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Kinetics, biochemical and factorial analysis of chromium uptake in a multi-ion system by *Tradescantia pallida* (Rose) D. R. Hunt

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ABSTRACT

Discharge of wastewater from electroplating and leather industries is a major concern for the environment due to the presence of toxic Cr^{6+} and other ions, such as sulfate, nitrate, phosphate, etc. This study evaluated the potential of *Tradescantia pallida*, a plant species known for its Cr bioaccumulation, for the simultaneous removal of Cr^{6+}, SO_4^{2-}, NO_3^-, and PO_4^{3-}. The effect of different co-ions on Cr^{6+} removal by *T. pallida* was examined following the Plackett-Burman design of experiments carried out under batch hydroponics conditions. The results revealed a maximum removal of 84% Cr^{6+}, 87% SO_4^{2-}, 94% NO_3^- and 100% PO_4^{3-} without any phytotoxic effect on the plant for an initial Cr^{6+} concentration in the range 5–20 mg L^{-1}. SO_4^{2-} and NO_3^- enhanced Cr uptake at a high initial Cr concentration (20 mg L^{-1}), whereas PO_4^{3-} did not affect Cr uptake both at high and low initial Cr concentrations. The Cr^{6+} removal kinetics in the presence of different ions was well described by the pseudo-second-order kinetic model which revealed that both biosorption and bioaccumulation of the metal played an important role in Cr^{6+} removal. Increase in the total carbohydrate and protein content of the plant following Cr^{6+} and co-ions exposure indicated a good tolerance of the plant toward Cr^{6+} toxicity. Furthermore, enhancement in the lipid peroxidation and catalase activity in *T. pallida* upon Cr^{6+} exposure revealed a maximum stress-induced condition in the plant. Overall, this study demonstrated a very good potential of the plant *T. pallida* for Cr^{6+} removal from wastewater even in the presence of co-ions.

KEYWORDS

antioxidant enzymes; chromium(VI) removal; Co-ions; kinetics; Plackett–Burman; *Tradescantia pallida*

Introduction

Chromium (Cr) is one of the most ecotoxic pollutant and is listed under the top twenty contaminants by the United States Environmental Protection Agency (Cervantes and Campos 2007). The deleterious effect of Cr^{6+} is linked to its high oxidizing potential, high solubility, and easy diffusion across the biomembranes (Peralta-Videa *et al.* 2009). It is an environmentally persistent transition metal and has the potential to induce mutagenic, carcinogenic, and teratogenic effects in biological systems (Chervona *et al.* 2012). Rapid industrialization within the last few years has led to uncontrolled release of pollutants into the environment, thereby posing a serious risk to the environmental health. Cr^{6+} is mainly released into the environment by leather tanning, textile processing, electroplating, mining operations, and pigment manufacturing industries, which discharge wastewater heavily contaminated with Cr^{6+}, SO_4^{2-}, and NO_3^- (Huang *et al.* 2009).

Besides the toxic effect of Cr^{6+}, the presence of NO_3^- and PO_4^{3-} ions in the wastewater poses a serious problem as they adversely affect the quality of the soil and water environment, mainly due to their very high solubility. Furthermore, high NO_3^- levels in drinking water cause methemoglobinemia in infants, and excess phosphate and nitrate in surface water contribute to eutrophication, adversely affecting aquatic life. Hence, in order to protect the ecosystem, it is imperative to bring these ions under permissible limits prior to their release into the environment (Rezaee *et al.* 2011).

In the last decade, much attention was given to the use of phyto-technology for the remediation of contaminated water and soil systems. Some plants are able to accumulate pollutants, and, therefore, can remove, transfer, and stabilize these pollutants from industrial wastewater and contaminated soil systems. These plants remove soluble ions by surface adsorption or bioaccumulation into their tissues or both (Vangronsveld *et al.* 2009).

Wetland plants are particularly suited for environmental detoxification of heavy metals as they can be grown directly on wastewater streams near industrial areas and can provide *in situ* bioremediation. Among the different plant species reported in the literature, *T. pallida* (wandering jew) has been suggested as an effective accumulator of chromium (536 and 449 $\mu g\ g^{-1}$ dry weight of roots and leaves, respectively) (Sinha *et al.* 2014). It is also a known bioindicator of environmental pollution and phytoremediates multiple trace elements (De Luccia 2012). However, simultaneous removal and the effect of SO_4^{2-}, NO_3^-, and PO_4^{3-} on Cr^{6+} uptake by *T. pallida* are still not clear. This is very important, mainly because effluent from electroplating and leather industries contains not only chromium but also

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other co-ions, viz. SO_4^{2-}, NO_3^-, and PO_4^{3}, in large amounts (Chen *et al.* 2010). Wastewater from industries such as leather tanning, textile processing, electroplating, mining, and pigment manufacturing is heavily contaminated with Cr^{6+} along with SO_4^{2-}, NO_3^-, and PO_4^{3-}, which leads to environmental deterioration (Huang *et al.* 2009). These ions are important for various industrial applications. For instance, chromium nitrate ($Cr(NO_3)_3$) is a widely used chemical in the preparation of chrome catalysts, in textile printing operations, and is used as a corrosion inhibitor. Ammonium sulfate ($(NH_4)_2SO_4$) and chromic sulfate ($H_{24}Cr_2S_3O_{24}$) are common salts of Cr that are employed for leather tanning and insulation. Besides they are used in the manufacture of green paints, inks, text dyes, and ceramics. Monosodium and disodium phosphates (Na_2HPO_4) perform many important functions in the cleaning, finishing, tanning, and dyeing of textiles and leather. Thus effluents from these industries contaminated with Cr^{6+} along with multi-ions need to be treated before discharge into the environment.

Co-presence and interaction between these co-ions may often exert a different effect on Cr uptake and translocation by *T. pallida*. This work addresses the use of an indigenous plant *T. pallida* for the simultaneous removal of Cr^{6+} and co-ions from wastewater. Further, it highlights the effect of each ion on Cr uptake and kinetics, which is an important parameter in understanding Cr removal from complex multi-ion systems. Therefore, the objective of this study was to (a) evaluate the effect of different co-ions, SO_4^{2-}, NO_3^-, and PO_4^{3-} on chromium uptake. (b) examine the Cr^{6+} uptake kinetic mechanisms using suitable kinetic models, and (c) analyze the biochemical and enzymatic changes in *T. pallida* for a better understanding of the oxidative stress and detoxification responses adopted by *T. pallida* due to its exposure to Cr^{6+} and co-ions.

Materials and methods

T. pallida and growth conditions

T. pallida plants were collected from an unpolluted site of North Guwahati, India. For selecting uniformly sized plants for this study, 4-cm-size stem cuttings were used and grown in hydroponic cultures for 15 days. Healthy plants with uniform length (13 ± 1 cm) were selected, thoroughly rinsed under running tap water in order to eliminate any remains of sediment, and, finally, transferred to clean plastic containers (15 plantlets in each experimental setup). Each of these containers was added with 2 l of 50% (v/v) Hoagland's solution (pH = 5.9) of composition (mM): 2.4 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 3.0 KNO_3, 1.0 $MgSO_4$, and 0.5 NaCl, 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 prepared using tap water. Plants were kept under controlled environmental conditions with a temperature regime of 25°C day/night, 14/10 hours light/dark period (1800 lux), and a relative humidity of 70–80% over a period of twelve days.

Effect of co-ions on Cr^{6+} uptake by *T. pallida*

Prior to the Cr^{6+} removal experiments, the plants were acclimatized in 50% (v/v) Hoagland solution (HS) for two weeks. Water lost via natural evaporation was replenished through periodic addition of distilled water. To study the effect of co-ions on Cr^{6+} uptake by *T. pallida*, a Plackett–Burman design comprising 12 treatments with four variables was chosen. Table 1 presents the different combination levels of the factors Cr^{6+}, SO_4^{2-}, NO_3^-, and PO_4^{3-} in this study. The low (−1) and high (+1) concentrations levels of each factor were chosen based on the concentration range in which these ions are commonly found in industrial effluent. Stock solution of 1000 mg L^{-1} each of Cr^{6+} and co-ions was prepared by dissolving (g L^{-1}) 1.631 g of KNO_3, 1.432 g of KH_2PO_4, 1.478 g of Na_2SO_4, and 2.828 g of $K_2Cr_2O_7$, respectively, in 50% (v/v) modified HS. These stock solutions were diluted to achieve a desired concentration of Cr^{6+} and co-ions in each treatment (Table 1). Preliminary experiments using *T. pallida* for Cr^{6+} removal revealed no significant difference in *T. pallida* root growth under aerated or non-aerated conditions. Therefore, aeration was provided only after every three days interval using aquarium pumps. Such mild-aerated and low-nutrition hydroponic conditions are key to the development of plant-based cost-effective treatment systems for chromium removal from wastewater.

Temperature, light/dark period, and relative humidity were maintained the same as mentioned earlier. The modified HS containing different ions of known concentrations was used in this study. A total of 12 experimental setups were chosen based on the initial concentration levels of these co-ions (Table 1). Experimental setups containing no plants but HS with different initial concentration of co-ions, served as a control in this study. Solution volume in the different setups was maintained

Table 1. Plackett–Burman experimental design matrix showing combination of Cr^{6+} and other co-ions in different experimental runs along with their removal by *T. pallida*.

Treatment	Cr^{6+}(mg L^{-1})	SO_4^{2-}(mg L^{-1})	NO_3^-(mg L^{-1})	PO_4^{3-}(mg L^{-1})	Cr^{6+}Removal (%)	SO_4^{2-}Removal (%)	NO_3^-Removal (%)	PO_4^{3-}Removal (%)
1	5	50	20.0	10.0	77.00	80.29	93.53	99.97
2	20	150	20.0	0.5	80.31	87.14	93.66	100.00
3	5	150	0.5	10.0	84.10	86.97	94.88	93.97
4	20	50	20.0	10.0	73.33	78.60	92.55	99.83
5	20	150	20.0	0.5	78.33	84.41	89.73	99.85
6	5	150	20.0	10.0	79.74	84.27	92.00	98.27
7	20	150	0.5	10.0	84.27	83.27	93.37	94.14
8	20	50	0.5	10.0	74.70	72.71	91.75	94.86
9	20	50	0.5	0.5	75.25	84.54	93.78	96.55
10	5	50	20.0	0.5	77.74	81.76	96.82	98.99
11	5	150	0.5	0.5	81.74	76.53	91.80	94.14
12	5	50	0.5	0.5	76.74	80.99	93.45	95.07

by adding solutions containing appropriate concentrations of the ions. For the analysis of the different co-ions, liquid samples from the experimental setups were taken everyday and their concentrations determined following the standard methods detailed later in the analytical methods section. Concentration of the co-ions from the experimental setups was compared with those from the corresponding control setups for monitoring their removal in the study and expressed as % removal (Eq. (1)).

$$\%\ \text{Removal} = \frac{(\text{Co C Re})}{\text{Co}} \times 100 \quad (1)$$

where C_o is the initial solution concentration of Cr^{6+} or the co-ions from the control setup (mg L^{-1}). C_e is the final solution concentration of Cr^{6+} or the co-ions from the experimental setup (mg L^{-1}). The Plackett–Burman design is based on a first-order polynomial equation (Tasharrofi *et al.* 2011) of the form:

$$Y = \beta_0 + \Sigma\ \beta_i X_i \quad (2)$$

This model is used to select the significant concentration of the ions affecting the response (% removal). In Eq. (2), Y is the response (% removal), β_0 is the model coefficient, and β_i is the linear coefficient, and X_i is the level of the independent variable. The statistical software package Minitab™ (version 16, PA, USA) was used for analyzing the experimental data.

Enzymatic and biochemical changes in *T. pallida* due to Cr^{6+} and co-ions exposure were examined using the plants subjected to the experimental conditions 2, 3, 7, and 11 (Table 1), as only in these treatments a high removal efficiency of Cr^{6+} and co-ions was observed. Catalase activity, lipid peroxidation, carbohydrate and protein content of the plant grown under these different conditions were compared with those of the plant grown in the absence of co-ions but in the presence of different initial Cr^{6+} concentrations in the range 5–20 mg L^{-1}. Plants grown only in 50% (v/v) HS without any Cr^{6+} or the co-ions served as the control in this biochemical and enzyme study.

Cr removal kinetics by T. pallida

The kinetics of Cr^{6+} uptake by *T. pallida* in the presence of the co-ions was studied by fitting the metal removal results obtained at different intervals of time (24 hours) to Lagergren's pseudo-first-order, Ho's pseudo-second-order, and Langmuir-type irreversible kinetic models. The pseudo-first-order rate expression is defined as in Eq. (3) (Mohanty *et al.* 2006)

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (3)$$

where, q_e and q_t, both expressed in mg g^{-1}, are the biosorption capacities at equilibrium and at time t (min), respectively, and k_1 (min^{-1}) is the pseudo-first-order rate constant. The values of k_1 and q_e were calculated from the slope and intercept, respectively, of a linear plot of log ($q_e - q_t$) versus t. The pseudo-second-order kinetic model, which is based on chemisorption theory, is represented by Eq. (4) (Roy *et al.* 2015).

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\ 2}} + \frac{1}{q_e} \quad (4)$$

where k_2 (g mg^{-1} min^{-1}) is the pseudo-second-order rate constant. The parameters q_e and k_2 were obtained from the slope and intercept, respectively, of a linear plot of t/q_t versus t.

The aforementioned models were employed to describe the biosorptive removal of Cr^{6+} whereas the Langmuir-type irreversible model was used to describe Cr^{6+} removal by bioaccumulation. This model is given by the Eq. (5).

$$C(t) = \frac{BC_0}{(AC_0 + B)\exp\left\{\left(\frac{B}{V}\right)(t - t_0)\right\} - AC_0} \quad (5)$$

$$A = \frac{k_3 V}{n} \quad (6)$$

$$B = k_3 q_{max} - k_3 q_0 - \frac{k_3 V C_0}{n} \quad (7)$$

where in equations 5–7, C (mg L^{-1}) is the residual Cr^{6+}concentration in the solution, q (mg g^{-1}) is the amount of Cr^{6+} sorbed onto *T. pallida*, q_{max} (mg g^{-1}) is the maximum Cr^{6+} sorption capacity, V (ml) is the liquid volume, n is the number of plants used, k_3 (g mg^{-1} min^{-1}) is the Cr^{6+} sorption rate constant, and t (min) is the experimental time. C_0 (mg L^{-1}), q_0 (mg g^{-1}), and t_0 (min) are the initial Cr^{6+} concentration in solution, initial amount of Cr^{6+} sorbed onto *T. pallida*, and initial time ($t = 0$), respectively. For solving the Eqs. (3)–(5), the mathematical software MATLAB™ 6.1 (The Mathworks, Inc.) was used.

Analytical methods

Concentration of Cr^{6+}, SO_4^{2-}, NO_3^-, and PO_4^{3-} in samples was determined using a UV–visible spectrophotometer (Cary 100, Varian, Australia) at every 24-h interval for the 12-day experimental period. Cr^{6+} concentration was analyzed by the diphenyl carbazide method (Chen *et al.* 2010) by measuring the absorbance of a color complex formed at 540 nm using a UV–visible spectrophotometer. The concentration of SO_4^{2-}, NO_3^-, and PO_4^{3-} ions was measured following the standard barium sulphate turbidity, salicylic acid and ascorbic acid methods, respectively (Cataldo *et al.* 1975; EPA 1983; APHA 1992).

For determining the catalase activity, the method described by Aebi *et al.* (1984) was followed. Fresh leaf samples 0.5 g) were homogenized in 5 mL of cold 200 mM sodium phosphate buffer (pH 7.8). The homogenates were centrifuged at 10,000 × *g* for 20 minutes 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 of 200 mM sodium phosphate buffer (pH 7.8), 1.0 mL of deionized water, and 0.3 mL of 0.1 M H_2O_2 prepared afresh prior to its use. The reaction mixture was then added with 0.5 mL of 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.

The lipid peroxidase activity was analyzed following the method described by Health and Packer (1968). Briefly, 0.5 g of powdered leaf tissue was homogenized in 20% trichloroacetic acid, containing 0.5% 2-thiobarbituric acid and heated at 95°C for 30 minutes. The thiobarbituric acid reactive substances concentration was measured as malondialdehyde (MDA; $\varepsilon = 155$ mM cm^{-1}) at OD_{532} and corrected for nonspecific turbidity at OD_{600} using a UV–visible spectrophotometer (Cary 100, Varian, Australia).

The total carbohydrate content was measured according to the anthrone method (Raunkjer *et al.* 1994) using glucose as the standard, whereas soluble protein content in the samples was measured according to Lowry's method (Lowry *et al.* 1951) using bovine serum albumin as the standard protein.

Results

Simultaneous removal of Cr^{6+} and co-ions from multi-ion system

Table 1 shows the removal of Cr^{6+}, SO_4^{2-}, NO_3^-, and PO_4^{3-} by *T. pallida* in the multi-ion system, which reveals that their removal efficiency varied depending upon their combination level in the respective treatments. Maximum removal of Cr^{6+}, SO_4^{2-} and NO_3^- were 84%, 87% and 94% respectively, corresponding to their initial concentrations of 20, 150, and 20 mg L^{-1} in the study depending upon their own initial concentration and that of the co-ions. For instance, the removal of Cr^{6+}, SO_4^{2-}, NO_3^- was maximum at a high initial concentration of 20, 150, and 20 mg L^{-1}, respectively. However, complete removal of PO_4^{3-} was achieved irrespective of its initial concentration (high or low). Further, these results clearly indicated that the removal of Cr^{6+} and co-ions depended on their initial concentration in the mixture. Thus, an overall removal efficiency of more than 70% for Cr^{6+} and co-ions was achieved in the multi-ion system.

Factorial analysis (ANOVA and Student's t test)

For a better understanding on the effect of Cr^{6+} and co-ions on each other removal, statistical analysis of the results were carried out in the form of analysis of variance (ANOVA) and Student's *t* test. The ANOVA result with a high Fischer's "*F*" value and a low probability "P" value of the regression model indicates its accuracy in explaining the variations in the results (Table 2). The values of the statistical parameters, namely Fischer's *F* value, probability P, standard error S, coefficient of determination (R^2), and adjusted R^2, collectively describe if the level means are significantly different from each other or not. Standard error is expressed in the same units as that of the response variable and it represents the standard distance between the experimental and the model-predicted values. The parameter value also indicates goodness of fit of the regression model used to describe the experimental results. Thus, a lower value of S obtained in this study indicates a very good accuracy of the model in predicting the experimental data. On the other hand, R^2 and adjusted R^2 values describe the amount of variation in the observed response values that is explained by the predictors. Therefore, a minimum *S* value and a maximum R^2 value indicate an accurate prediction ability of the model in this study (Roy *et al.* 2015).

Table 2. Analysis of variance (ANOVA) of removal of Cr^{6+} and co-ions by *T. pallida* in the multi-ion system.

Term	Cr^{6+} Removal [a]		SO_4^{2-} Removal [b]		NO_3^- Removal [c]		PO_4^{2-} Removal [d]	
Values	P	F	P	F	P	F	P	F
Main effect	0.01	6.98	0.0	0.13	0.00	1.39	0.0	52.8
Cr^{6+}	0.01	11.6	0.88	10.02	0.6	0.3	0.05	5.61
SO_4^{2-}	0.00	14.74	0.55	0.38	0.16	2.37	0.01	9.54
NO_3^-	0.02	7.89	0.87	0.03	0.18	2.18	0.01	2.99
PO_4^{3-}	0.06	4.71	0.75	0.11	0.43	0.69	0.12	3.05

S	PRESS	R-Sq	R-Sq (pred)	R-Sq (adj)
a 0.74	8.4918	97.96	94.12	91.52
b 0.40	4.45	97.14	92.0	88.0
c 0.85	5.93	94.19	93.0	92.3
d 0.58	7.04	96.79	90.57	94.96

Moreover, values of the predicted residual error sum of squares presented in Table 2 confirmed that the regression-based model was highly accurate in predicting the experimental results. To further understand which of the individual factors (Cr^{6+} and co-ions) in the multi-ion system played a significant role in their removal, Student's *t* test was performed, which is used as a tool to check the significance of the regression coefficient of the parameters. The estimated coefficients of the individual effect of Cr^{6+} and co-ions are presented in Table 3, in which the associated t and p values were used to check their significance. Any variable with a p value less than 0.05 was found significant for the removal of Cr^{6+} and co-ions. The positive and negative effects of the factors on each other's removal were determined by their respective estimated *t*-values.

Effect of co-ions on Cr^{6+} removal by *T. pallida* was significant as revealed by their respective t and p values (Table 3). The ion SO_4^{2-} exhibited a highly significant positive effect with a *t* value of 3.84 and a p value of less than 0.05 (Table 3, Figure 1). NO_3^- also positively affected Cr^{6+} uptake by *T. pallida* with a t value of 2.81 and p value of 0.02. On the other hand, PO_4^{3-} showed no significant effect on Cr uptake (*t*-value = 2.17 and p value = 0.06). The removal of ions SO_4^{2-} and NO_3^- was rather found to be independent of the presence of any of the co-ions as their respective p-values were insignificant (p-value >0.05). PO_4^{3-} removal was found to be positively affected by Cr^{6+} (*t*-value = 2.37 and p-value = 0.05); however, it was negatively affected by the presence of SO_4^{2-} (*t*-value = −1.54 and p-value = 0.016). Overall, it can be inferred that an increase in SO_4^{2-} and NO_3^- concentration affected Cr^{6+} removal. On the other hand, the PO_4^{3-} removal was positively affected by the presence of Cr^{6+}.

Furthermore, the Pareto analysis was carried out to visualize the effect of Cr^{6+} and co-ions on each other's removal in the multi-ion system. In the Pareto chart (Figure 1(a–d)) horizontal bars indicate the magnitude and effects due to the individual factors, and parameters which extend past the vertical reference line were considered the significant ones ($\alpha = 0.05$). As shown

Table 3. Student's *t* test of the model coefficient for Cr^{6+} and co-ions' removal by *T. pallida* in the multi-ion system (a) Cr^{6+}, (b) SO_4^{2-}, (c) NO_3^- and (d) PO_4^{3-}.

(a)

Term	Effect	Coef	SE Coef	T	P
Constant		79.38	0.59	132.8	0.0
Cr^{6+}	−0.92	−0.46	0.59	7.74	0.46
SO_4^{2-}	4.59	2.29	0.59	3.84	0.00
NO_3^-	3.36	−1.68	0.59	2.81	0.02
PO_4^{3-}	2.6	1.3	0.59	2.17	0.06

(b)

Term	Effect	Coef	SE Coef	T	P
Constant		82.39	1.18	69.75	0.0
Cr^{6+}	0.35	0.17	1.18	0.15	0.88
SO_4^{2-}	1.46	0.73	1.18	0.62	0.55
NO_3^-	−0.4	−0.2	1.18	−0.17	0.87
PO_4^{3-}	0.76	0.38	1.18	0.32	0.75

(c)

Term	Effect	Coef	SE Coef	T	P
Constant		93.08	0.24	378.41	0.0
Cr^{6+}	0.27	0.14	0.24	0.55	0.6
SO_4^{2-}	−0.76	−0.38	0.24	−1.54	0.16
NO_3^-	−0.73	−0.36	0.24	−1.48	0.18
PO_4^{3-}	0.41	0.20	0.24	0.83	0.43

(d)

Term	Effect	Coef	SE Coef	T	P
Constant		97.01	0.16	574.33	0.0
Cr^{6+}	0.8	0.4	0.16	2.37	0.05
SO_4^{2-}	−1.04	−0.38	0.24	−1.54	0.016
NO_3^-	4.69	−0.36	0.24	−1.48	0.18
PO_4^{3-}	−0.59	0.20	0.24	0.83	0.43

in these figures, the co-ions SO_4^{2-} and NO_3^- significantly affected Cr^{6+} uptake by *T. pallida*.

Cr^{6+} removal kinetics and its modeling

Figure 2 shows a gradual uptake of Cr^{6+} and co-ions by *T. pallida* throughout the experimental period. Although some variation in the percentage removal of Cr^{6+} and co-ions was observed in each of the treatments, the removal, in general, was slow and steady for Cr^{6+} and the co-ions. For a better understanding of the kinetics of Cr^{6+} removal by *T. pallida*, the experimental results were fitted to three well-known kinetic models found in the literature, *i.e* irreversible, Lagergen's first-order and Ho's second-order kinetics. The values of the estimated kinetic model parameters and the coefficient of determination (R^2) due to these models, obtained using actively growing *T. pallida* plants, are presented in Table 4.

These results reveal that Cr^{6+} removal by *T. pallida* followed pseudo-second-order kinetics more accurately than the irreversible or pseudo-first-order kinetics, which is in agreement with the literature (Espinoza *et al.* 2009; Gupta *et al.* 2013). The maximum estimated biosorption capacity (3.04 mg/g) (Table 4) obtained using this model also matched well with the maximum Cr^{6+} removal efficiency

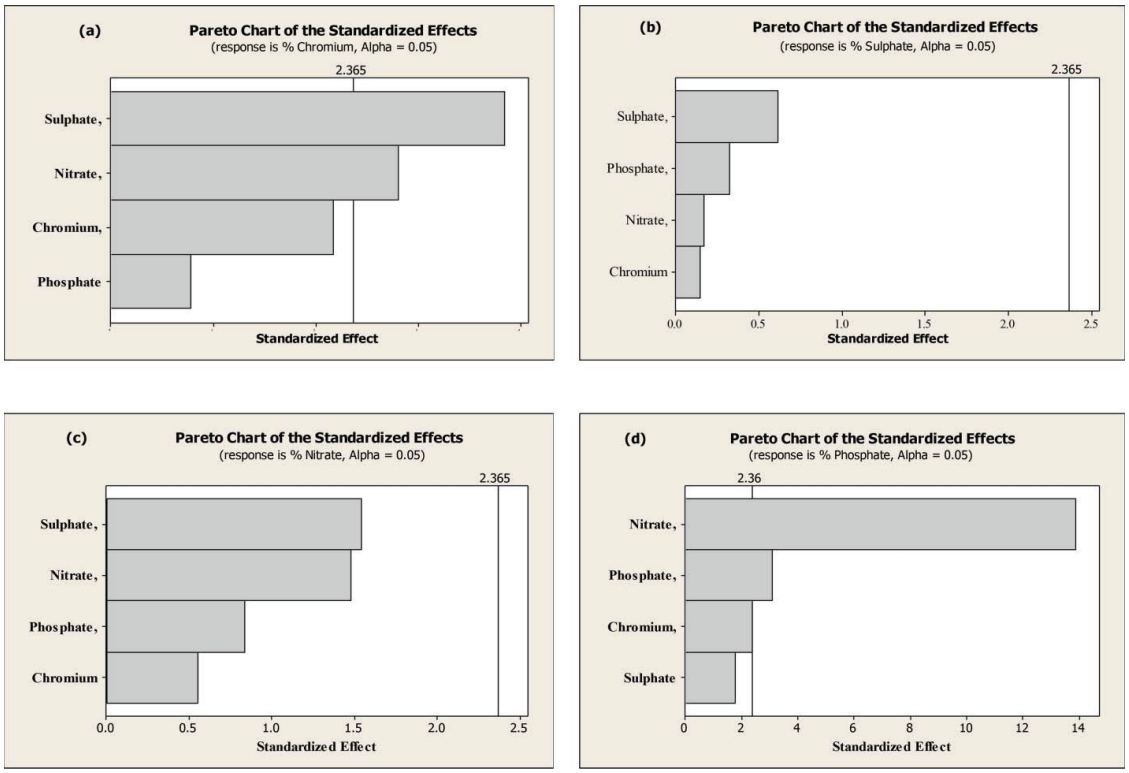

Figure 1. Pareto chart showing the effect of Cr^{6+} and co-ions on each other removal by *T. pallida* in the different treatments: (a) Cr^{6+} removal, (b) SO_4^{2-} removal, (c) NO_3^- removal, and (d) PO_4^{3-} removal (vertical line shows significance cutoff at p value less than 0.05).

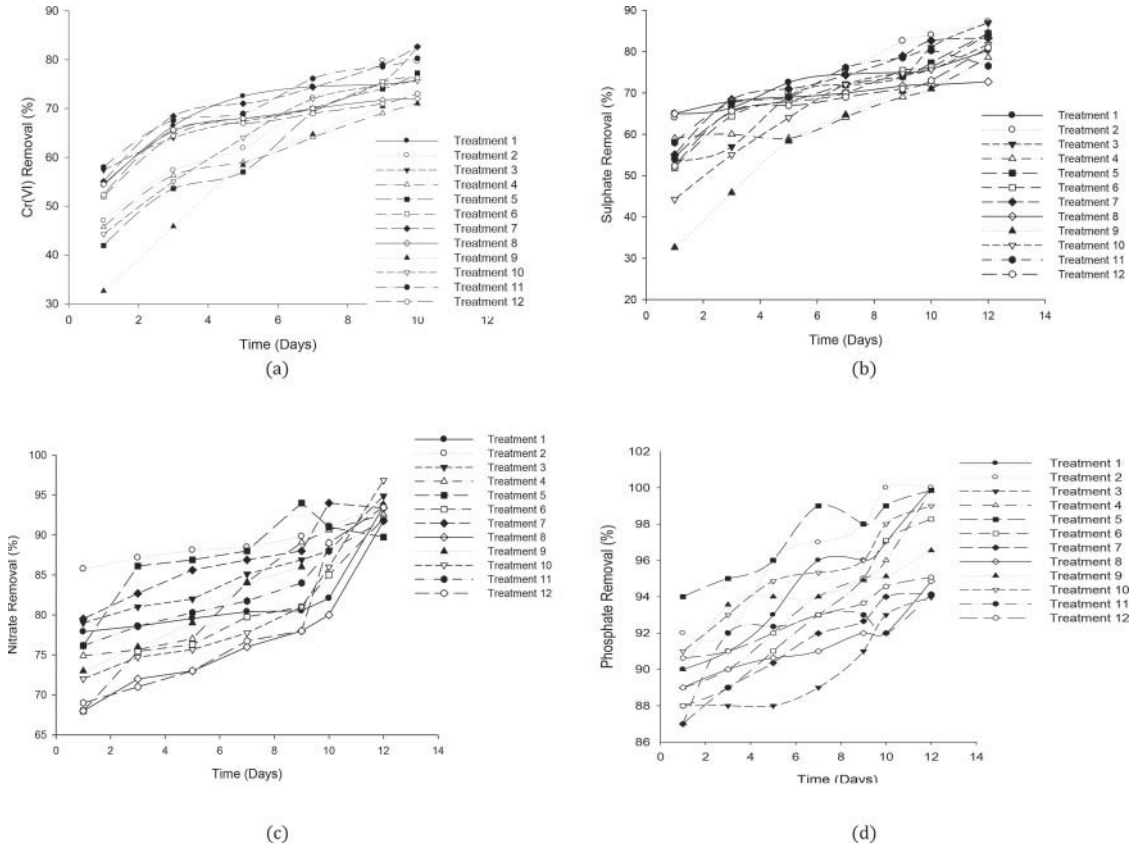

Figure 2. Time profile of Cr^{6+} and co-ions removal by *T. pallida* in the different treatments: (a) Cr^{6+}, (b) SO_4^{2-}, (c) NO_3^- and (d) PO_4^{3-}.

(84.27%) obtained in treatment 7 (Table 1). Further, the effect of sulfate on Cr^{6+} removal was observed both on the experimental Cr^{6+} removal (Figure 1a) and estimated pseudo-second-order biosorption capacity value (q_{max}) (Figure 3).

Enzymatic and biochemical analysis

In the Plackett–Burman design (Table 1), high initial Cr^{6+} concentration was used in the treatment nos. 7 and 2, whereas low initial Cr^{6+} concentration was used in the treatment nos. 3 and 11. Therefore, plant biomass taken from these respective treatments was subjected to the analyses of catalase and lipid peroxidation activity every alternate day for an experimental period of 12 days. Maximum catalase activity was observed in the exp run nos. 7 and 2 with a relatively low enzyme activity in the exp run nos. 3 and 11 (Figure 4a). The least activity was observed in the control plant and in the presence of only 5 mg L^{-1} Cr^{6+} initial concentration (in the absence of co-ions). Lipid peroxidation was estimated by malondialdehyde (MDA) content, which increased with a high initial Cr^{6+} concentration in the absence of co-ions. The value was lower in the case of the control plant (Figure 4b). The total sugar content was high in all the treatments and a maximum value was observed at a low initial Cr^{6+} concentration. The sugar content was also the least in the plant exposed to only 20 mg L^{-1} of Cr^{6+} (in the absence

Table 4. Estimated kinetic model parameters of Cr^{6+} removal by *T. pallida* in the different treatments.

			Irreversible		Pseudo first order			Pseudo second order	
Treatment	q_{max}(mg g^{-1})	Rate constant (k_1) (g mg^{-1} min^{-1})	R^2	q_{max}(mg g^{-1})	Rate constant (k_2) (min^{-1})	R^2	q_{max}(mg g^{-1})	Rate constant (k_3)(g mg^{-1} min^{-1})	R^2
1	0.2136	0.2936	0.8656	0.2237	0.4467	0.7689	0.1887	4.7769	0.9955
2	1.1659	0.0536	0.8221	0.7650	0.3286	0.7367	2.86345	0.7654	0.9778
3	0.1896	0.3291	0.8873	0.1048	0.8768	0.8560	1.2244	1.1568	0.9732
4	1.0360	0.0603	0.8145	0.8669	0.4386	0.8348	1.1237	1.1679	0.9568
5	0.9854	0.0634	0.8097	0.9786	0.2478	0.7932	2.9756	0.7986	0.9324
6	0.2586	0.2412	0.8965	0.2678	0.5863	0.7332	1.1854	0.9864	0.9560
7	0.8678	0.0720	0.7898	0.8670	0.3258	0.7187	3.0478	0.7789	0.9588
8	0.6472	0.0965	0.8123	0.6890	0.2896	0.6987	1.4327	1.0964	0.9568
9	0.7896	0.0821	0.8026	0.656	0.3103	0.7860	1.4795	0.9446	0.9455
10	0.3210	0.1948	0.8676	0.1540	0.7689	0.7684	0.1789	4.8970	0.9899
11	0.2285	0.2735	0.8432	0.1865	0.7423	0.7457	1.3842	0.9875	0.9776
12	0.1180	0.5274	0.8321	0.2011	0.4867	0.7556	0.2012	5.237	0.9876

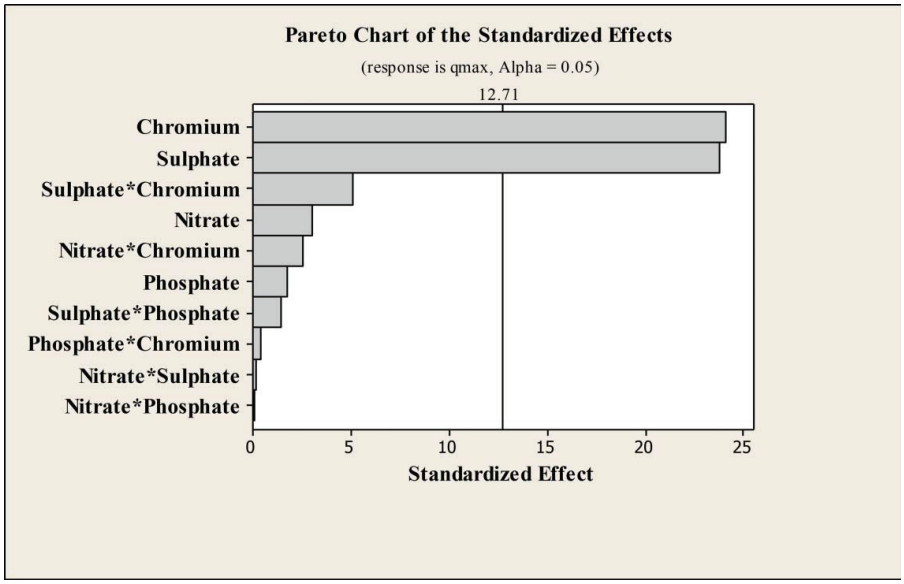

Figure 3. Pareto chart showing the effect of Cr^{6+} and co-ions on the estimated maximum Cr^{6+} uptake capacity (q_{max}) of *T. pallida* in the multi-ion system (vertical line shows significance cut-off at p value less than 0.05).

of co-ions) and in the control plant (Figure 4c). Total protein content was low in the first 2–3 days of experiments in all the treatments, following this experimental time period, the protein content significantly increased in exp run nos. 2, 3, 7, and 11. The protein content was also low with 5 and 20 mg L^{-1} of Cr^{6+} in the absence of the co-ions (Figure 4d).

Discussion

Cr^{6+} removal efficiency varied in the presence of different concentrations of co-ions (SO_4^{2-}, PO_4^{3-}, and NO_3^{-}) (Figure 2a). Sulfur is an essential nutrient for plants and is predominantly taken up by the roots as SO_4^{2-} ions (Buchner *et al.* 2004). In plants, most of the sulfur is incorporated into organic

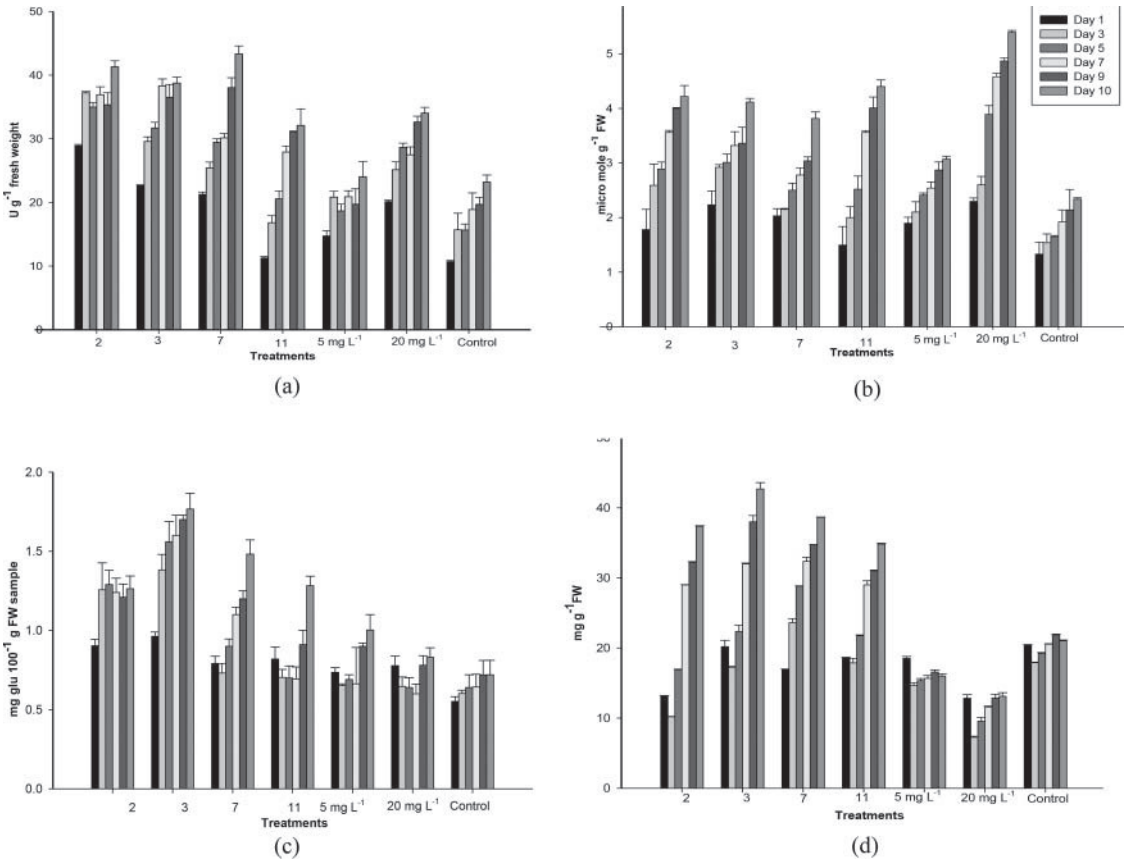

Figure 4. Biochemical and enzyme activity of *T. pallida* in the different treatments: (a) catalase, (b) malondial-dehyde, (c) carbohydrate content and (d) protein content.

compounds, including cysteine, which are used for the production of antioxidants such as glutathione and phytochelatin (Davidian *et al.* 2010). These compounds further improve Cr^{6+} tolerance in plants through complexation and/or sequestration into vacuoles (Leustek and Saito, 1999). These compounds are also vital to all plant proteins and certain plant hormones. NO_3^- and PO_4^{3-} are other plant macronutrients essential for plant growth. NO_3^- is used to produce chlorophyll and proteins (Amtmann *et al.* 2009). Besides, it is a principal component of DNA, and therefore plays an important role in plant reproduction. Similarly, PO_4^{3-} present in water is utilized by plants for routine metabolism and in the synthesis of DNA and proteins (Nakanishi *et al.* 2014). In the present study, Cr^{6+} uptake increased along with increase in the initial NO_3^- concentration which may be attributed to the plant's mechanism to tolerate Cr toxicity. Although PO_4^{3-} did not show any significant effect on Cr uptake it was found that PO_4^{3-} uptake increased along with increase in an initial Cr^{6+} concentration which may be attributed to the plants' mechanism to overcome the Cr toxicity and to maintain its nutrient homeostasis.

In this study, among the different co-ions, SO_4^{2-} enhanced Cr^{6+} removal the most (Figures 1 and 3, Table 2), which is in agreement with previous studies, which report that Cr^{6+} is transported in *Brassica juncea* by a non-specific anion carrier, that is involved in transporting SO_4^{2-} (Schiavon *et al.* 2012). Further, Cr^{6+} removal by *T. pallida* was found to be dose dependent as with other plant species such as *Amaranthus viridis* (Liu *et al.* 2008), *Citrullus* (Dube *et al.* 2003). It has been reported that most plants do not have specific transporters for Cr^{6+} uptake mainly because it is not an essential nutrient. In this study, a high initial SO_4^{2-} concentration increased the accumulation of Cr^{6+} inside the cells, which suggests that a high number of SO_4^{2-} transporters are engaged at a high SO_4^{2-} concentration for simultaneous uptake of SO_4^{2-} and Cr^{6+} occurred (Shaven et al. 2012). In a variety of plant species, Cr^{6+} entry takes place through the sulfate ABC transporter, which is up-regulated following CrO_4^{2-} exposure (Vita *et al.* 2014). In *T. pallida*, a substantial amount of Cr^{6+} was previously found to bioaccumulate in shoots as well as in roots, which could be attributed to the sulfate transporters present in the plant system (Kaszycki et al. 2005). At an initial Cr^{6+} concentration of 20 mg L^{-1}, the effect of PO_4^{3-} on Cr^{6+} removal was negligible (Figure 1a, Table 1), which is due to the fact that PO_4^{3-} ions act as an essential nutrient for plants, and, hence, it is assimilated over chromate by the plant (López-Bucio *et al.* 2014). In the present study, Cr^{6+} uptake increased along with an increase in the initial PO_4^{3-} concentration which may be attributed to the plant's mechanism to tolerate Cr toxicity and to maintain the plant's nutrient homeostasis (Qian *et al.* 2013). A similar inference was drawn by López-Bucio et al. (2014), who reported that Cr induced the expression of PO_4^{3-} transporters in *Arabidopsis thaliana*. Several other reports have also shown that mineral nutrients, *e.g.* PO_4^{3-}, attenuate Cr^{6+} toxicity by decreasing Cr absorption and increasing the absorption of beneficial ions in plants such as *Raphanus sativus* L. (Sayantan *et al.* 2013) and *Pteris vittata* (de Oliveira *et al.* 2015). These authors also confirmed that a reduced Cr toxicity in plants is due to an increase in PO_4^{3-} uptake with an increase in Cr^{6+} concentration. Further, SO_4^{2-} showed negative effect on PO_4^{3-} removal (Table 3d). This may be attributed to the alteration in the expression of *SULTR2:1* transporter, which increases SO_4^{2-} translocation at a low concentration of PO_4^{3-} (Rouached 2011). The uptake of PO_4^{3-} ions increased along with an increase in an initial Cr^{6+} concentration in order for the plant to tolerate Cr toxicity and to maintain nutrient homeostasis (Alam 1999). The kinetic rate constants obtained from irreversible, pseudo-first and second-order models are given in Table 4. The values of R^2 for the pseudo-second-order model is relatively high (>0.995), and the adsorption capacities calculated by the model are also close to those determined experimentally. Thus, the Cr^{6+} removal kinetics followed the pseudo-second-order kinetic model, suggesting that the initial Cr^{6+} sorption by *T. pallida* is reaction controlled, involving chemisorption, *i.e.* Cr^{6+} was initially bound to the surface of *T. pallida* for its subsequent uptake (Sinha *et al.* 2015). The advantage of the pseudo-second-order equation for estimating the q_{max} values is its high accuracy with minimum experimental errors. The integral form of the model, represented by the Eq. (4), predicts that the ratio of the time to Cr^{6+} removed amount (t/qt) should be a linear function of time (Ho *et al.*, 2006). Both theoretical investigations and the experimental studies indicate that the value of k_2 depended on the initial Cr^{6+} concentration in the liquid phase.

Catalase efficiently scavenges the free radicals, particularly H_2O_2, produced during stress, thereby preventing membrane lipids, proteins, and nucleic acids from being damaged (Tamás *et al.* 2008). In this study, catalase activity was maximum at a high initial Cr^{6+} concentration of 20 mg L^{-1} and in the presence of the co-ions (exp run nos. 7 and 11), revealing a maximum stressed condition of the plant due to the elevated level of Cr^{6+} (Figure 4a). Furthermore, the increased MDA content in the presence of 20 mg L^{-1} of Cr^{6+} clearly indicated oxidative stress in *T. pallida* (Figure 4b). Increase in the sugar content due to the presence of Cr^{6+} and co-ions (Figure 4c) shows that it helps the plant to regulate osmotic stress, thereby preventing damage to its biomolecules and membrane (Najafian *et al.* 2012). It has been reported that exposure to chromate results in overexpression of genes related to carbohydrate metabolism (glyoxylate metabolism, citrate cycle, pyruvate metabolism, glycolysis, gluconeogenesis) (Couée *et al.* 2006). Negative correlation between initial Cr^{6+} concentration and protein content could be observed in the absence of the co-ions (Figure 4d). Whereas in the presence of co-ions, protein content gradually increased after day 3, suggesting that SO_4^{2-}, NO_3^-, and PO_4^{3-} helped the plant to overcome the initial stress condition. These co-ions act as macronutrients for plant growth and metabolism. Their initial concentrations used in this study were well below their toxic range for plants. Hence, it is quite expected that an increase in their concentration within the normal range will lead to an increased plant growth. This could be explained based on the fact that supplementation with NO_3^- helps the plant to synthesize more protein, which is a nitrogenous biomolecule. Similarly, SO_4^{2-} is incorporated with cysteine and other organic molecules, which play a key role in the generation of antioxidants like glutathione and phytochelatin. These compounds get complexed and/or sequestrated into vacuoles that

further help the plant toward Cr tolerance (Brychkova *et al.* 2012).

Conclusions

This study demonstrated that the bioremoval of Cr^{6+} by *T. pallida* was enhanced by the presence of co-ions, viz. SO_4^{2-}, and NO_3^-, and PO_4^{3-}. In this multi-ion study, PO_4^{2-} was removed with a high efficiency followed by NO_3^-, SO_4^{2-} and Cr^{6+}. Biochemical analyses of the plant indicated that its tolerance toward Cr^{6+} may be related to high constitutive levels of carbohydrates and catalase activity. The Cr^{6+} removal kinetics by *T. pallida* followed the chemisorption-based pseudo-second-order kinetics. Overall, this study not only demonstrated a very high removal of Cr^{6+} by *T. pallida*, but also an excellent tolerance toward the metal in presence of the co-ions.

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References

Aebi H. 1984. Catalase in vitro. Methods Enzymol 105:121–126.

Alam SM. 1999. Nutrient uptake by plants under stress conditions. Handbook of plant and crop stress 2:285–313.

Amtmann A, Armengaud P. 2009. Effects of N, P, K and S on metabolism: new knowledge gained from multi-level analysis. Curr Opin Plant Biol 12(3):275–283.

Brychkova G, Yarmolinsky D, Fluhr R, Sagi M. 2012. The determination of sulfite levels and its oxidation in plant leaves. Plant Sci 190:123–130.

Buchner P, Takahashi H, Hawkesford MJ. 2004. Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. J Exp Bot 55(404):1765–1773.

Cataldo DA, Maroon M, Schrader LE, Youngs VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6(1):71–80.

Cervantes C, Campos-Garcia J. 2007. Reduction and efflux of chromate by bacteria. Molecular microbiology of heavy metals. Heidelberg: Springer-Verlag, p. 407–419.

Chen S, Yue Q, Gao B, Xu X. 2010. Equilibrium and kinetic adsorption study of the adsorptive removal of Cr (VI) using modified wheat residue. J Coll Interf Sci 349(1):256–264.

Chervona Y, Arita A, Costa M. 2012. Carcinogenic metals and the epigenome: understanding the effect of nickel, arsenic, and chromium. Metallomics 4(7):619–627.

Couée I, Sulmon C, Gouesbet G, El Amrani A. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57(3):449–459.

Davidian JC, Kopriva S. 2010. Regulation of sulfate uptake and assimilation—the same or not the same? Mol Plant 3(2):314–325.

De Luccia TP. 2012. Determination of heavy metals in the bioindicator plant *Tradescantia pallida* var. Purpurea. J Environ Eng Ecol Sci 2050–1323:1–4.

de Oliveira LM., Lessl J. T., Gress J., Tisarum R., Guilherme L. R., Ma L. Q. 2015. Chromate and phosphate inhibited each other's uptake and translocation in arsenic hyperaccumulator *Pteris vittata* L. Environ Pollut 197:240–246.

Dube BK, Tewari K, Chatterjee J, Chatterjee C. 2003. Excess chromium alters uptake and translocation of certain nutrients in *citrullus*. Chemosphere 53(9):1147–1153.

Espinoza-Quiñones FR, Módenes AN, Costa Jr IL, Palácio SM, Szymanski N, Trigueros DE, Silva EA. 2009. Kinetics of lead bioaccumulation from a hydroponic medium by aquatic macrophytes *Pistia stratiotes*. Water Air Soil Pollut 203(1–4):29–37.

Health RL, Packer G. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biopys 125(1):189–198.

Ho Y S. 2006. Second-order kinetic model for the sorption of cadmium onto tree fern: a comparison of linear and non-linear methods. Water Res 40(1):119–125.

Huang X, Liao X, Shi B. 2009. Adsorption removal of phosphate in industrial wastewater by using metal-loaded skin split waste. J Hazard Mater 166(2):1261–1265.

Gupta VK, Pathania D, Sharma S, Agarwal S, Singh P. 2013. Remediation of noxious chromium (VI) utilizing acrylic acid grafted lignocellulosic adsorbent. J Mol Liquids 177:343–52.

Kaszycki P, Gabryś H, Appenroth KJ, Jaglarz A, Sedziwy S, Walczak T, Koloczek H. 2005. Exogenously applied sulphate as a tool to investigate transport and reduction of chromate in the duckweed *Spirodela polyrhiza*. Plant Cel Environ 28(2):260–268.

Leustek T., Saito K. 1999. Sulfate transport and assimilation in plants. Plant Physiol 120(3):637–644.

Liu D, Zou J, Wang Jiang W. 2008. Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. Bioresour Technol 99 (7):2628–2636.

López-Bucio J, Hernández-Madrigal F, Cervantes C, Ortiz-Castro R, Carreón-Abud Y, Martínez-Trujillo M. 2014. Phosphate relieves chromium toxicity in *Arabidopsis thaliana* plants by interfering with chromate uptake. BioMetals 27(2):363–370.

Lowry OH, Rosenburg JJ, Farr AL, Randall R J. 1951. Estimation of protein with the Folin–phenol reagent. Biol Chem 193:265–270.

Methods for Chemical Analysis of Water and Wastes. 1983. EPA-6004-79-020, USEPA, Method 375.4.

Mohanty K, Jha M, Meikap BC, Biswas M. 2006. Biosorption of Cr (VI) from aqueous solutions by *Eichhornia crassipes*. Chem Eng J 117 (1):71–77.

Najafian M, Kafilzadeh F, Azad HN, Tahery Y. 2012. Toxicity of Chromium (Cr) on growth, ions and some biochemical parameters of *Brassica napus L*. World Appl Sci J 16(8):1104–1109.

Nakanishi TM, Thibaud MC. 2014. Phosphate import in plants: focus on the PHT1 transporters. Evol Transport Plants 235.

Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J. 2009. The biochemistry of environ-mental heavy metal uptake by plants: implications for the food chain. International Journal of Biochemistry and Cell Biol 41:1665–1677.

Qian H, Sun Z, Sun L, Jiang Y, Wei Y, Xie J, Fu Z. 2013. Phosphorus availability changes chromium toxicity in the freshwater alga *Chlorella vulgaris*. Chemosphere 93(6):885–891.

Rezaee A, Hossini H, Masoumbeigi H, Darvishi Cheshma Soltani R. 2011. Simultaneous removal of hexavalent chromium and nitrate from wastewater using electrocoagulation method. IJESD 2:294–298.

Roy AS, Hazarika J, Manikandan NA, Pakshirajan K, Syiem MB. 2015. Heavy Metal Removal from Multi-ion System by the Cyanobacterium *Nostoc muscorum*: Kinetics and Interaction Study. Appl Biochem Biotechnol 175(8):3863–3874.

Raunkjer K, Jacobsen TH, Nielson PH. 1994. Measurement of pools of protein, carbohydrates and lipids in domestic wastewater. Water Res 28:251–262.

Rouached H. 2011. Multilevel coordination of phosphate and sulfate homeostasis in plants. Plant Signal Behav 6(7):952–955.

Schiavon M, Galla G, Wirtz M, Pilon-Smits EA, Telatin V, Quaggiotti S, Malagoli M. 2012. Transcriptome profiling of genes differentially modulated by sulfur and chromium identifies potential targets for phytoremediation and reveals a complex S–Cr interplay on sulfate transport regulation in *B. juncea*. J Hazard Mater 239:192–205.

Schmitt D, Müller A, Csögör Z, Frimmel FH, Posten C. 2001. The adsorption kinetics of metal ions onto different microalgae and siliceous earth. Water Res 35(3):779–785.

Sinha V, Pakshirajan K, Chaturvedi R. 2014. Chromium (VI) Accumulation and Tolerance by *Tradescantia pallida*: Biochemical and Antioxidant Study. Appl Biochem Biotechnol 173(8):2297–2306.

Sinha V, Pakshirajan K, Chaturvedi R. 2015. Evalution of Cr(VI) exposed and unexposed plant parts of *Tradescantia pallida* (ROSE) D.R.Hunt. for Cr removal from wastewater by biosorption. Int J Phytoremediation 17:1204–1211.

Standard Methods for the Examination of Water and Wastewater, 18th Edition, AWWA, APHA, WPCF; Water Pollution Control Federation, Washington, DC, 1992.

Sayantan D. 2013. Amendment in phosphorus levels moderates the chromium toxicity in *Raphanus sativus* L. as assayed by antioxidant enzymes activities. Ecotoxicology and environmental safety 95:161–170.

Tamás L, Dudíková J, Durceková K, Huttová J, Mistrík I, Zelinová V. 2008. The impact of heavy metals on the activity of some enzymes along the barley root. Environ Exp Bot 62:86–91.

Tasharrofi N, Adrangi S, Fazeli M, Rastegar H, Khoshayand MR, Faramarzi MA. 2011. Optimization of chitinase production by *Bacillus pumilus* using Plackett-Burman design and response surface methodology. Iran J Pharmaceut Res 10(4):759.

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Mench M. 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16(7):765–794.

Viti C, Marchi E, Decorosi F, Giovannetti L. 2014. Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. FEMS Microbiol Rev 38 (4):633–659.