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Chromium(VI) Accumulation and Tolerance by *Tradescantia pallida:* Biochemical and Antioxidant Study

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Abstract *Tradescantia pallida* (Wandering jew)—a succulent perennial herb—was screened to be a potent chromium (Cr) accumulator. Its ability to grow under Cr stress was examined by studying biochemical changes and physiological response of the plant in presence of 5–20 mg L^{-1} Cr(VI) concentration in hydroponic environment for up to ca. 90 days. Average Cr(VI) bioaccumulation in plant roots reached about 408 μg g^{-1} dry weight (dw) after 30 days and up to 536 μg g^{-1}dw after 60 days of culture. Biochemical changes in the plant exposed to Cr(VI) indicated a reduction in the total carbohydrate and protein content. Furthermore, lipid peroxidation, catalase, peroxidase and ascorbate peroxidase activity were measured in different parts of the plant exposed to Cr(VI). Increased activities of these enzymes showed their important role in overcoming the Cr-induced oxidative stress on the plant.

Keywords Phytoremediation · Bioaccumulation · *Tradescantia pallida* · Chromium · Antioxidants · Lipid peroxidation

Introduction

Heavy metal contamination of soil, aqueous waste stream and groundwater poses a major threat to the environment worldwide and can cause serious health hazards. Water pollution by chromium (Cr) is of considerable concern, as it is one of the most toxic heavy metals that attenuate the environment [1]. The occurrence of Cr in environment is primarily due to anthropogenic activities, particularly due to their widespread use in industries such as electroplating, leather tanning, metal finishing, nuclear power plant, textile, steel production, catalyst, pigment manufacturing, metal corrosion inhibitor and chromate preparation.

Cr is largely found in either +3 or +6 oxidation states and is unstable in other oxidation states [2]. Cr(VI) is most toxic and exists as CrO_4^{-2}, $Cr_2O_7^{-2}$ or $HCrO^{-4}$ depending on its solution pH. It has the ability to oxidise biological molecules by diffusing through cell membranes. Cr(VI) can cause renal dysfunction as well as chronic alterations in the nervous system and gastrointestinal tract [3]. Cr(VI) is a potential carcinogen, mutagen and teratogen

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[4], and its uncontrolled discharge into the environment is a serious matter of concern affecting water quality, thereby presenting a direct hazard to human health. Considering these reasons, Cr(VI) must be removed from constituent wastewater prior to discharge into the aquatic environment.

Conventional and advanced treatment technologies used for the removal of Cr from wastewater streams include different methods such as redox processes, ion exchange, coagulation, cementation, electro-winning, reverse osmosis, membrane separation, electrolysis, chemical precipitation and adsorption [5]. However, most of these technologies suffer from one or more significant disadvantages such as incomplete metal removal, energy intensive, metal specific, generation of toxic sludge and other waste products and low efficiency, especially at metal concentrations commonly found in the wastewater [6]. Thus, there is a need for more effective and novel techniques to remove Cr from wastewaters.

Phytoremediation is a novel clean-up technology, which is of great interest from ecological and economical points of view and is a renewable alternative to the previously tested remediation techniques [7, 8]. It is a promising method especially for treating large-scale, low-level Cr contamination [9]. Certain plant species are capable of accumulating metals in their various parts, a process referred to as phytoextraction that can be used to remove metals from soil and water. However, important aspects which must be considered when choosing the best phytoremediator species for a specific location and level of contamination are the biomass production ability and the ecology of the species [10]. In this respect, phytoremediation using native and wild plant species becomes imperative as no special growth conditions are required in the process. Furthermore, Cr is less adverted compared with other heavy metals and only a few plants have been investigated till date for its removal from wastewater. Moreover, there is a complete lack of comprehensive information on Cr tolerance mechanism in plants, which is essential to gain understanding and, therefore, applicability of the Cr bioaccumulation process.

Thus, specific objectives of this study were to: (a) screen different plant species for Cr(VI) bioaccumulation, (b) analyse Cr(VI) accumulation in different plant parts viz. roots, shoots and leaves exposed to different Cr(VI) initial concentration ranging from 0 to 20 mg L^{-1} and (c) determine the plants oxidative feedback in response to a high Cr(VI) concentration exposure.

Materials and Methods

Screening of Different Plants for Cr Accumulation and Tolerance

Hydroponic culture experiments were carried out to evaluate and compare Cr(VI) tolerance among different plant species. The different plant species used for this investigation are presented in Table 1. These plants were collected from a non-polluted site in North Guwahati, India. The plants selection was based on their minimal nutrient requirement, non-medicinal or non-edible use, easy propagation within a small time period and a profuse root system.

The effect of different initial concentration of Cr(VI) ranging from 5 to 20 mg L^{-1} was examined to evaluate their tolerance capability. For this experiment, healthy plants with uniform weight were thus selected, thoroughly rinsed with running tap water in order to eliminate any remains of sediment and finally transferred to clean plastic containers (15 plants per container). Each of these containers was added with 2 L of 50 % Hoagland's solution of composition (mM): 2.4 Ca $(NO_3)_2$, 1.0 KH_2PO_4, 3.0 KNO_3, 1.0 $MgSO_4$ and 0.5NaCl and (μM) 23.1 H_3BO_3, 4.6 $MnCl_2$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 0.052 H_2MoO_4 and 44.8 Fe-EDTA complex with tap water. Plants cultivated in Hoagland solution without Cr(VI) served as

Table 1 Different plant species screened for Cr bioaccumulation and tolerance in this study

Scientific name	Common name	Family	Type	Habitat
Tradescantia pallida	Wandering jew	Commelinaceae	Herbaceous perennial	Tropical and semi-tropical areas
Gnaphalium luteoalbum	Jersey Cudweed	Asteraceae	Annual herb	Cosmopolitan
Alternanthera philoxeroides	Alligator weed	Amaranthaceae	Perennial aquatic herb	Water bodies

control. All these experiments were carried out under controlled environmental conditions with a temperature regime of 25 °C day/night, 14/10 h light/dark period (1,800 lux) and a relative humidity of 70–80 %.

Final selection of the plant for Cr accumulation and tolerance was based on visual changes on the plants grown in the presence of Cr and their tolerance index based on their wet weight (calculated according to the following equation [11]:

$$\text{Tolerance Index} = \frac{\text{mean biomass of plants treated with Cr(VI)}}{\text{mean biomass of control plants}} \times 100$$

Tradescantia pallida and Growth Conditions

Based on the previous screening experiment, *T. pallida* was investigated further for Cr(VI) bioaccumulation and tolerance. Following collection of the whole plants, they were immediately transferred to polystyrene pots containing 1 L of tap water and stored for 3 days under outdoor condition (recuperation period). Healthy plants with uniform weight were then selected, thoroughly rinsed with running tap water in order to eliminate any remains of sediment and finally transferred to clean plastic containers (15 plants per container) with 2 L each of 50 % Hoagland's solution.

Different concentration of Cr(VI): 0 (control), 5, 10 and 20 mg L^{-1} was supplied as potassium dichromate ($K_2Cr_2O_7$) from its stock solution (100 mg L^{-1}). The pH of Cr solution and control tap water ranged between 6.8 and 7.0. The nutrient solution in each container was replaced with a fresh medium every 4 days during the experiments. Growth conditions of the plants were the same as noted earlier in 'Screening of Different Plants for Cr Accumulation and Tolerance'. However, the plants leaves were kept away from contact with the nutrient culture solutions to avoid Cr precipitation on the same.

For analysis of different *T. pallida* plant parts for Cr accumulation, plants were harvested after 30 days of treatment, and its leaves, shoots and roots were separated. The plant parts were then blotted dry on a filter paper before final drying at 70 °C for 2 days to determine their dw. For estimation of total Cr content, plant parts were harvested after 30 and 60 days of Cr treatment, thoroughly rinsed with distilled water to remove any adsorbed metal on the root surface prior to digestion and analysis by spectrometry as detailed later (in '*Tradescantia pallida* and Growth Conditions'). For estimation of enzyme activities, plant parts were harvested after 30, 60 and 90 days treatment and analysed for catalase, peroxidase, APX and lipid peroxidation as detailed under *Antioxidant Enzyme Assay*. Moreover, protein and carbohydrate content was determined to observe biochemical changes in the plant parts.

Analytical Methods

Measurement of Cr Content in Plant Root and Aerial Parts

Cr content in *T. pallida* tissues was determined from 0.5 g (dw) of leaves, shoots and roots following wet digestion of the plant parts in an acid mixture containing HNO_3/$HClO_4$ in the ratio 3:1 (USEPA 3051method). This acid digestion step was carried out at 110 °C for 15 min. Cr determination was carried out by atomic absorption spectrophotometry (AA240, Varian, the Netherlands) and Cr content was expressed as micrograms per gram dw.

Estimation of Lipid Peroxidase Activity

The lipid peroxidase activity was determined following the method described by Heath and Packer [12]. Briefly, 0.5 g of powdered tissue was homogenised in 20 % trichloroacetic acid (TCA), containing 0.5 % 2-thiobarbituric acid and heated at 95 °C for 30 min. The thiobarbituric acid reactive substances (TBARS) concentration was measured as malondialdehyde (MDA; ε=155 mM cm^{-1}), which was determined at OD_{532} and corrected for nonspecific turbidity at OD_{600} using a ultraviolet (UV)–visible spectrophotometer (Cary 100, Varian, Australia).

Antioxidant Enzyme Assay

Catalase Assay Catalase activity was determined according to method described by Aebi [13]. About 0.5 g fresh leaf samples was homogenised in 5 mL of cold 200 mM sodium phosphate buffer (pH 7.8), using chilled mortar and pestle. The homogenates were centrifuged at 10,000×*g* for 20 min at 4 °C, and the supernatant was assayed for catalase activity by using a UV–visible spectrophotometer (Cary 100, Varian, Australia). The reaction mixture (2.8 mL) contained 1.5-mL 200-mM sodium phosphate buffer (pH 7.8), 1.0-mL deionised water and 0.3-mL 0.1-M H_2O_2 prepared afresh prior to its use. The reaction mixture was then added with 0.5 mL enzyme extract, and the enzyme activity was measured by monitoring the decrease in absorbance at 240 nm due to H_2O_2 consumption. One unit of catalase activity was defined as change in absorbance of the mixture at 240 nm min^{-1} g^{-1} of fresh weight.

Peroxidase Assay The peroxidase activity was determined based on the oxidation of guaiacol, which was measured by an increase in the absorbance at 470 nm [14]. The reaction mixture (total volume, 1 mL) contained 25 mM phosphate buffer (pH 7.0), 1.0 mM H_2O_2 (30 %), 0.05 % guaiacol and 0.1 mM EDTA and enzyme extract. The reaction was initiated by adding H_2O_2, and the increase in the absorbance was monitored at 470 nm (ε=26.6 mM cm^{-1}) at 1-min intervals up to 4 min. One unit of peroxidase activity was defined as the change in absorbance at 470 nm min^{-1} mg^{-1} fresh weight of leaf.

Ascorbate Peroxidase Assay Ascorbate peroxidase (APX) activity was measured according to a method described by Leonardis et al. [15]. Two-millilitre reaction mixture for this assay contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.3 mM ascorbate, 0.1 mM H_2O_2 and 100 μL enzyme extract. The reaction was initiated by the addition of H_2O_2, and oxidation of ascorbic acid was estimated by following the decrease in absorbance at 290 nm.

APX activity was calculated using the molar extinction coefficient (ε=2.8 mM cm^{-1}) and was expressed as units per milligram of protein.

Determination of Total Carbohydrate and Protein Content

Total carbohydrate content was measured according to anthrone method [16] using glucose as the standard, whereas the soluble protein content in the samples was measured according to Lowry's method [17] using bovine serum albumin (BSA) as the standard protein.

Results

Screening of Cr Tolerance in Different Plant Species

T. pallida showed the maximum tolerance capacity to Cr(VI) among all the plants tested for screening. At an initial Cr(VI) concentration of 10 mg L^{-1}, *Alternanthera philoxeroides* and *Gnaphalium luteoalbum* showed toxic symptoms at the end of 30 days in the form of leaf necrosis as revealed by yellowish, dark brown spots and acute burning on the edges of the leaves compared with the respective control plants. Dry weight of the treated plants (root and aerial parts) was also significantly reduced as compared with the control.

Figure 1 shows the Cr tolerance index for the plant species investigated, in which the horizontal line indicates a tolerance index of 50 % that is generally considered the minimum desired biomass production for plants growing under heavy metal-stressed condition. The tolerance index was the lowest for *A. philoxeroides* (35 %). Although *G. luteoalbum* (58 %) showed tolerance higher than 50 %, the aforementioned toxicity symptoms were still observed. Therefore, only *T. pallida* that showed the highest tolerance index (87 %) across all its parts along with no toxicity symptoms was finally selected for further experiments.

Cr Accumulation by *T. pallida*

Analysis of Cr content in *T. pallida* revealed a high metal accumulation in the plant roots compared with that in the plant's shoots and leaves, which was not observed in the control plants (Fig. 2a). Cr accumulation also increased in all the tissues with an increase of Cr initial

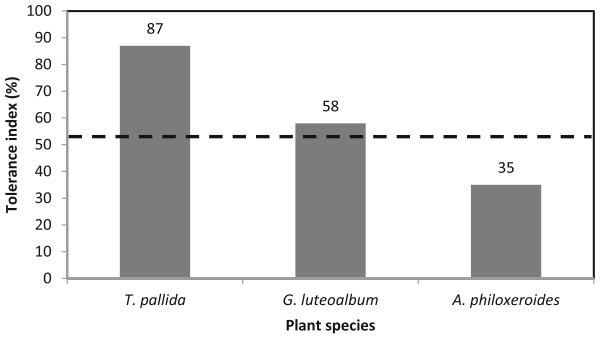

Fig. 1 Cr(VI) tolerance index of different plant species (Cr(VI) initial concentration=10 mg L^{-1} and Cr(VI) exposure period=30 days)

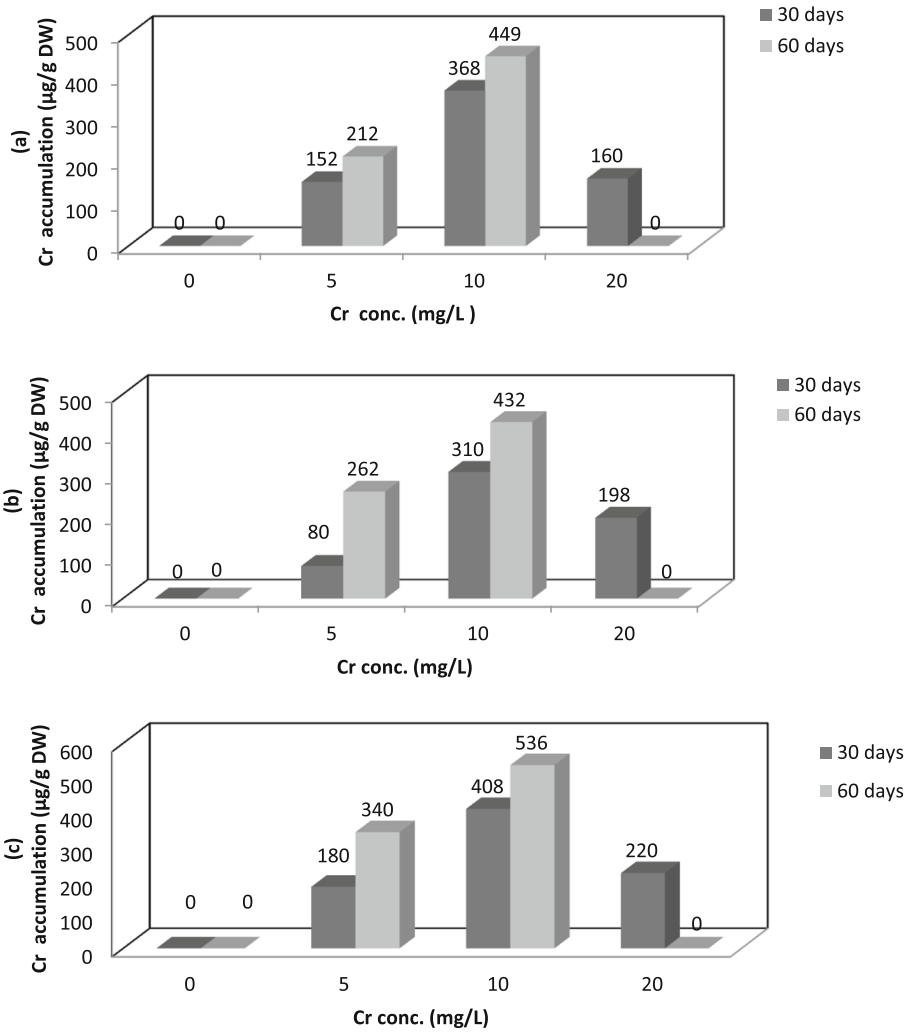

Fig. 2 Total Cr uptake by *T. pallida* at different initial concentration over 30 and 60 days period: **a** leaves, **b** shoots and **c** roots

concentration in the nutrient solution upto 10 mg L^{-1}. However, at 20 mg L^{-1} Cr initial concentration, the plant showed severe phytotoxic symptoms with a reduced Cr accumulation value, particularly after 60 days exposure period. Maximum accumulation values for the plant's roots, leaves and shoots were 536, 449 and 432 µg g^{-1} dw, respectively, at 10 mg L^{-1} Cr initial concentration in the medium (Fig. 2). Thus, Cr accumulation levels in *T. pallida* treated with 10 mg L^{-1} Cr(VI) was in the order: roots>leaves>stem.

Effect of Cr on *T. pallida* Biochemical and Antioxidant Defence System

Compared with Cr(VI)-untreated *T. pallida*, Cr(VI)-exposed plant revealed a higher activity of lipid peroxidation (Table 2, lipid peroxidation), catalase activity (Table 2, catalase activity), peroxidase (Table 2, peroxidase activity) and APX (Table 2, APX activity) enzymes. In

Table 2 Changes in biochemical and antioxidant enzyme activity in *T. pallida* due to Cr(VI) exposure: lipid peroxidation, catalase activity, peroxidase activity and APX activity

Experimental period (days)	Roots		Shoots		Leaves	
	Control	Cr(VI) treated	Control	Cr(VI) treated	Control	Cr(VI) treated
Malondialdehyde (μM g^{-1} fresh weight)						
30	8.3±0.4	39.7±0.3	7.6±0.2	28.6±0.3	9.5±0.2	26.3±0.2
60	20.2±0.6	54.1±0.2	16.9±0.5	31.3±.3	15.4±0.4	37.2±0.3
90	22.8±0.4	57.5±0.4	14.2±0.3	28.9±0.3	18.6±0.4	40.1±0.3
Catalase (μM H_2O_2 destroyed mg^{-1} protein min^{-1})						
30	5,342±10.9	5,923±15.4	4,198±18.2	5,605±21.2	5,853±12.1	6,854±21.2
60	5,809±11.1	6,107±13.0	4,634±14.3	5,490±17.4	6,352±10.8	6,912±21.2
90	6,566±21.0	7,650±11.3	4,823±19.2	5,499±12.2	6,031±22.4	7,696±19.0
Peroxidase (U mg^{-1} protein)						
30	5,267±21.3	8,952±17.3	4,339±21.3	6,971±21.3	4,108±19.4	5,877±18.4
60	6,209±16.6	9,977±21.6	4,987±17.4	7,085±27.7	5,977±14.3	8,568±13.5
90	6,744±14.5	9,806±18.4	4,768±19.4	7,100±11.6	6,132±12.8	8,892±15.4
Ascorbate peroxidase (U mg^{-1} protein)						
30	2,006±26	3,500±14	1,407±11	3,148±15	2,210±18	3,865±19
60	2,783±18	9,800±13	1,922±18	8,554±23	2,630±21	8,317±22
90	2,976±13	9,823±25	2,314±17	9,439±16	2,965±14	9,709±12

Results presented are average of three samples analysed with standard deviation±SD (n=3)

addition, the Cr(VI)-treated plants showed high activity of lipid peroxidation, peroxidase activity in its roots than in the other plant parts following the 90-day experimental period. Maximum catalase activity was, however, observed in the plants leaves.

Results of carbohydrate and protein analysis revealed that carbohydrate content was higher in roots and leaves of Cr-treated plants compared with that in the control plants (Table 3). Conversely, protein content was lower in the Cr-treated plants as compared with that in the control plant parts (Table 3). Moreover, the protein content was significantly reduced in the leaves as compared with that in the stem and roots of the Cr(VI)-treated plants.

Table 3 Change in carbohydrate and protein content in *T. pallida* plant parts following treatment with Cr(VI) (initial Cr(VI) concentration=10 mg L^{-1} and exposure period=90 days)

Roots		Shoots		Leaves	
Control	Cr(VI) treated	Control	Cr(VI) treated	Control	Cr(VI) treated
Carbohydrate content (mg/100 mg of dry weight of plant part)					
1.8783±0.1604	2.1058±0.1681	1.7549±0.0921	1.8139±0.1541	2.4814±0.2357	3.4231±0.1284
Protein content (mg/g of fresh weight of plant part)					
13.519±0.4541	12.961±0.9983	7.937±0.4230	7.233±0.1069	19.805±0.5512	15.533±0.5910

Results presented are average of three samples analysed with standard deviation±SD (n=3)

Discussion

During the screening of different plants for Cr(VI) tolerance, results showed that Cr(VI) reduced the dw of all the plants indicating its harmful effect on development and growth of the different plant species. The Cr toxic effect was more pronounced in *A. philoxeroides* and *G. luteoalbum* than in *T. pallida*. This may be due to the inhibition of cellular division and damage to the root systems of the plants by Cr(VI). It has been reported that heavy metals cause acute decrease in photosynthesis rate and hinders its translocation in plants [18].

Cr Tolerance Mechanism

T. pallida plants treated with Cr(VI) showed reduced growth rate (Fig. 1), a high oxidative damage (Table 2) and increased peroxidation of membrane lipids compared with those in the control plants. All these effects could be attributed to free radicals production under Cr-stressed conditions, which results in the oxidative damage. Cr(VI) is a known oxidising agent that upon reduction to Cr(III) inside the plant cell produces free radicals, such as singlet oxygen, hydroxyl ions and hydrogen peroxide, which causes its toxicity at the cellular level. However, because only total Cr (Cr(III)+Cr(VI)) was measured in this study, further experiments are necessary to validate this aspect. Recently, several reports have demonstrated the dual role of reactive oxygen species (ROS) in plants subjected to abiotic stress [19]. For instance, membrane destabilisation is generally attributed to lipid peroxidation due to an increased production of ROS. Thus, in the present study, enhancement in lipid peroxidation due to Cr(VI) exposure (Table 2, lipid peroxidation) suggests that Cr caused oxidative damage to the cell, particularly the membrane lipids due to ROS generation.

The above ROS generation further helps in signalling activation of defence responses. For instance, various antioxidant enzymes viz., catalase, peroxidase and APX act synchronously to quench the free radicals produced. In chloroplasts, ascorbate–glutathione cycle is the major defence system by which H_2O_2 is scavenged and converted to H_2O and O_2 [20]. The main enzymes in this pathway are APX and glutathione reductase enzymes, which uses ascorbate and glutathione as oxidoreductants, H_2O_2 as an electron acceptor and nicotinamide adenine dinucleotide phosphate ($NADP^+$) as an electron donor. These enzymes are also strictly compartmentalised and act in a highly coordinated manner. Thus, the increased activities of these antioxidant enzymes in the present study is attributed to the plants defence mechanism to counteract the toxicity induced by Cr(VI). Moreover, high increase in APX activity (Table 2, APX activity) suggests that APX is up-regulated under Cr(VI)-induced oxidative stress and plays a major role in the detoxification of H_2O_2 [21]. These results also indicate that APX was more efficient in destroying H_2O_2 than the other antioxidant enzymes.

The soluble sugar content presented in Table 3 revealed that lower concentrations of Cr(VI) increased the soluble sugar content in all plant parts. This is mainly due to a high starch accumulation in Cr(VI)-exposed leaves as it improves the resistance of the plant's photosynthetic apparatus and lowers the starch export from the mesophyll. In addition, an enhancement in the carbohydrate supplements helps in defence of the plant's biomolecules and membranes under stress conditions [22].

The decrease in the protein content of Cr(VI)-exposed plant (Table 3) suggests that proteins are easily susceptible to oxidative stress damage induced by Cr(VI) in these plants. This is in agreement with [23], who observed a decrease in soluble protein content of *Lupinus albus* in the presence of heavy metals.

Cr Uptake Mechanism

Phytoremediation using aquatic plants are highly efficient in absorbing metals and metalloids from their surrounding water [24]. This bioremoval process involves the following two phenomena: (i) biosorption which is an initial fast, reversible and metal-binding process and (ii) bioaccumulation which is a slow, irreversible and ion-sequestration step [25, 26]. Cr(VI), which is an oxyanion, can easily cross the cell membrane following the same mechanism by which phosphates and sulphates are transported inside by the phosphate-sulphate transport carrier. Conversely, plants uptake Cr(III) cations by a passive process following their exchange with cationic functional groups present on their cell wall. In this study, the different *T. pallida* plant parts showed very high bioaccumulation of Cr, particularly in the roots (Fig. 2c). Thus, Cr uptake mechanism by *T. pallida* roots could be proposed to be either direct Cr(VI) uptake or via its reduction to Cr(III) or both. However, further investigations using Cr(III) need to be performed to determine the precise Cr uptake mechanism by this plant. Following uptake by the plant's roots, Cr gets stored in the root vacuoles as a natural defence mechanism adopted by hyperaccumulator plants, which is then transported to aerial parts of the plant. This in part as well explains the higher accumulation of Cr(VI) by the roots as compared with that by other parts (shoots and leaves) of the plant. A high Cr dose of 20 mg L^{-1} for 60 days, however, proved toxic to the plant possibly due to a high energy demand of the plant tissues to maintain its metabolism under such adverse conditions, which in turn led to a reduced Cr uptake by the plant.

Considering the plants good bioaccumulation and tolerance to Cr, this study showed that *T. pallida* can be successfully applied to treat Cr containing industrial effluents, which, however, needs to be researched further.

Conclusions

T. pallida, compared with *A. philoxeroides* and *G. luteoalbum*, showed good tolerance for Cr(VI). Cr uptake by *T. pallida* was also significant at a low initial concentration. A good correlation between Cr accumulation and the plant's biochemical and antioxidant enzyme system was observed. This study thus contributed to understanding the Cr(VI) tolerance and accumulation by *T. pallida*. Furthermore, this plant can be used in remediating Cr contaminated system.

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