50 % inhibition of NBT reduction under assay conditions. For the assessement of superoxide dismutase (SOD) enzyme activity, samples were macerated in 0.1 M phosphate buffer (pH 7.5) and centrifuged at 10,000 g for 20 min at 4°C. Supernatant was used then analysed for enzyme activity. The assay mixture for SOD activity contained Tris-HCl buffer, Phenazine methosulfate (PMS), NBT and NADH and cell free extract. The reaction was terminated by adding glacial acetic acid and absorbance was taken at 560nm.

Catalase activity was determined following the method of Sinha.[12] The assay mixture was composed of 0.1 M Phosphate buffer, potassium dichromate: glacial acetic acid (1:3), 0.2 M H_2O_2 and enzyme extract.The absorbance was taken at 570 nm. One unit of enzyme activity was defined as the amount of enzyme which catalyzed the oxidation of 1 mmole H_2O_2 per minute under assay condition.

Metal Analysis and Accumulation Factors in Plants

Dried samples of plants (aboveground and belowground parts) were ground into fine powder and 0.5 g of each sample was digested using HNO_3 and $HClO_4$ (5:1 v/v) by heating on an

electric hot plate at 80-90°C till a clean solution was obtained. The samples were cooled and filtered through Whatman 42 filter paper, and diluted to 50 ml using deionised water and analyzed for total Cr using atomic absorption spectroscopy (AAS-Agilent 280 FS AA).[13] Different concentrations of Cr were prepared from the standard solution (1000 mg/l ; Sigma-Aldrich) using deionized water for dilution and a calibration curve was drawn using the known concentrations and absorbance data. Cr Concentration in the samples were read from the calibration curve using its absorbance. For background correction a blank without the analyte was run in each analytical batch.

Bioaccumulation factor (BAF) was calculated as: Cr concentration in plant shoot/ Cr concentration in soil.[14] Bioconcentration factor (BCF) was calculated as ratio of the metal concentration in plant roots to that in soil.[15] Phytoextraction capacity of the plants was the total metal extracted from the soil by the plant, and was calculated as the product of biomass of plant parts and metal concentration in plant parts. The total metal concentration in soil was determined following Allen[16] by digesting 0.5g of dried and sieved soil samples with concentrated HNO_3, H_2SO_4 and $HClO_4$ (5:1:1) at 80°C and digested samples were then filtered through Whatman 42 filter paper and filtrates were diluted to 50 ml with deionized water.

Statistical Analysis

The data were analyzed to test the statistical significance of differences in various parameters in response to different Cr concentrations using one way ANOVA, followed by Tukey's test for comparison of individual means using SPSS software (20.0 version).

Table 1: Biomass of the plant species exposed to different chromium concentrations in soil

Plant species	Dose	Dry biomass (g/plant)			
		Aboveground		Belowground	
	(mg kg^{-1}soil)	30 d	60 d	30d	60 d
C.indica	Control	10.05 ± 0.64	14.38 ± 0.50	15.14± 0.93	17.12 ± 0.62
	Cr_{250}	9.08 ± 0.83	8.65 ± 0.64***	12.70 ± 0.59**	11.79± .67***
	Cr_{500}	6.91 ± 0.70**	6.23 ± 0.94***	10.55 ± 0.31***	8.21 ± 0.22***
	Cr_{750}	4.41 ± 0.36***	2.6 ± 0.42***	7.9 ± 0.67***	4.24 ± 0.19***
N.exaltata	Control	14.62 ± 0.98	18.43 ± 0.31	12.86 ± 0.68	14.85 ± 0.73
	Cr_{250}	15.65 ± 0.55**	13.76 ± 0.41***	11.12 ± 0.83*	8.78 ± 0.75***
	Cr_{500}	12.22 ± 0.41***	10.21 ± 0.21***	9.36 ± 0.32***	7.44 ± 0.49***
	Cr_{750}	8.49 ± 0.43*	6.23 ± 0.31***	8.79 ± 0.37 ***	6.59 ± 0.35***
S.trifasciata	Control	25.65 ± 1.16	27.28 ± 1.15	9.54 ± 0.45	11.97 ± 0.75
	Cr_{250}	26.35 ± 1.62	22.92 ± 1.03***	8.26 ± 0.09*	9.20± 0.56***
	Cr_{500}	23.82 ± 1.67	19.05 ± 0.64***	7.41 ± 0.49***	6.68 ± 0.29***
	Cr_{750}	17.17 ± 0.19***	13.17 ± 0.19***	5.68 ± 0.47***	3.8 ± 0.92***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Results

Plant Response to Varying Cr Concentrations in Soil

All the plant species grew very well in the greenhouse in Cr contaminated soils in pots. *S. trifasciata* was found to have maximum above ground biomass (25g) while *C.indica* had maximum below ground biomass (25g) under control conditions (Table 1). In comparison to control, Cr_{250} did not show any significant adverse effect on aboveground biomass of *Canna* and *Nephrolepis*, while in case of *Sansevieria*,Cr_{250} as well as Cr_{500} had

no adverse effect till 30d. However, a significant ($p< 0.05$) decline in aboveground biomass was seen at later stage (60d) in response to all Cr concentrations for all the species. The decline was more in *C. indica*, (32-86%), followed by *N. exaltata* (25-66%) and *S.trifasciata* (16-52%). Thus, tolerance to high Cr contamination in soil in the order *Sansevieria>Nephrolepis>Canna* in terms of aboveground biomass.

Table 2. Photosynthetic pigment concentrations in the plant species in response to chromium

		Chlorophyll (mg g-*1* fresh wt.)							
Plant species	**Dose (mg kg^{-1}soil)**	**Chla**		**Chlb**		**Total Chl**		**Carotenoids**	
		30 d	**60 d**	**30 d**	**60 d**	**30 d**	**60d**	**30 d**	**60 d**
C.indica	Control	2.03 ± 0.13	2.25 ± 0.19	1.63 ± 0.15	2 ± 0.15	3.66 ± 0.13	4.25 ± 0.06	0.71 ± 0.03	0.89 ± 0.03
	Cr_{250}	2.05 ± 0.04	1.21±0.13***	1.55 ± 0.07	1.13±0.19***	3.61 ± 0.10	2.34±0.06***	0.69±0.02	0.9 ± 0.02
	Cr_{500}	0.93±0.06***	0.73±0.08***	1.12 ± 0.06**	0.54±0.11***	2.06 ±0.13***	1.27±0.12***	0.45 ± 0***	0.5 ±0.02***
	Cr_{750}	0.86±0.07***	0.62 ± 0.1***	0.55±0.19***	0.20±0.13***	1.41 ±0.12***	0.83±0.08***	0.23±0.02***	0.31±0.14***
N.exaltata	Control	3.16 ± 0.14	3.24 ± 0.03	2.33 ± 0.14	2.43 ± 0.1	5.49 ± 0.02	5.67 ± 0.07	0.88 ± 0.02	0.93 ± 0.04
	Cr_{250}	3.24 ± 0.09	1.72±0.06***	2.17 ± 0.05	1.53 ± 0.3**	5.41± 0.04	3.25±0.26***	0.87 ± 0.01	0.79 ± 0.02
	Cr_{500}	1.8 ± 0.06***	1.53±0.06***	1.56±0.06***	0.81±0.1***	3.36± 0.01***	2.34±0.16***	0.79 ±0.01*	0.74±0.02*
	Cr_{750}	1.21±0.12***	1.08±0.11***	1.08±0.17***	0.55±0.33***	2.29±0.19****	1.63±0.33***	0.48 ±0.01***	0.3± 0.03***
S.trifasciata	Control	3.44 ± 0.1	3.46 ± 0.68	2.36 ± 0.14	2.78 ± 0.18	5.79 ± 0.06	6.24 ± 0.67	1.71 ± 0.05	1.75 ± 0
	Cr_{250}	3.55 ± 0.10	3.31 ± 0.12	2.62± 0.19	1.95±0.12***	6.17± 0.10***	5.25 ± 0.06*	1.05 ±0.02***	0.98±0.04***
	Cr_{500}	3.23 ± 0.05	3.31 ± 0.03	2.91 ± 0.11**	2.08 ± 0.05	6.14 ± 0.12***	5.12 ± 0.02*	0.93 ±0.02***	0.83±0.01***
	Cr_{750}	2.98±0.09***	1.48±0.18***	1.27±0.05***	0.76± 0.3***	4.11 ± 0.12***	2.16±0.14***	0.71 ±0.02***	0.52±0.02***

Values are mean ± standard deviation (n=3), significant difference w.r.t control is represented as * $p < 0.05$, **$p<0.01$, ***$p<0.001$

Belowground biomass of all the species, however, decreased on exposure to Cr from the beginning that became more pronounced at later stage. There was a decline of 51- 83% for *C.indica*, 31- 55% for *N.exaltata* and 52- 80% for *S. trifasciata* at different concentrations on 60d, Thus, *Nephrolepis* showed better tolerance to the metal than the other two ornamental species in terms of belowground biomass.

Leaf Chlorophyll and Carotenoid

Leaf chlorophyll was not affected by Cr concentrations up to 250 mgkg $^{-1}$, but concentrations higher than this(Cr_{500},Cr_{750}) had a significant ($p<0.05$) effect on leaf chlorophyll and carotenoid concentrations (Table 2) as compared to respective control in case of *Canna* and *Nephrolepis*. On the other hand, leaf chlorophyll in *Sansevieria* was not affected significantly even by high Cr concentrations in soil.

Total chlorophyll concentrations (mgg^{-1}fw) on 30 and 60d, were more in *S. trifasciata*, (5.8- 6.2), followed by *N. exaltata* (5.5 and 5.7) and were lowest in *C. indica* (3.7-4.3) under control conditions. On exposure to Cr (250 to 750mg kg -1), total chlorophyll (mgg^{-1}fw) was 3.61 to 0.83(*C. indica*), 5.41 to 1.63 (*N.exaltata*) and 6.17 to 2.16(*S.trifasciata*), showing a major decline at Cr_{750}.

While Chla was affected in these species, when exposed to a concentration exceeding Cr_{250}, a significant decline was observed in Chl b even at Cr_{250}. While the decline was 46% in *C. indica* and *N. exaltata* at Cr_{250}, and 67-72% at Cr_{750}, there was an increase in chlorophyll concentration in *S.trifasciata* on exposure up to 500 mg kg $^{-1}$ in initial days, particularly. Chl b response to Cr also followed the same trend (Table2).

Decrease in leaf carotenoid content was also observed in the presence of high concentrations of chromium. Decline in carotenoid recorded for *C. indica*, *N.exaltata* and *S.trifasciata* was 58, 62 and 70%, respectively at Cr_{750} on 60d exposure, while at lower Cr concentrations the pigment was not adversely affected.

Table 3: Catalase activity in test plant species

Plant species	Dose (mg kg^{-1}soil)	Catalase activity (µ moles of H_2O_2 consumed min^{-1} g^{-1} FW)			
		Aboveground		Belowground	
		30 d	60 d	30d	60 d
C. indica	Control	1.41 ± 0.01	1.43 ± 0.06	1.42 ± 0.01	1.45 ± 0.01
	Cr_{250}	1.35 ± 0.01	0.91 ± 0.02***	1.31 ± 0.01	0.80 ± 0.01***
	Cr_{500}	1.31 ± 0.01***	0.87 ± 0.02***	1.22 ± 0.01***	0.71 ± 0.01***
	Cr_{750}	1.27 ± 0.01***	0.78 ± 0.00***	1.09 ± 0.01***	0.66 ± 0.02***
N. exaltata	Control	1.15 ± 0.02	1.16 ± 0.01	1.48±0.01	1.50 ± 0.02
	Cr_{250}	0.96 ± 0.01***	0.90 ± 0.01***	1.2 ± 0.02	0.70 ± 0.01***
	Cr_{500}	0.99 ± 0.01***	0.86 ± 0.02***	1.29 ± 0.02***	0.62 ± 0.01***
	Cr_{750}	0.86 ± 0.02***	0.77 ± 0.01***	1.39 ± 0.05**	0.49 ± 0.01***
S.trifasciata	Control	1.44 ± 0.02	1.46± 0.01	1.32 ± 0.02	1.33 ± 0.01
	Cr_{250}	1.19 ± 0.01	1.04 ± 0.01***	1.05± 0.01***	1.00 ± 0.00***
	Cr_{500}	1.25 ± 0.02***	0.90 ± 0.01***	1.08 ± 0.01***	0.92 ± 0.007***
	Cr_{750}	1.14 ± 0.01***	0.79 ± 0.01***	0.96± 0.01***	0.70 ± 0.01***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Antioxidative Enzyme Activities

Activities of certain anti-oxidative enzymes are required by plants to overcome the oxidative stress. Table 3 shows how the concentration of chromium and duration of exposure influenced catalase activity of the three plant species. Catalase activity was

significantly higher ($p<0.05$) on 30d as compared to that on 60d. Catalase activity in aboveground parts of *C. indica* was marginally less (5%) than that in control, while at Cr_{250}, in *N.exaltata* and *S.trifasciata*, it was 16-17% less than that at control on 30d. However, at Cr_{750}, the decline became more prominent(34- 46%), in aboveground parts for *C. indica*, *N.exaltata* and *S.trifasciata* on 60d. Same trend of decline was observed for belowground parts of the plants with (8-21%) at Cr_{250} on 30d and much greater (47-68%) at Cr_{750}.

Table 4: Superoxide dismutase activity in test plant species

Superoxide dismutase activity (μg^{-1}fw) in plant parts					
Plant species	**Dose (mg kg^{-1}soil)**	**Aboveground**		**Belowground**	
		30 d	**60 d**	**30d**	**60 d**
C. indica	Control	4.82 ± 0.24	5.19 ± 0.21	8.99 ± 0.34	9.25 ± 0.12
	Cr_{250}	8.86 ± 0.15***	5.94 ± 0.13***	10.10 ± 0.36	9.18 ± 0.33
	Cr_{500}	6.05 ± 0.28	4.61 ± .18	7.26 ± 0.60**	5.63 ± 0.24***
	Cr_{750}	2.89 ± 0.54***	2.06 ± 0.19***	4.12 ± 0.48***	3.27 ± 0.18***
N. exaltata	Control	9.80±0.05	9.95 ± 0.23	6.28 ± 0.62	6.56±0.06
	Cr_{250}	10.49 ± 0.19	10.00 ± 0.23	11.67 ± 0.13***	8.95 ± 0.35***
	Cr_{500}	8.67 ± 0.23**	8.15 ± 0.31***	8.99 ± 0.34***	8.69 ± 0.32***
	Cr_{750}	6.22 ± 0.41***	4.81± 0.46***	7.14± 0.18	4.82 ± 0.25***
S. trifasciata	Control	5.51 ± 0.33	5.52±0.51	7.07 ± .40	7.80 ± 0.83
	Cr_{250}	7.45 ± 0.25***	6.44 ± 0.07*	8.70 ± 0.28***	7.48 ± 0.32
	Cr_{500}	5.82 ± 0.12	5.06 ± 0.41	7.72± 0.15	6.00 ± 0.10**
	Cr_{750}	4.35 ± 1.51***	3.14 ± 1.29***	5.54 ± 0.10***	4.16 ± 0.19***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Another antioxidant enzyme Superoxide dismutase on exposure to Cr_{250}, however showed increased activity in all the three species (Table 4). At Cr_{250}, SOD activity was boosted by 84% in in aboveground parts of *Canna*, and by 7-35% in the other two species, whereas it was increased by 86% in belowground parts of *Nephrolepis*, and 12-23% in the others.

But, at higher concentrations Cr_{750}, there was a significant decrease in the activity of Superoxide dismutase($p<0.05$) compared to control in all the species showing 43-60% decline in aboveground and 27-64% decline in belowground parts, as compared to their control. A boost in superoxide dismutase activity in aboveground parts in response to a mild metal stress (Cr_{250}) indicates its likely role in stress tolerance at low Cr concentration.

Metal Accumulation

The plants were investigated for their Cr phytoremediation capability by testing accumulation of the metal in aboveground and belowground parts, separately (Table 5). Uptake of the metal progressively increased in the test plants with increase in concentration and duration of exposure. There was significantly higher concentration of the metal in both above and belowground parts of each species ($p<0.05$) as compared to respective controls all through.

In *C.indica*, Crconcentrations in the belowground parts were 675- 1848 μg g^{-1}, while that in above groundparts were 141- 557 μg g^{-1}. Total Cr accumulation in *N.exaltata* ranged from 475 to 2136 μg g^{-1} in belowground and 130 to 607 μg g^{-1} in aboveground parts. In *S.trifasciata*, Cr was

accumulated in the range of 223-1101 µg g^{-1} in belowground parts and 72- 386 µg g^{-1} in above ground parts. Thus, highest accumulation of Cr was recorded in belowground part of *N.exaltata* at Cr 750 (60 d).

Table 5: Metal content in the three ornamental species after metal exposure

Cr concentration(µg g^{-1}drywt.) in plant parts					
Plant species	**Dose (mg kg^{-1}soil)**	**Aboveground**		**Belowground**	
		30 d	**60 d**	**30d**	**60 d**
C. indica	Control	9.54 ± 0.23	9.92 ± 0.26	18.52 ± 0.26	18.55 ± 0.18
	Cr_{250}	141.31 ± 2.39***	217.13± 16.95***	674.87± 8.98***	916.24 ± 3.00 ***
	Cr_{500}	224.31 ± 3.72***	331.67± 11.16***	1144.5±37.36***	1351.07 ± 31.4***
	Cr_{750}	254.03 ± 25.76***	556.7 ± 26.44***	1446.6±37.72***	1847.4±32.46***
N. exaltata	Control	5.18 ± 0.15	5.24 ± 0.23	12.65 ± 0.12	12.76 ± 0.12
	Cr_{250}	130.63 ± 16.92***	276.47± 53.18***	475.5±23.19***	654.27±32.08***
	Cr_{500}	218.4 ± 7.11***	366.7 ± 55.1***	826.1±11.35***	1093.9 ± 50.8***
	Cr_{750}	458.33 ± 35.5***	607.07± 85.23***	1131.9±110****	2136.07 ± 140***
S. trifasciata	Control	18.62 ± 0.36	18.82 ± 0.3	14.77 ± 0.08	14.83 ± 0.12
	Cr_{250}	71.79 ± 5.71***	124.8 ± 16.38***	222.9 ± 25.7***	367.3 ± 17.88***
	Cr_{500}	176.6 ± 10.63***	231.49 ± 10.2***	332.8 ± 35.8***	601.55 ± 131.6***
	Cr_{750}	203.87 ± 8.5***	386.1 ± 15.9***	569.3 ± 19.3***	1101.1 ± 46.2***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Table 6: Phytoextraction capacity for Cr (mg/plant) in test plant species

Phytoextraction capacity (mg/plant)					
Plant species	**Dose (mg kg^{-1}soil)**	**Aboveground**		**Belowground**	
		30 d	**60 d**	**30d**	**60 d**
C. indica	Control	0.14 ± 0.01	0.195 ± 0.01	0.39 ± 0.05	0.47 ± 0.05
	Cr_{250}	1.90 ± 0.07***	1.88 ± 0.28***	10.80 ± 0.65***	11.41 ± 0.36***
	Cr_{500}	1.70± 1.35***	2.06 ± 0.33***	15.51 ± 0.71***	11.53 ± 0.85***
	Cr_{750}	1.12 ± 0.84***	1.45 ± 0.29**	11.44 ± 1.23***	7.82 ± 0.22***
N. exaltata	Control	0.08 ± 0.01	0.10 ± 0.01	0.2217 ± 0.03	0.275 ± 0.01
	Cr_{250}	2.05 ± 0.33***	3.80 ± 0.78***	8.62 ± 0.55***	9.64 ± 0.24***
	Cr_{500}	2.66 ± 0.11***	3.74 ± 0.60***	12.67 ± 0.38***	14.71 ± 1.16***
	Cr_{750}	3.89 ± 0.48***	3.77 ± 0.44***	13.17 ± 1.28***	20.50 ± 2.06***
S.trifasciata	Control	0.48 ± 0.03	0.51 ± 0.02	0.24 ± 0.01	0.28 ± 0.01
	Cr_{250}	1.89 ± 0.19***	2.87 ± 0.44***	2.52 ± 0.50***	3.38 ± 0.37***
	Cr_{500}	4.21 ± 0.49***	4.41± 0.21***	3.35 ± 0.37***	3.99 ± 0.74***
	Cr_{750}	3.50± 0.15***	5.08 ± 0.16***	4.73 ± 0.20***	4.17 ± 0.93***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Phytoextraction Capacity of Test Plant Species

The phytoextraction capacity differed significantly ($p<0.001$) among plant species and the metal concentrations in the soil. Total Cr extracted was significantly more in belowground parts than in the aboveground parts of *Canna* and *Nephrolepis* (Table 6). *N. exaltata* plants had the highest phytoextraction (20.5 mg) in its belowground parts (60d). Though *C. indica* also showed good phytoextraction of Cr in its belowground biomass (15.5mg) on 30d, it declined with time. This was because the overall belowground biomass decreased in this species at 60d, though metal concentration was high. On the other hand, *S.trifasciata* showed almost comparable extraction of the metal in aboveground parts (5.08mg) and belowground parts (4.7mg) at Cr_{750} indicating that the plants tend to translocate a balanced proportion of the metal taken up by the roots to the shoots. The overall Cr phytoextraction by these species was up to 24, 17.7 and 9.2mg per plant in *Nephrolepis*, *Canna* and *Sansevieria*, respectively.

Chromium Accumulation Factors

Bioaccumulation and bioconcentration factors for the metal in the three species are shown in Table 7. Bioaccumulation factor (BAF) indicates the ability of the plants to tolerate and accumulate metals in the aboveground parts in relation to the metal concentration in soil.[14] BAF in all the test species was > 1 except for *N.exaltata* (1.07) at Cr_{250} after 60d. BAF for *N. exaltata* lies in the range from 0.14-1.07, for *C.indica* it is 0.55 to 0.84 and for *S.trifasciata* it is 0.27 to 0.50.The BAF values tended to increase as Cr concentrations in soil increased, but remained< 1, indicating that none of these speciesact as metal accumulator. BAF values show that these plant species have lesser tendency to accumulate Cr in aerial parts of the plants indicating low translocation.

Table 7: Chromium accumulation Factors (BAF and BCF) in the plant species

Plant species	Dose (mg kg^{-1}soil)	Accumulation factors			
		Bioaccumulation factor		Bioconcentration factor	
		30 d	60 d	30d	60 d
C. indica	Control	0.25 ± 0.01	0.26 ± 0.01	0.48 ± 0.01	0.49± 0.01
	Cr_{250}	0.55 ± 0.01***	0.84 ± 0.07***	2.62 ± 0.03***	3.54 ± 0.01***
	Cr_{500}	0.43 ± 0.01***	0.64 ± 0.02***	2.21 ± 0.07***	2.61 ± 0.06***
	Cr_{750}	0.33 ± 0.03**	0.72 ± 0.03***	1.89 ± 0.04***	2.40 ± 0.04***
N. exaltata	Control	0.14 ± 0.01	0.14 ± 0.01	0.33 ± 0.01	0.33 ± 0.01
	Cr_{250}	0.51 ± 0.07***	1.07 ± 0.21***	1.84 ± 0.09***	2.54 ± 0.12***
	Cr_{500}	0.42 ± 0.01***	0.71 ± 0.11**	1.60 ± 0.02***	2.12 ± 0.1***
	Cr_{750}	0.60 ± 0.05***	0.79 ± 0.11***	1.47 ± 0.14***	2.78 ± 0.15***
S. trifasciata	Control	0.49 ± 0.01	0.49 ± 0.01	0.39 ± 0.01	0.39 ± 0.01
	Cr_{250}	0.29 ± 0.02***	0.48 ± 0.06	0.86± 0.10***	1.42 ± 0.07***
	Cr_{500}	0.34 ± 0.02***	0.45 ± 0.02	0.64 ± 0.07**	1.16 ± 0.26***
	Cr_{750}	0.27 ± 0.01***	0.50 ± 0.02	0.74 ± 0.03***	1.43 ± 0.06***

Values are mean ± standard deviation (n=3), significant difference w.r.t. control is represented as * p <0.05, **p<0.01, ***p<0.001

Bioconcentration factor (BCF) indicating metal accumulation in belowground parts of the plants in relation to the concentration of the metal in soil Yoon[15] showed that all the three species had a potential for phytoremediation. The values of BCF for *C.indica* is much higher (1.89 to 3.54) as compared to that of *S.trifasciata* (0.64 to 1.16) and *N.exaltata* (1.47 to 2.78) as may be seen in Table7. BCF values >1 indicate good phytoremediation potential of plants at different concentrations of chromium. Bioconcentration factor (BCF) values between 0.1 to 1 indicates that the plant species is a moderate

accumulator, while that with BCF value greater than one suggests that it is metal accumulator. Thus, all the species have potential for bioremediation of Cr by concentrating the metal in belowground parts.

Discussion

Plant species in the present study, though showed a significant decline in aboveground biomass at higher Cr concentrations on 60d, and in belowground biomass all through, yet it is noteworthy that the plants continued to grow and thrive even under the harsh metal contaminated conditions. In the present study, *Nephrolepis* showed good biomass of belowground parts while *Sansevieria* showed high aboveground biomass in the presence of the metal.

Presence of Cr in concentrations greater than 250 mg kg^{-1} in the soil had significant impact on major photosynthetic pigments like Chlorophyll a, b, total chlorophyll and carotenoids in all the plants, while lower concentrations had no significant effect on the pigments. However, all plants showed early symptoms of senescence when exposed to the metal, thereby showing some loss of chlorophyll at 60 d of sampling. Thylakoidmembranes of chloroplasts are reported be break down due to Cr toxicity resulting in decrease in chlorophyll level in plants.[17] Decrease in chlorophyll a and b content in *Brassica oleracea* (L.), a plant used in bioremediation, was reported by Ozdener[18] on Cr exposure. Several studies show that Cr stress leads to oxidative stress and a decrease in total chlorophyll, chlorophyll a and b.[19, 20] Though Cr is known to have toxic effects on plant chlorophyll, but a slight promoting effect on chlorophyll has also been reported sometimes,[21] as also in case of *Sansevieria* in the present study. Since successful growth of a plant species is governed largely by its photosynthetic pigments, hence effect of the metal on these pigments is important. Photosynthetic pigments in the test plant species were quite resistant to Cr, and even increased under low concentrations (< 250 mg kg^{-1}), which suggests suitability of their use in bioremediation.

Metals are known to cause oxidative stress in plants and there are some enzymes that have anti-oxidative action to protect the cells from damage. In metal stressed plants antioxidant enzyme activities are highly variable and depend on various factors like plant species, metal concentration and its duration of exposure.[22] Amongst antioxidant enzymes, catalase activity which has potential to scavenge H_2O_2 was found to decline in both time as well as dose dependent manner in all the test plant species. Though this enzyme is often reported to help against the oxidative stress induced by heavy metals, reduced CAT activity has been reported in different plant species exposed to chromium.[23, 24] No direct role of catalase was thus observed in combating the oxidative stress caused by chromium at higher concentration in the test species. However, superoxide dismutase (SOD) which is the first enzyme in the detoxification process of free radicles showed increased activity in both aboveground and belowground parts of plants in all test plant species at lower concentrations of Cr. An elevated level of SOD shows an active antioxidant defense system. A boost in superoxide dismutase activity in response to a mild metal stress (Cr_{250}) indicates its role in stress tolerance. Several studies have shown earlier an increase in superoxide dismutase activity in higher plants due to oxidative stress induced by Cr.[25] Decreased superoxide dismutase activity at Cr_{750} in the test species could be due to excess production of reactive oxygen species in the presence of high concentration of the metal, as suggested by Dazy.[23] Decline in SOD activity with increase in concentration of heavy metals in comparison to their lower concentration has been reported earlier by Sinha.[26] The results show that none of the three ornamental species studied here have a robust anti-oxidative defense enzyme against Cr toxicity especially at 750 ppm concentration.

Phytoextraction capacity of the test plant species was calculated to know the actual uptake of the heavy metal in plant biomass. The phytoextraction was found to be more in belowground parts as compared to aboveground parts of *Nephrolepis* and *Canna* and the reverse in *Sansevieria*, which had overall lesser phytoextraction capability for the metal. Cr accumulation factor for aboveground parts was found to be less than 1, both for *Sansevieria* and *Canna* indicating low transfer of the metal to shoots. Low translocation of Cr to metabolically more active aboveground parts of all test plants at high Cr concentration (Cr_{750})indicates restricted transportation of Cr to aerial parts.

The test species responded in a dose dependent as well as time dependent manner showing maximum accumulation of Cr (60 d) when the soil metal

concentration was 750ppm. All the species showed higher Cr concentration in the belowground than in aboveground parts indicating low translocation. Significant accumulation of Cr in belowground parts of plants indicates that these species tend to take up the metal from contaminated soils, but store it mostly in the belowground tissues, which is a mechanism of phytostabilization of the metals. High Cr accumulation in roots have been reported earlier by some researchers[27, 28] and in *Sesbania Cannabina* on treatment with fly ash.[29]

Greater accumulation of Cr in roots or rhizomes seem to be helpful for the plant as high levels of the metal in shoots and leaves can interfere with the major metabolic activities of the plant. Immobilization of Cr in the vacuoles of the root cells is a natural response of some plants to reduce toxic impacts on the plant.[24] Since Cr is known to be very toxic in nature, it affects plant metabolism and growth. Reduced plant biomass at high Cr concentration is reported for some oil yielding plant species like *Brassica* and *Jatropha*.[30,31] For uptake of metal ions, the plants need to spend extra energy because of which there is decrease in plant biomass with increasing concentration of Cr.[32]

Similar findings were reported by other researchers with higher BCF in roots than shoots for Cr metal in *Sesbania virgate*.[33] Similar findings of poor translocation of Cr from root to shoots have been reported in aquatic plant *Eichhornia crassipes*. Lytle[34] reported that Cr (VI), which is a more toxic form of the metal gets reduced to Cr(III) and is retained in roots some tolerant plant species. Thus, the metal is phytostabilized in the belowground plant parts in a less toxic form. Enzymes such as Fe (III) reductase are known to reduce Cr(VI) to Cr(III) in belowground parts.[35] Phytoextraction capacity, indicating efficiency of a species for heavy metal removal from the soil, depends upon the metal concentration in the plant and its biomass.[36]

Bioconcentration factor (BCF) between 0.1 to1.0 indicates that the plant species is a moderate accumulator, while plants with BCF values >1 suggests that it is metal accumulator.[37,38] In the present study, all the three species show BCF >1 suggesting that these can be used for phytoremediation of Cr from soil. *Canna indica* and *Nephrolepis exaltata* show excellent phytoremediation potential. The tendency of these plant species to concentrate Cr in the below ground parts(rhizome or roots) and limited translocation to aerial parts is of great advantage, as there would be lesser chances of movement of the toxic metal through herbivory.

Conclusion

All three test plant species (*S.trifasciata*, *N.exaltata* and *C. indica*) were found to take up Cr from the contaminated soils and accumulate. The plant species showed excellent tolerance to low Cr pollution in terms of biomass and photosynthetic pigments. High superoxide dismutase activity in all the three plant species up to 500ppm concentration of the metal suggested that anti-oxidative action of this enzyme helps combat the oxidative stress caused by the metal. These herbaceous perennial ornamental plants could successfully grow in the contaminated soil, remove the toxic metal, and accumulate the same mainly in the belowground parts, with restricted translocation to aerial parts, except that in *S.trifasciata*, which had relatively more Cr accumulation in aboveground parts. Possibilities of transfer of Cr through insect herbivory and food chain are reduced when the metal accumulation is mainly in belowground parts. Use of ornamental plant species has thus a great potential for sustainable phytoremediation of chromium contaminated soils in aesthetic way.

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Conflict of Interest

The authors do not have any conflict of interest.

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