

Comparative accounts of chromium accumulation in three ferns under hydroponic system

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Hydroponic experiments were carried out to demonstrate the chromium bioaccumulation potential of three ferns viz. *Diplazium esculentum* (Retz.) Sw., *Pteris cretica* L. and *Polystichum squarossum* (D. Don) J. Sm. Plants were exposed to seven levels of Cr (0, 25, 50, 100, 300, 600 and 1000 $\mu\text{g ml}^{-1}$) for 10 days. All three test species accumulated significant Cr content in comparison to control. The result showed the increasing trends in fresh biomass (25-30%) up to 600 $\mu\text{g ml}^{-1}$ concentration in *P. cretica* and *P. squarossum*. Although, *D. esculentum* showed necrosis even after 1 day exposure; necrosed biomass contained significant Cr than control but significant decrease in chlorophyll and carotenoid content indicates its least tolerance capacity. It has also been revealed that chlorophyll content was not affected by Cr exposure up to 300 $\mu\text{g ml}^{-1}$ concentrations in *P. cretica* and *P. squarossum* and the carotenoid content showed the significant increase of 35 to 74% at higher dose from 300 and 1000 $\mu\text{g ml}^{-1}$ in comparison to the control. Therefore, it has been observed that *P. cretica* and *P. squarossum* are new Cr hyperaccumulator with efficient tolerance capacity. It also revealed that *P. cretica* has highest tolerance capacity to Cr ions amongst the three test species which often grows rapidly, densely and easily adapt to artificial cultivation and can be exploited for treatment of tannery and other Cr contaminated wastelands.

Key words: Ferns, phytoremediation, photosynthetic pigments, biomass.

Introduction

Chromium is one of the toxic heavy metals widely used in electroplating, leather tanning, cement, dyeing, metal processing, wood preservatives, paint and pigments, textile, steel fabrication and canning industries. These industries produce large quantities of toxic effluents.¹ According to an estimate, in India 2000-3200 tones of elemental chromium escaped into the environment from the tanning industry alone. It occurs in several oxidation states ranging from Cr (III) to Cr (VI), with trivalent and hexavalent state being the most stable and common in terrestrial environments and its uptake and toxic effects depends on oxidation state.² Toxic metals affects the central nervous system, kidney, liver and gastrointestinal system, and it also directly and indirectly cause diseases such as anemia, encephalopathy, hepatitis, and the nephritic syndrome.³ The Bureau of Indian Standard permits discharge of effluents containing only 0.1 $\mu\text{g ml}^{-1}$ of Cr to surface water. A wide range

of physical and chemical processes are available for the removal of heavy metals from wastewater, such as electro-chemical precipitation, ultrafiltration, ion exchange and reverse osmosis.⁴ A major drawback with precipitation is sludge production. Therefore, there is a need for low cost natural resources for removal of such toxic metal from environment. Many plants have been known to accumulate heavy metals and other nutrients from contaminated water and can be exploited for polishing of wastewater. However, selection of any particular species for removal of metal ions from polluted sites will also depend on its growth, nature and amount of biomass, and level of metal concentration in the environment.

To evaluate the potential of fern species for tolerance and accumulation of heavy metals, use of hydroponic float system could be a simple and economical method.⁵ Most of literature concerning metallophytes is centered on angiosperms and very few reports are available on ferns. For instance, the comparison between *Adiantum capillus-veneris* and *Microsorium punctatum* has been reported for Cr accumulation and antioxidant responses.⁶ Similarly,

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Adiantum capillus-veneris and *Pteris vittata* has been reported for arsenic hyper accumulation.⁷ Our previous study also reported that heavy metals accumulated by fern species growing on fly ash.⁸⁻⁹ The species of *Azolla* have recently been reported as Cr hyperaccumulators¹⁰. Besides arsenic hyperaccumulation has been reported in Brake fern *Pteris vittata*.¹¹⁻¹²

During survey of fern and fern allies of western Himalaya, it has been observed that the three species namely *Diplazium esculentum* (Retz.) Sw., *Pteris cretica* L. and *Polystichum squarrosum* (D. Don) J. Sm. were growing naturally in different localities of Himachal Pradesh, India as well as in vicinity of industrial wastes also without showing any visual toxicity symptoms. In this context, it was considered worthwhile to explore how these ferns were able to grow in industrially polluted sites and their phytoremediation potential. As Cr is one of the potential contaminants of industrial wastes, the present study was undertaken with an objective to determine the degree of Cr accumulation capability of these fern and their tolerance abilities by measuring biomass and photosynthetic pigments, which are the basic steps regarding metabolic adaptations of plants growing in heavy metal stress conditions.

Materials and methods

The chromium metal standard solution was purchased from Merck (Germany). The distilled water (18MΩ cm) was obtained from Millipore (Direct Q₃) system. The chemical used for digestion and analysis such as nitric acid (HNO₃) and perchloric acid (HClO₄) were obtained from Merck and hydrochloric acid (HCl) was from Ranbaxy. The calibration curve of Cr metal was prepared by appropriate dilution of stock standard solution (1000 µg ml⁻¹). Filter papers were obtained from Qualigens (0.45µm), 615A, Germany.

Plant materials and treatments

Three Himalayan fern species namely *Pteris cretica*, *Polystichum squarrosum* and *Diplazium esculentum* of equal age group were collected from botanical garden of institute. All the three targeted fern species were hydroponically acclimatized for 10 d in 0.2% Hoagland nutrient solution. Further, the acclimatized plants were transferred into 1 % Hoagland nutrient solution containing 0, 25 (23), 50 (47), 100 (96), 293 (300), 600 (593) and

1000 (992) µg ml⁻¹ chromium as K₂Cr₂O₇ (Merck) in triplicate. After removing the moisture content of salt the actual weight of Cr salt has been depicted inside bracket. The experimental set up was kept in a culture room under controlled condition with 14-h light period at temperature and humidity of 25 ± 2⁰ C and 65-70% respectively. The hydroponic cultures were aerated with air bubbler used inside aquarium. The plants were harvested after 10 d of chromium treatment. Harvested plants were washed with double distilled water, blotted dried and separated into the above (fronds) and below ground (roots including rhizomes) parts. Both plant parts were oven dried at 80⁰ C and the oven dried plant tissue (frond and root) of treated and control plants (*Pteris cretica*, *Polystichum squarrosum* and *Diplazium esculentum*) were digested in mixture of concentrated nitric acid (HNO₃) and perchloric acid (HClO₄) (V/V 3:1) at 140⁰C for Cr analysis. The Cr measurements in frond and root samples were performed with Atomic Absorption Spectrophotometer (Shimadzu AA6300). Biomass of chromium treated and control plants were observed by recording the fresh weight of the whole plant and were expressed on fresh weight (FW) basis. For estimation of photosynthetic pigments, plant material (0.3 g) was ground in 80% ice-cold acetone in dark and centrifuged (4⁰ C) (Eltek-RC 4100 F) at 10,000 rpm for 10 minutes. The absorbance of supernatants was measured on UV- visible spectrophotometer (Genesis-20, Thermospectronic). Chlorophyll and Carotenoid contents were calculated using the formula given by Arnon¹³ (1949) and Duxbury and Yentsch¹⁴ (1956) respectively.

Results

The impacts of various Cr concentrations on fern species within certain exposure time was evaluated to study the Cr induced growth behavior in forms of plant biomass, induced oxidative stress by measuring chlorophyll, and carotenoid content. The target species *P. cretica*, *P. squarrosum* and *D. esculentum* were exposed to Cr concentration of 23-992 µg ml⁻¹ for 10 days. All the three test species survived at lower concentration of Cr from 23-96 µg ml⁻¹ while at higher concentration (> 96 µg ml⁻¹) *D. esculentum* began to develop necrosis even after 1 day of exposure. The other two species *P. cretica* and *P. squarrosum* did not show any visual phytotoxicity symptoms at higher concentration of Cr up to 593 µg ml⁻¹. Further, increase in concentration of Cr (992 µg ml⁻¹), caused

necrosis from margin and tip of leaf frond in both species. Chromium accumulation by the three target fern species at different Cr treatment are depicted in Fig. 1, 2 and 3. All the three species showed increased accumulation of Cr, when the concentration of Cr in the medium was increased. Among the three species, *P. cretica* showed highest level of Cr in root (21.2 mg g^{-1}) and shoot (10.3 mg g^{-1})

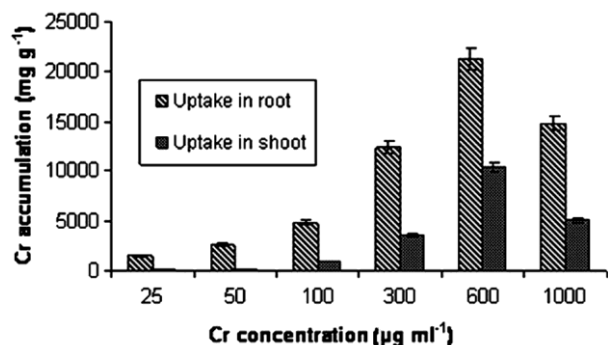


Fig. 1—Accumulation of chromium by *P. cretica* at different concentrations

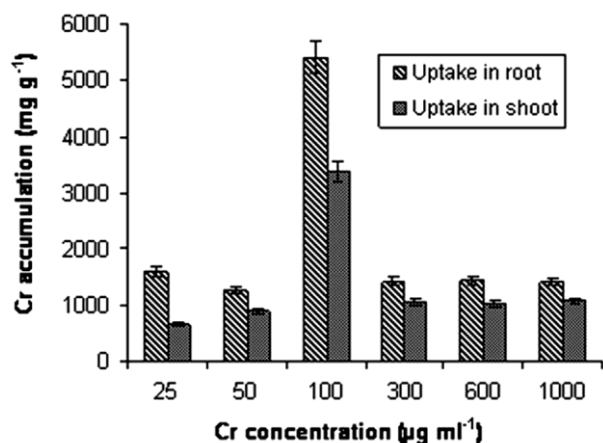


Fig. 2—Accumulation of chromium by *D. esculentum* at different concentrations

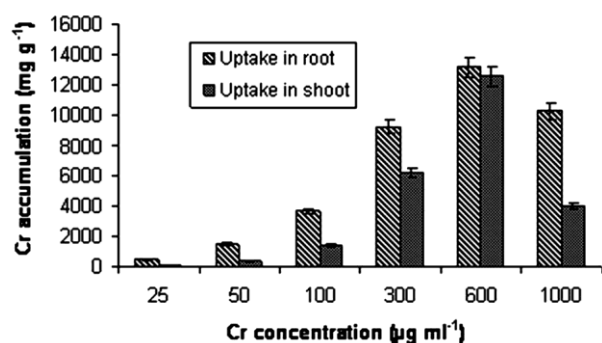


Fig. 3—Accumulation of chromium by *Polystichum squarrosus* at different concentrations

dry biomass. *P. squarrosus* also accumulated almost same amount of Cr in root (13.2 mg g^{-1}) and shoot (12.6 mg g^{-1}) at $593 \mu\text{g ml}^{-1}$. However, *D. esculentum* necrosed after one day exposure of Cr, though the dry necrosed biomass containing significant amount of Cr in root (5.4 mg g^{-1}) and shoot (3.4 mg g^{-1}) at $96 \mu\text{g ml}^{-1}$. Biomass of above ground parts was increased by 35 % in *D. esculentum* up to $96 \mu\text{g ml}^{-1}$ Cr concentration but at higher level, it started diminishing. Biomass of below ground part enhanced up to 12.7% in comparison to control. The biomass of *P. cretica* was significantly increased by 25% in below as well as above ground parts, as compared to control at $293 \mu\text{g ml}^{-1}$ Cr concentration. The *P. squarrosus* showed higher biomass in root (41%) than the frond (23%). There was no significant change in the biomass of the test species at $593 \mu\text{g ml}^{-1}$ and decreased by further increasing Cr concentration ($992 \mu\text{g ml}^{-1}$) (Table 1). Root biomass in these species enhanced, which could be due to hydroponics system in which all metabolic activities are governed by root. The chlorophyll a, b and total chlorophyll content in all species showed no significant variation up to $96 \mu\text{g ml}^{-1}$ Cr concentration within certain exposure time. However, the chlorophyll content reduced in *D. esculentum*, *P. cretica* and *P. squarrosus* at Cr concentration of 293, 593 and $992 \mu\text{g ml}^{-1}$ after 10 d exposure (Table 2). *D. esculentum* showed interveinal necrotic symptoms and also reduction in biomass. Such typical symptoms of Cr- toxicity were not observed in other two species. However, due to exposure of 593- $992 \mu\text{g ml}^{-1}$ Cr, the concentration of total chlorophyll in both fern species was significantly reduced by 33% and 41% compared to that of the control (Table 2). In the case of *D. esculentum*, total chlorophyll content decreased by 24% up to $96 \mu\text{g ml}^{-1}$. The decline of chlorophyll concentration implies Cr-induced internal stress in both species.

Chromium exposure for 10 d reduced the carotenoid content of *P. cretica* and *P. squarrosus* at lower dose of exposure up to $96 \mu\text{g ml}^{-1}$ but at higher dose of 593 and $992 \mu\text{g ml}^{-1}$, it increased in comparison to the control (Fig. 4). Chromium stress increased carotenoid content in *P. cretica* and *P. squarrosus* species at higher dose of 293, 593 and $992 \mu\text{g ml}^{-1}$; it increased significantly by 25, 35, 74% and 20, 26, 32% respectively. The higher level of carotenoid in *P. cretica* and *P. squarrosus* showed its greater tolerance capacity to Cr ions. But in the case

Table 1—Effect of chromium on growth of fern species

Cr level in Medium ($\mu\text{g ml}^{-1}$)	Biomass on fresh wt. (g) basis ^a					
	<i>Pteris cretica</i>		<i>P. squarrosus</i>		<i>Diplazium. esculentum</i>	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	2.25 $\pm$ 0.05	1.88 $\pm$ 0.06	1.15 $\pm$ 0.07	1.67 $\pm$ 0.07	2.05 $\pm$ 0.19	1.68 $\pm$ 0.05
23	2.41 $\pm$ 0.11	2.01 $\pm$ 0.14	1.23 $\pm$ 0.02	1.79 $\pm$ 0.10	2.19 $\pm$ 0.07	1.98 $\pm$ 0.11
47	2.59 $\pm$ 0.053	2.15 $\pm$ 0.06	1.37 $\pm$ 0.03	1.91 $\pm$ 0.09	2.23 $\pm$ 0.03	2.18 $\pm$ 0.04
96	2.65 $\pm$ 0.04	2.27 $\pm$ 0.07	1.53 $\pm$ 0.04	1.97 $\pm$ 0.11	2.31 $\pm$ 0.06	2.27 $\pm$ 0.08
293	2.82 $\pm$ 0.08	2.35 $\pm$ 0.06	1.62 $\pm$ 0.03	2.05 $\pm$ 0.15	-	-
593	2.74 $\pm$ 0.07	2.22 $\pm$ 0.04	1.57 $\pm$ 0.13	1.83 $\pm$ 0.04	-	-
992	2.10 $\pm$ 0.06	1.55 $\pm$ 0.06	1.27 $\pm$ 0.07	1.58 $\pm$ 0.11	-	-

^aThe value represent the mean $\pm$ SD (n=3)

Table 2—Chlorophyll content after chromium stress

Exposure time	Chromium exposure ($\mu\text{g ml}^{-1}$)	Fern species	Chlorophyll a*	Chlorophyll b*	Total Chlorophyll
10 days	Control	PC	3.48 $\pm$ 0.35	1.13 $\pm$ 0.08	4.61
		PS	2.11 $\pm$ 0.05	0.67 $\pm$ 0.05	2.78
		DE	2.54 $\pm$ 0.06	1.03 $\pm$ 0.05	3.75
		PC	3.40 $\pm$ 0.04	1.27 $\pm$ 0.04	4.67
		PS	2.19 $\pm$ 0.02	0.78 $\pm$ 0.03	2.97
		DE	2.67 $\pm$ 0.03	1.15 $\pm$ 0.05	3.82
		PC	3.55 $\pm$ 0.13	1.35 $\pm$ 0.06	4.9
		PS	2.27 $\pm$ 0.05	0.88 $\pm$ 0.04	3.15
		DE	2.49 $\pm$ 0.07	0.75 $\pm$ 0.07	3.24
	23	PC	3.49 $\pm$ 0.04	1.21 $\pm$ 0.04	4.7
		PS	2.21 $\pm$ 0.06	0.91 $\pm$ 0.02	3.12
		DE	2.15 $\pm$ 0.07	0.69 $\pm$ 0.15	2.84
	47	PC	3.11 $\pm$ 0.08	0.97 $\pm$ 0.07	4.08
		PS	1.91 $\pm$ 0.01	0.55 $\pm$ 0.05	2.46
		DE	NS	NS	NS
	96	PC	2.68 $\pm$ 0.04	0.89 $\pm$ 0.04	3.57
		PS	1.57 $\pm$ 0.07	0.49 $\pm$ 0.07	2.06
		DE	NS	NS	NS
	293	PC	2.43 $\pm$ 0.04	0.67 $\pm$ 0.09	3.1
		PS	1.25 $\pm$ 0.07	0.39 $\pm$ 0.02	1.64
		DE	NS	NS	NS
	593	PC	2.43 $\pm$ 0.04	0.67 $\pm$ 0.09	3.1
		PS	1.25 $\pm$ 0.07	0.39 $\pm$ 0.02	1.64
		DE	NS	NS	NS
	992	PC	2.43 $\pm$ 0.04	0.67 $\pm$ 0.09	3.1
		PS	1.25 $\pm$ 0.07	0.39 $\pm$ 0.02	1.64
		DE	NS	NS	NS

*The value represent the mean $\pm$ SD (n=3) PC- *Pteris cretica* PS - *Polystichum squarrosus* DE- *Diplazium esculentum* NS: Not survived

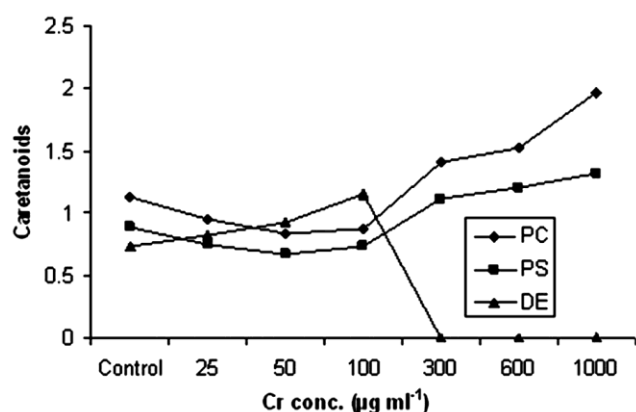


Fig. 4—Effect of chromium stress on carotenoid content of three fern species

of *D. esculentum* it remained unchanged at very lower dose up to 23-47 $\mu\text{g ml}^{-1}$ but started to decrease at higher dose at 96 $\mu\text{g ml}^{-1}$. Carotenoid content was significantly lower in *D. esculentum* in comparison to *P. cretica* and *P. squarrosus* with or without Cr.

Discussion

Chromium is one of the micro-nutrients for plants but becomes toxic at higher concentrations. In the present study, *P. cretica* accumulated highest amount of Cr at the higher concentration provided in the growth medium. *Diplazium esculentum* was able to tolerate 100 $\mu\text{g ml}^{-1}$ Cr concentration without physiological and biochemical changes. Although, metal accumulation (Cr) in *D. esculentum* was almost

high up to $96 \mu\text{g ml}^{-1}$ but it did not survive at higher concentration and its interveinial necrosis (in it) indicates that it is not tolerant to Cr even for 1 day exposure. This suggests that the plant has least tolerance to Cr stress. The root accumulated more Cr than the leaf in all the test species, after treatments. This was probably due to reduction of Cr (VI) to Cr (III), which reduces its mobility from root to shoot.¹⁵⁻¹⁶ It has been reported that Cr (III) readily forms complexes with $-\text{COOH}$ groups which inhibit the translocation of metal from root to shoot.¹⁷ The accumulation and distribution of heavy metals in plant shoot and root are important factors to evaluate the role of plants in the remediation of metal contaminated soil.¹⁸ A low metal distribution to the shoots compared with the roots was found in most plant species and metal elements, both in plants grown in hydroponic culture and those collected from metal contaminated sites.¹⁹ The chelating agents were used to increase the accumulation of heavy metals in fern species.²⁰ Chromium stress increased the biomass of the *P. squarrosus* and *P. cretica* by 22-25%, in shoot and 25-40 % in root at $293 \mu\text{g ml}^{-1}$ Cr concentration. Further increase in the Cr stress, reduced the biomass. Similar reduction in biomass of *Vallisneria spiralis* plants exposed to varying concentrations of Cr has been reported.¹⁵ *D. esculentum* showed increased biomass up to $96 \mu\text{g ml}^{-1}$. Biomass of all the three test species increased at lower doses but decreased on exposure to higher doses due to Cr stress. *P. cretica* and *P. squarrosus* species have remarkable high Cr accumulation in its dry biomass. Growth changes are the first signs of metal toxicity in plants grown under heavy metal stress.²¹

The total chlorophyll, chlorophyll *a*, and chlorophyll *b* contents reduced in *D. esculentum*, *P. cretica* and *P. squarrosus* at higher concentration of Cr. This might be attributed to the toxicity of chromium to chlorophyll biosynthesis of the test plant. The impaired δ -aminolaevulinic acid dehydratase activity leading to reduced photosynthetic pigments has been observed in chromium-treated *Nymphaea alba*.²² Besides, lipid peroxidation also causes degradation of the photosynthetic pigments.²³ The decline in concentration of photosynthetic pigments was observed after 6 day Cr treatment to mung bean.²⁴ Chlorophyll content decreased as a marked effect of various concentrations of different Cr compounds (Cr (III) and Cr (VI)) in *Triticum aestivum*.²⁵ The decrease in the chlorophyll (*a*, *b*)

owing to Cr indicates that Cr toxicity possibly reduces size of the peripheral part of the antenna complex.²⁶ The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants under Cr stress.² The effect of Cr on growth and Chlorophyll content of *Wolffia globosa* has been reported.²⁷ Therefore, as efficient Cr hyperaccumulator *P. cretica* and *P. squarrosus* must also have efficient defense symptoms to mitigate Cr-induced oxidative stress. Carotenoids are a class of natural fat-soluble pigments found in plants, where they play a critical role in the photosynthetic process as well as membrane associated antioxidant activity. Increased carotenoid contents provide membrane bound stability to ferns.²⁸ Chromium also reduced carotenoid content in *Oscimum tenuiflorum* which serve as accessory pigment for photosynthesis and also protected the plants from photo-oxidation.²⁹ Similar observations have also been made in other plants exposed to chromium.²²

Conclusion

In conclusion, the result showed that *P. Cretica* and *P. squarrosus* are new Cr hyperaccumulator with an extraordinary accumulation capacity for chromium. It also revealed that *P. cretica* has highest tolerance capacity to Cr ions among the three test species. It often grows rapidly, densely, and easily adapts to artificial cultivation. Moreover, *P. Cretica* and *P. squarrosus* species grow in terrestrial environment and therefore, these species has great potential to rehabilitate Cr contaminated wastelands (soil). It can also provide a new resource for exploring the biochemical mechanism of chromium hyperaccumulation and detoxification. However, future studies at metabolic and molecular level with these species are necessary for better understanding of their responses to Cr.

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