Continuous removal of Cr(VI) from wastewater by phytoextraction using *Tradescantia pallida* plant based vertical subsurface flow constructed wetland system

Vibha Sinha, N. Arul Manikandan, Kannan Pakshirajan[*], Rakhi Chaturvedi

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India

ABSTRACT

This study investigated the performance of *Tradescantia pallida* in continuous removal of Cr(VI) from contaminated water using a laboratory scale vertical subsurface flow (VSSF) constructed wetland system. The effect of different hydraulic residence time (HRT), i.e. 1, 2 and 3 day HRT, was first studied at an influent Cr(VI) concentration of 20 mg/L. Later experiments were carried out at different influent pH (7 and 4) and at different influent Cr(VI) concentration (20 and 30 mg/L), but with the same HRT of two days. Best results were achieved for 2 d HRT and for an influent pH of 7 with a maximum Cr(VI) removal efficiency of 97.2–98.3% and a maximum total Cr removal efficiency of 86–88.2%. Cr(VI) was found to be uniformly distributed in different segments of the wetland units. Compared with the control unit in which no plants were grown, the units with the plant biomass showed substantially high amount of Cr(VI) in its soil (63–68%). Bioconcentration factor and translocation factor values ranged from 16.45 to 23.21 and 0.37 to 0.90 respectively, which revealed the excellent Cr uptake potential of *T. pallida*. The highest accumulated Cr concentration (464.33 mg/kg) was found in plants grown in the system at pH 7.0, whereas the minimum accumulated Cr concentration (249.21 mg Cr/kg) was in plants grown in the system at pH 4.0. Furthermore, 3–4% of the total Cr input into the system was accumulated in the plants at the end of the operation. All these results revealed an excellent performance of the *T. pallida* plant based constructed wetland system for continuous removal of Cr(VI) from contaminated water.

1. Introduction

Hexavalent chromium (Cr(VI)) is one of the most noxious metal contaminating the soil and water resources. Cr containing compounds get discharged into the environment mainly from anthropogenic sources due to its diverse commercial uses. They are widely used in industries such as leather tanning, metallurgical operation, steel production, electroplating, pigment and textile manufacturing, wood preservation, and chromate preparation (Barrera-Díaz et al., 2012). Among the different oxidative states, Cr(III) and Cr(VI) forms predominate and are found to be stable in aqueous environment (Cainelli and Cardillo, 2012). Cr(VI) is a potential carcinogen and is 500 times more harmful than Cr(III) which is relatively insoluble (Jin et al., 2016). The high aqueous solubility of Cr(VI), bioavailability and its persistent property pose a serious environmental concern (Dhal et al., 2013). The World Health Organization has set the Cr(VI) permissible limit in surface waters to be below 0.05 mg/L, whereas total Cr, containing Cr(III) and other oxidation states, are permitted to be below 2 mg/L due to their oxidation potential to Cr(VI) (WHO, 1993). Therefore, an efficient method to treat Cr(VI) contaminated water prior to its discharge is mandatory to satisfactorily address the environmental and public health concerns.

Conventional treatment methods for Cr containing wastewater employs energy-intensive physico-chemical processes such as ion exchange, reverse osmosis and advanced oxidation reduction processes (Lofrano et al., 2013). Due to the high cost, generation of secondary toxic sludge and high energy demand of these physio-chemical methods, biological treatment method seems to be more advantageous.

Recently, constructed wetlands (CWs) have been successfully applied to be efficient and low cost treatment method for secondary treatment of industrial effluents loaded with heavy metals and other contaminants (Wu et al., 2016; Ge et al., 2016). CWs act as

an engineered ecosystem where substrate, plants and its associated microbial community function simultaneously in a controlled environment (Chyan et al., 2016). However, the potential of CWs has not been sufficiently tested to treat Cr(VI) contaminated systems, such as effluent from tanneries and metallurgical industries. Such effluents vary in both pH as well as Cr(VI) concentrations, which can adversely affect plant growth and Cr removal in CWs. This work addresses the use of an indigenous plant in constructed wetlands for the continuous removal of Cr(VI) from wastewater. Further, it highlights the significance of wastewater pH, which is an important parameter affecting metals chemistry and speciation, on Cr(VI) removal in constructed wetland system.

The main aim of this work is to investigate the role of *Tradescantia pallida* in continuous Cr(VI) removal using laboratory scale CWs. This plant species was earlier shown to be Cr(VI) tolerant and efficient in the uptake of Cr(VI) and other metals, but in batch hydroponics system (Sinha et al., 2014). It is a rapidly colonizing plant species and easily adapts to a wide range of pH (3.5–10.5), temperature and light conditions (Paiva et al., 2003). The present work describes the performance of *T. pallida* in continuous Cr(VI) removal at different hydraulic retention time (HRT) and pH. Cr(VI) concentration in CWs microcosms comprising soil, sand and *T. pallida* plants was determined to understand the mechanism and distribution of Cr removal in the CWs.

2. Materials and methods

2.1. Experimental set up

Two identical sized laboratory scale wetland systems of dimensions 0.3 m length, 0.15 m width and 0.5 m depth each were operated in vertical subsurface mode. These units were constructed out of Perspex material. A schematic of the set up used is shown in Fig. 1. Beds were filled with soil (up to 15 cm), sand (up to 10 cm) and gravel (up to 10 cm) of two different particle sizes (5–7 mm and 3–4 mm). Sand and gravels were washed thoroughly with water prior to use. At the base of the units, bigger size gravel was placed up to a height of 5 cm to prevent any clogging. The top of the

wetland unit was left open. Three sampling ports (4 mm internal diameter) at the height of 0 (P1), 20 (P2) and 30 (P3) cm from the bottom were provided for samples collection from different depths of the units. Void volume of the units was measured by draining the units and determining the water volume of each tank. Table 1 presents the characteristics of the wetland units.

2.2. Start-up and continous Cr(VI) experiments

Continuous experiments were carried out using the wetland system operated under different hydraulic retention time (HRT), pH and influent Cr(VI) concentration conditions. Plantlets of height 5 cm ± 2 each were collected from North Guwahati, India, and planted in the unit. The unit was initially supplied with only tap water to acclimatize the plants to the CW environment. Cr(VI) containing influent was prepared by dissolving 2.83 g of potassium dichromate ($K_2Cr_2O_7$) in 1 L tap water. This stock solution (1000 mg/L) was suitably diluted to achieve the desired influent Cr(VI) concentration in the experiments. For adjusting the pH of Cr(VI) containing feed, 1 N HCl/1 N NaOH was used. Thereafter, Cr(VI) solution (pH = 5.6) at a concentration 20 mg/L was continuously fed into the units using peristaltic pumps to achieve three different HRT (3, 2 and 1 days). The water level was maintained 5 cm below the soil surface in order to ensure/maintain a subsurface flow. All the CW units were operated under laboratory conditions with a light/dark period of 16/8 h. The other parameters, such as relative humidity and temperature, were only monitored and not controlled. Whereas the relative humidity was within 70–80%, the temperature varied between 23 and 32 °C. Samples were collected periodically from the influent and the effluent points for Cr(VI) analyses. A similar unit of the same dimensions, containing only soil, sand and gravel, but without the *T. pallida* plant species served as the control.

Among the different parameters, besides Cr(VI) concentration and HRT, wastewater pH is also known to significantly affect Cr(VI) removal by plants due to its effect on Cr chemistry and speciation. To study the effect of pH, on the performance of the CW system, *T. pallida* plants were grown in two CW units and acclimatized as
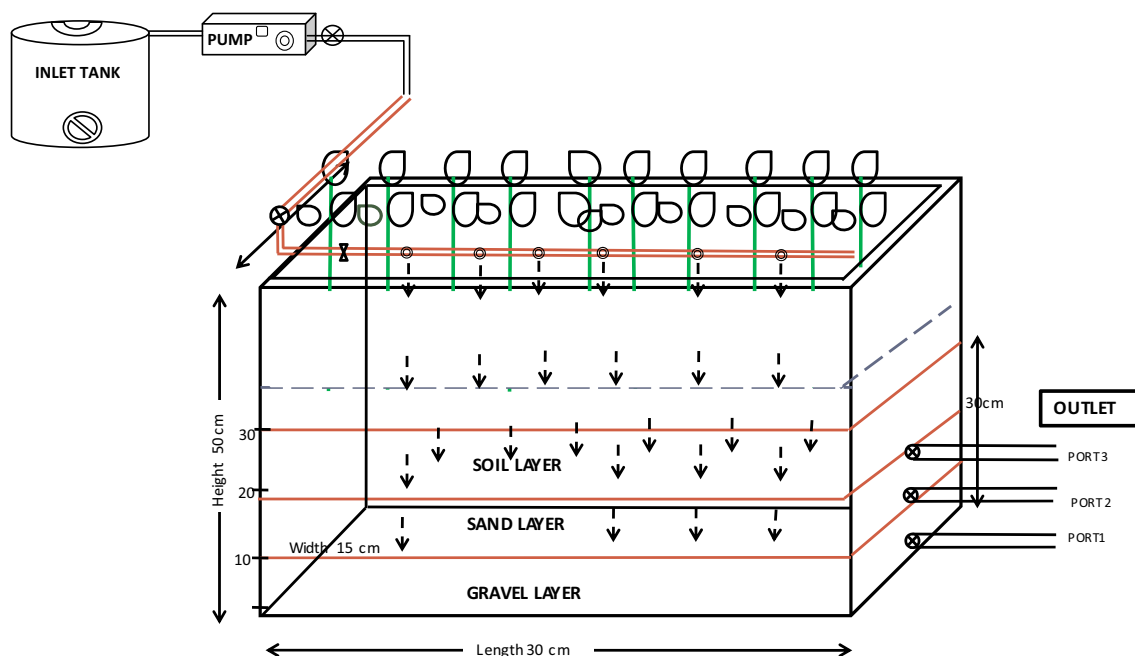

Fig. 1. Schematic of the vertical flow constructed wetland experimental setup used in this study.

Table 1
Wetland design and characteristics.

	Dimension/effective size (cm)	Volume/Capacity	Area of veg (no.of stems)	Working volume	Porosity/root depth
Main unit	30 × 20 × 40 cm (l × w × d)	5.5 L	450 cm², 24 plantlets	3.5 L 3.7 L	
Treatment zone/media type					
Plant	*Tradescantia pallida*	–	24		8-10 cms
Soil	Garden soil (Alluvial)	2.5 kg	–	–	
Coarse sand	125-150 μm	3 kg	–		
Fine gravel	Size: passed through 6 retained by 4 (3–4 mm)	2 kg	–	–	
Large gravel	Size: passed through seive no. 10 retained by 8 (5–7 mm)	3 kg	–		

before, but operated at a different influent pH (Dhal et al., 2013; Wu et al., 2016) and different influent Cr(VI) concentration (20 and 30 mg/L) with 2 days HRT. Cr(VI) concentration in effluents from tanneries and electroplating industries usually varies in the range 0.05–9.36 mg/L (Sharma and Adholeya, 2011). Further, Cr(VI) concentrations in the range 0.05–20 mg/L has been reported in the literature for studying Cr(VI) removal in constructed wetland system using different plant species (Chandra et al., 2009; Gowd and Govil, 2008). Similarly, an HRT value of 1–7 days has been reported in the literature to study continuous Cr(VI) removal in constructed wetlands (Sultana et al., 2014, 2015). Hence, based on the literature available on constructed wetland system for Cr(VI) removal by different plant species, the Cr(VI) concentration and HRT values were adopted as found suitable for this study. All these continuous experiments at the respective afore-mentioned conditions (HRTs and pH) were carried out for at least three consecutive steady state values, and results reported are average of three steady state values and duplicate sample analysis. The initial pH of the Cr(VI) containing influent was adjusted by HCl (2 N) or NaOH (2 N) or both.

2.3. T. pallida growth and Cr bioremoval

At the end of the continuous Cr(VI) removal experiments using the *T. pallida* based CW system, the plant growth was analysed in terms of its height, fresh weight, dry weight and chlorophyll contents. For measuring the plant height, measurements were taken from the root-stem intersection to the growing tip of the stem. Fresh weight and dry weight was measured according to the Standard Methods (Gowd and Govil, 2008), for which plants were air-dried and fresh weight was determined. To measure the dry weight, plants were dried at 80 °C for 32 h till constant weight was observed.

Water content of the plant was determined by measuring its relative water content (RWC), obtained using Eq. (1).

$$RWC = \frac{FW - DW}{FW} \qquad (1)$$

where FW is the fresh weight and DW is the dry weight of plant at the end of the experimental period.

Cr(VI) phytoextraction potential of *T. pallida* was measured by calculating its bioconcentration factor (BCF) and translocation factor (TF) at the end of the experimental period. BCF was calculated according to Eq. (2).

$$BCF = \frac{C_{Plant}}{C_{Medium}} \qquad (2)$$

where C_{Plant} and C_{Medium} are the Cr concentration in *T. pallida* (mg Cr/kg of *T. pallida* biomass) and in the medium, respectively.

Translocation factor (TFs/r) was expressed as the ratio of Cr(VI) concentration in the plant shoot and root biomass and was

calculated according to Eq. (3).

$$TFs/r = \frac{C_{Shoot}}{C_{Root}} \qquad (3)$$

where C_{Shoot} and C_{Root} are the concentration of Cr in *T. pallida* shoots (mg Cr/kg) and roots (mg Cr/kg), respectively.

Total chlorophyll content was measured on a FW basis according to the Standard Methods (Lichtenthaler, 1987).

2.4. Extraction and analysis of Cr(VI) and total Cr

For the extraction of Cr(VI) from soil, sand and gravel, the alkaline digestion method was followed (USEPA 3060 method). The samples (2.5 ± 0.10 g each) were air dried and digested with 50 ml of 0.28 M Na_2CO_3/0.5 M NaOH solution and heated at 95 °C for 60 min. For Cr(VI) determination in the effluent, samples (10 ml each) were neutralized with 25 ml extraction solution containing either 2% NaOH or 3% Na_2CO_3 and treated with 2 ml 0.1% KMnO4 and 0.75 ml 6 N H_2SO_4 to remove any reducing agents. The samples were then acidified with 7.8 ml of 6 N H_2SO_4 followed by addition of 2 ml of 0.25% 1, 5-diphenyl carbazide and making up the final volume to 100 mL. The solution was then mixed well and kept for 10 min. Cr(VI) content in the samples was finally analysed by measuring the absorbance of a colour complex formed at 540 nm using a UV visible spectrophotometer (Cary 100, Varian, Australia) (USEPA 7196 method) (Vitale et al., 1993).

For extraction of total Cr, acid digestion method was used. Soil, sand and gravel samples were air dried and digested with HCl:HNO₃:H₂SO₄ in the ratio 3:1:1 (v/v) at 95 °C on a hot plate. Plant parts were thoroughly rinsed with distilled water, oven-dried at 70 °C for 48 h and digested with HNO₃: HClO₄ in the ratio 3:1 at 110 °C for 1hr following the USEPA 3051 method (USEPA (United States Environmental Protection Agency), 1995). The digested extract was suitably diluted with de-ionized water and analysed by AAS spectrometry (AA240, Varian, the Netherlands) as per the Standard Methods. Cr(III) concentration was calculated by subtracting Cr(VI) concentration from the total Cr concentration.

3. Results

3.1. Effect of HRT on Cr(VI) removal

Fig. 2 presents influent and effluent concentrations of Cr(VI) for control and planted CW units at three different HRTs. During the initial phase of the experiments, the units were operated under three-day HRT. Thereafter, the HRT was reduced to two-day and then to one-day and operated continuously for the remaining period. At three-day HRT, the mean effluent value for Cr(VI) in the planted and control CW units operating at an influent concentration of 20 mg/L was found to be 0.27 and 0.34 mg/L, respectively, which is well below the recommended value of 0.5 mg/L. Upon reducing the HRT to two-day, the mean effluent value was found to
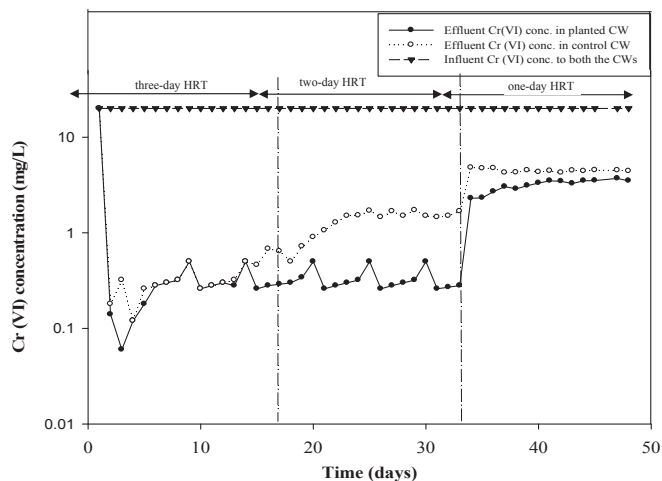
Fig. 2. Effect of different HRT on the Cr(VI) removal by *T. pallida* in control and planted CW units.

be 0.337 mg/L in the planted unit, and it increased to 1.23 mg/L in the control unit. Whereas, at one-day HRT, the Cr(VI) removal efficiency significantly decreased in both the units with mean effluent concentrations of 2.96 mg/L and 4.29 mg/L, in the planted and control CW units, respectively. Thus, planted unit showed 3 fold increase in Cr(VI) removal as compared to the control unit. These results also revealed that Cr(VI) removal efficiencies were very high and stable for two-day and three-day HRTs. Maximum Cr(VI) removal in the range 97.2–98.3% was achieved in the planted unit for two and three-day HRT. Least Cr(VI) removal in the range 73.24–77.9% was observed in the control unit operated at one-day HRT.

3.2. Effect of metal loading rate on Cr(VI) removal

From Fig. 2 it is apparent that the performance of the wetlands largely depends upon the influent flow rate or HRT in the wetlands. Therefore, the estimated values of Cr(VI) removal rate and Cr(VI)

removal efficiency were re-plotted versus Cr(VI) loading rate and the data is shown in Fig. 3. Almost 98% of Cr(VI) removal efficiency is observed for both 6.67 and 10 mg/L/day Cr(VI) loading rate. A further increase in the Cr(VI) loading rate (20 mg/L/day) greatly reduced the Cr(VI) removal efficiency to 88% in the case of the planted CW and to 77.8% in case of unplanted system. On the other hand, the Cr(VI) removal rate steadily increased with an increase in the Cr(VI) loading rate. In case of planted system, a maximum Cr(VI) removal rate of 16.5 mg/L/day was observed for a maximum Cr(VI) loading rate of 20 mg/L/day. For the same Cr(VI) loading rate, the control CW showed a removal rate of only 15.5 mg/L/day.

3.3. Effect of pH on Cr(VI) and total Cr removal

Fig. 4 shows the result of Cr(VI) and total Cr removal for two-day HRT in the CW units operated at pH 4 and 7, respectively. During the first 45 days of the experiments both the units were operated at an influent concentration of 20 mg/L each. Thereafter, the influent
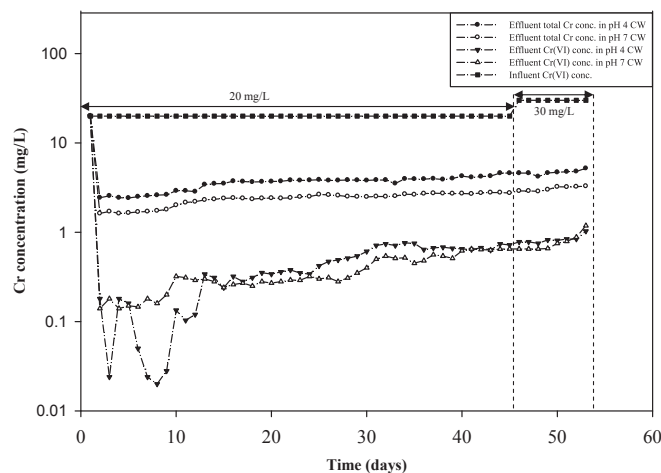

Fig. 4. Effect of influent pH on the Cr(VI) and total Cr removal by *T. pallida* in the respective CW units.
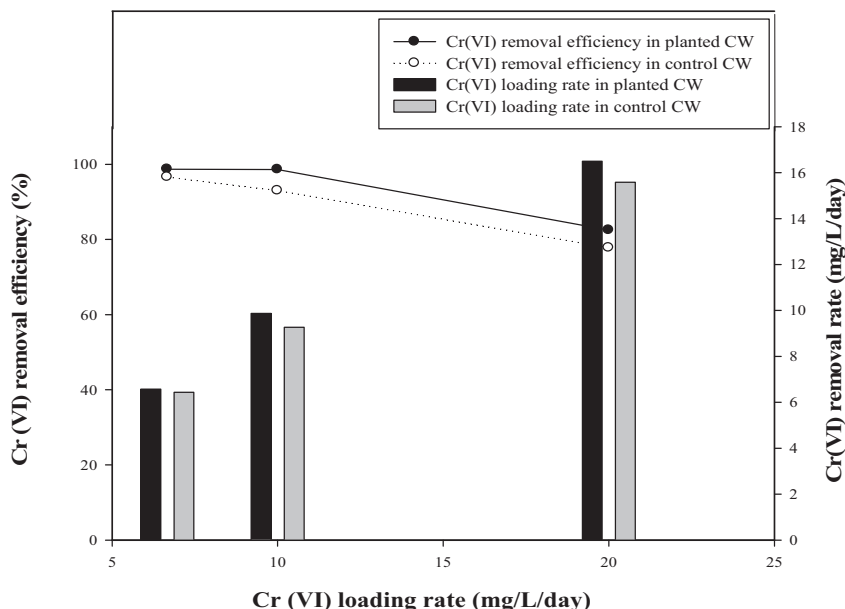

Fig. 3. Effect of Cr(VI) loading rate on Cr(VI) removal in control and planted system.

concentration was increased to 30 mg/L and operated continuously for the next seven days. For an influent pH of 4 and 7, the mean effluent Cr(VI) value was found to be 0.441 mg/L and 0.379 mg/L, respectively, at an influent concentration of 20 mg/L. For the same influent concentration, mean effluent value of total Cr was found to be 3.59 mg/L and 2.352 mg/L at pH 4 and 7, respectively. At an influent concentration of 30 mg/L, the mean effluent Cr(VI) value was found to be 0.907 mg/L and 0.896 mg/L at pH 4 and 7, respectively. For the same influent concentration, the mean effluent value of total Cr was 4.86 mg/L and 3.173 mg/L at pH 4 and 7, respectively. These results reveal that the maximum Cr(VI) removal efficiency is observed in the planted unit operated at an influent pH of 7, in which case maximum 97.2–98.3% Cr(VI) and 86.0–88.2% total Cr removal was achieved.

To determine the Cr(VI) reduction rate under pH 4 and pH 7 conditions in the soil of the CW units, amount of Cr(VI) and Cr(III) was determined at regular intervals. Fig. 5 shows percentage amount of Cr species vs. treatment time in respective CW units. Results show that initial pH of the influent has a significant effect on Cr(VI) reduction rate as the time required to achieve 50% reduction of Cr(VI) at pH 7 was very less as compared to that shown by the pH 4 CW unit. After the first 30 day of experimental period, percentage of Cr(III) in total Cr was significantly higher in the pH 7 CW (81.24%) as compared to that in the pH 4 CW (58.7%). Hence, Cr(VI) reduction rate is found to be faster in the pH 7 CW as compared to pH 4 CW. After 50 days of treatment time, complete Cr(VI) reduction occurred in both pH 7 and pH 4 CWs.

3.4. Cr accumulation and distribution in the CW

During the experiments carried out to study the effect of different HRT on Cr(VI) removal by the CW units (planted/Control), the total Cr(VI) mass loading into the system was 962.49 mg each. Fig. 6 presents the distribution of total Cr in each fraction shown as a percentage of total Cr loaded into the units over the entire experimental period. In general, a different distribution pattern of Cr was observed in the planted CW as compared with that in the control CW. These results revealed that the soil in these CW acts as the main sink for Cr, sequestering up to 40–68.41% of the total Cr supplied to the system. On the basis of the plant biomass produced and the bed constituent weight, the amount of total Cr accumulated by the whole plant (dry weight basis), soil, sand and gravel in the planted CW is estimated to be 55.68, 552.575, 242.73 and 46.7 mg, respectively. Whereas in the control CW, the Cr accumulated by the soil, sand and gravel is estimated to be 471, 194 and 48.23 mg respectively.
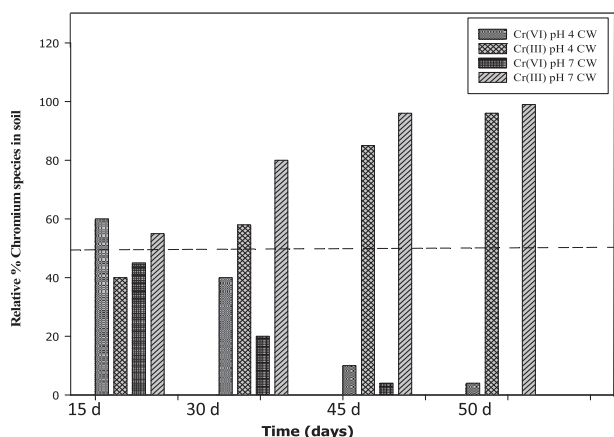

Fig. 5. Effect of influent pH on Cr(VI) reduction rate in the respective CW units.

During the experiments to study the effect of influent pH on Cr(VI) removal by the CW units (pH 4/pH 7), Cr(VI) mass loading in the system was 1785 mg. For the pH 4 CW unit, amount of total Cr accumulated by the whole plant (dry weight basis), soil, sand and gravel is estimated to be 35.69, 408.57, 292.73 and 46.7 mg respectively. Whereas in the case of pH 7 CW unit, Cr accumulated by the plant, soil, sand and gravel is found to be 61.32, 618, 264 and 45.23 mg, respectively. All these results clearly reveal that the *T. pallida* plant biomass played a highly significant role in Cr retention capacity in the soil fraction (62.7–68.41% of the total Cr inflow).

Total Cr accumulation in the plant parts made a small contribution to the mass balance, and soil was found to be the most important component in the system for Cr retention. In pH 7 CW unit, a high Cr accumulation was observed in the plant roots (754 μg Cr/g dry wt.) compared to that in the shoots and leaves (318 and 321 μg Cr/g dry wt.) of the plant.

Fig. 6 presents the percentage distribution of Cr in each of the CW units at the end of the study. Total Cr distribution in the wetlands revealed that nearly 3% of the total input Cr was bioaccumulated within the plant biomass, of which 69% was found in the plant roots, whereas 13% and 18% were sequestered within the plant shoots and leaves, respectively, 67–69% of total Cr was retained in the soil fraction, 16–20% were deposited within the sand bed and the remaining 6% may be attributed to volatilization effect, which needs to be confirmed further.

3.5. Effect on T. pallida growth

To analyze the effect of influent pH on Cr accumulation and plant growth, *T. pallida* plant's weight, height and chlorophyll content were determined at the end of experimental period (Table 2). As control for this study, a planted CW unit with only tap water (pH 5.6 and no Cr(VI)) was used as the influent. The values of BCF in the different CW units ranged from 16.45 to 23.21. Maximum Cr(VI) uptake was observed with the unit supplied with influent at pH 7 and BCF value was 23.21. TFs for roots to shoots ranged from 0.37 to 0.90 indicating that maximum Cr accumulated by *T. pallida* was mostly retained in the plant roots.

The Relative Water Content (RWC) of plants was determined and found to be significantly high in the CW unit receiving influent at pH 7 (77.44%) and pH 4 (73.9) as compared to the control CW unit (65.54). Total chlorophyll amount in the plants from the different CW units revealed that pH had no significant effect on this parameter. Furthermore, no toxicity in terms of growth rate and chlorophyll content was observed in the plants grown in the different CW units, suggesting a high tolerance of *T. pallida* for the phytoremediation of Cr(VI) at a wide pH range.

4. Discussion

T. pallida plant biomass based CW system showed a high percentage of Cr removal along with better retention capacity of Cr than the control CW. In this study, 97.2–98.3% Cr(VI) and 86–88.2% total Cr removal efficiency was achieved which is significant and comparable with Cr removal efficiency reported in the literature. For instance, CWs planted with *Leersia hexandra* Swartz showed a maximum removal efficiency of 99% for a low influent Cr(VI) concentration of 2.5 mg/L (Liu et al., 2014). In another study, 72% Cr removal was achieved with *Phragmites australis* planted CW, in which the bed consisted of gravel of different sizes (Fibbi et al., 2012). In the control CW without the plant biomass, adsorption, reduction and precipitation governed the major mechanisms for Cr removal from the influent. Whereas, in case of *T. pallida* plant based CW systems, both bioaccumulation and significantly enhanced rate
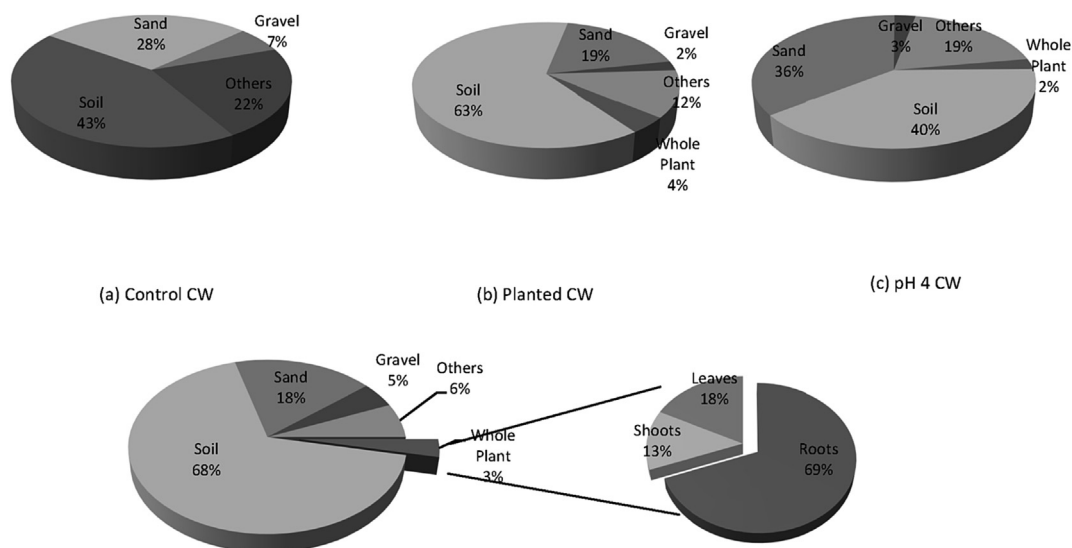
Fig. 6. Distribution of total Cr in the wetland systems (a) Control CW (b) Planted CW (c) pH 4 CW (d) pH 7 CW. The mass of Cr in each fraction is presented as a percentage of the total amount of Cr loaded into the wetland over the experimental period.

Table 2
Growth and bioremoval potential parameters of *T. pallida* plants from the different CW units at the end of the study.

CW	Fresh. Wt (g)	Dry. Wt (g)	RWC (%)	Height (cms)	Total chloro (mg/g)	BCF	TFs/r	TFr/soil
Control CW	24.6 ± 3.25	8.2 ± 0.91	65.83	35 ± 3.5	1.532	–	–	–
Planted CW	22.8 ± 2.74	6.1 ± 0.89	72.21	34 ± 4.30	1.484	20.92	0.425	1.3
pH 4 CW	23.0 ± 2.83	6 ± 0.75	73.9	32 ± 4.8	1.481	16.45	0.377	0.93
pH 7 CW	22.6 ± 2.30	5.1 ± 0.67	77.44	33 ± 6.3	1.4909	23.21	0.90	2.504

Results presented are average of three samples analysed with standard deviation ± SD (n = 3)
*RWC = relative water content, BCF = bioconcentration factor, TFs/r = translocation factor from root to shoot, TFr/soil = translocation factor from soil to root.

of adsorption coupled with reduction are attributed to the significantly high Cr removal observed in this study. As reported by many authors, plants are known to reduce Cr(VI) to Cr(III), which is a thermodynamically stable form of Cr in soil (Duarte et al., 2012; Rascio and Navari-Izzo, 2011). In a recent study, more than 90% of Cr(VI) was found to be in the form of Cr(III) state in the effluent water of all the CW systems planted with different plant species known for phytoremediation potential (Ranieri and Gikas, 2014). This Cr(VI) reduction is attributed to the plant roots which enhance aerobic condition in the soil, produces organic matter (El Zahar Haichar et al., 2014; DalCorso et al., 2013) and secretes root exudates containing carboxylic acids, citric acid, malic acid, carbohydrates and proteins necessary for Cr(VI) reduction and sequestration in the soil (UdDin et al., 2015). In addition, precipitation of the less soluble $Cr(OH)_3$ occurs, thereby aiding in its retention within the soil.

Fig. 2 revealed that Cr removal increased with HRT. It is evident from the results that even at a low HRT of two days, Cr removal from 20 mg/L initial Cr(VI) concentration, was always below the permissible discharge limit for total Cr and Cr(VI) (0.05 mg/L) (Förstner and Wittmann, 1979). Further at two-day HRT, least variation was observed throughout the experimental period in the planted system as compared to control. Hence, it could be well said that the *T. pallida* plant based CW systems is successful for achieving acceptable treatment results even at a low HRT. Probable reason for the high Cr removal is due to its rapid and initial biosorption in the wetland unit. It is well reported that Cr(VI) biosorption followed by its retention in the media (soil and sand) play a major role in such wetland systems for its removal. Cr distribution and retention results shown in Fig. 6 as well confirmed the high Cr

removal obtained using the CW system. Both, pH 4 and pH 7 CW units, showed significantly high removal of Cr(VI) from the influent. Whereas, total Cr removed was high in case of pH 7 CW unit indicating an increased Cr(VI) reduction efficiency and retention capacity than the pH 4 CW unit. This is mainly because of the fact that at neutral pH value (pH 7), Cr(III) is immobilized more effectively in the soil matrix as insoluble complexes than at acidic pH (pH 4). Cr(III) is reported to form insoluble $Cr(OH)_3$ in the pH range 6–9 (Ksp, 6.7×10^{-31}) that it remains tightly adsorbed to the soil. Further, Cr(III) remains relatively insoluble at the pH values (pH 6.95–7.59) and hence, gets retained in the soil (Barrera-Díaz et al., 2012).

In the pH 4 CW unit, the primary Cr(VI) removal mechanism is attributed to the adsorption of Cr(VI) ions. It is already reported that Cr(VI) adsorption increases at a faster rate as pH decreases. At low pH values, the hydronium ions surrounds the surface of soil matrix that strongly binds with the anionic Cr(VI) species (CrO_4^{2-}, $Cr_2O_7^{2-}$). Under acidic soil conditions, Cr(VI) reduction to Cr(III) is reported to occur in lesser proportion as compared with that under neutral or alkaline soil conditions (Gupta and Bhattacharyya, 2011).

Further, it is reported that in soil, low pH values result in higher K_d values for Cr (Cainelli and Cardillo, 2012). Hence, at acidic pH, Cr(VI) along with other metals such as Si and Al undergo leaching from soil and gets deposited in the sand forming a thick orange colored complex, as also observed in this study using the pH 4 CW unit.

Analysis of Cr distribution in the CW showed that in all the units, maximum retention was due to its adsorption and reduction in the soil which can be attributed to the high redox potential value of Cr(VI) (above +1.3 V at standard conditions) (Gu et al., 2012). The

basic process for Cr detoxification is the transformation of Cr(VI) to Cr(III). In this CW system almost all the Cr(VI) retained by the soil was reduced to Cr(III) that is less toxic and insoluble. Recently, a sufficient number of studies have been carried out by different researchers for the safe remediation/disposal of Cr(VI) contaminated soil and plants. In a recent study, Cr accumulated *T. pallida* plant biomass was found as an efficient biosorbent for Cr (Sinha et al., 2015). Also desorption of Cr from loaded biosorbent using a number of eluents such as 1% HNO$_3$, EDTA (0.1 M) and tap water (pH 9) has been successfully evaluated by many authors (Fathy et al., 2015). Many studies have reported enhanced Cr(VI) reduction process by amending the Cr loaded soil with indigenous microbes and genetically engineered microorganisms (Shao et al., 2014; Narayani and Shetty, 2013). An in situ bioremediation of Cr(VI) contaminated soil by supplementing with various carbon sources and organic amendments have also been reported to be an effective method (Krishna and Philip, 2005). In another study, an ex situ treatment of Cr(VI) contaminated soil was carried out using a bioreactor-biosorption system involving reduction, leaching and adsorption of Cr(III) (Bolan et al., 2013). Thus, by adopting any of these suggested methods, Cr loaded soil and plants from the constructed wetland system can be disposed off safely.

In plants, Cr uptake is directly related to Cr availability and mobility. Maximum BCF $_{soil/root}$ value was found at pH 7 since at near neutral pH values, Cr(VI) remains in soluble form and, thus, it is more bioavailable than at low pH values. The fact that accumulation was maximum in *T. pallida* roots could be attributed to this adsorption from the soil. It has been reported that plants not only help in Cr uptake but also play a major role in its reduction and retention within the soil on which they grow (Prado et al., 2010). In this study, results of Cr distribution in the constructed wetland revealed a high retention of Cr in the soil as compared with that in the control unit without any plants. Although Cr accumulation by the plant is less (2–4%), Cr removal in terms of μg removed per g of biomass is very high, i.e., 464 μg/g in comparison with the Cr uptake values reported in the literature. In pH 7 CW, Cr(VI) reduction increased with time (Fig. 4) suggesting the role of microbial community associated with the plant roots and soil in the CW, which needs to be confirmed further. Whereas in the pH 4 CW, adsorption and leaching of Cr occur at fast rate due to which Cr remains less available for plants and its microcosm to act.

Thus, these studies reveal that the reduction and retention of Cr in planted units were strongly pH dependent, being this parameter the most significant where acidic pH values of the influent favoured Cr(VI) adsorption and neutral pH of the influent enhanced both reduction and total Cr removal in the respective CWs.

The high Cr removal efficiency combined with extremely low construction and operation cost of *T. pallida* based CW system is an effective solution for treatment of Cr laden wastewater from tanneries, metallurgical, textile and other industries. This approach is mostly suited for developing countries where wastewater treatment is a major problem due to fast urbanization and industrialization. Moreover, it is an ecologically sustainable bioremediation method that can restore Cr-contaminated soil with no toxic sludge generation as against the other energy intensive physico-chemical treatment methods.

5. Conclusions

This study demonstrated that wastewater containing Cr(VI) can be treated using *T. pallida* plants based subsurface CWs under continuous operation mode. The planted CW unit showed better removal in terms of both Cr(VI) and total Cr removal as compared to the control CW unit. Further, this study showed the importance of pH of the influent which controls the Cr removal mechanism in the CW. The pH 7 CW removed Cr(VI) ions with 95–97% Cr(VI) removal efficiency at two-days HRT. In case of total Cr, high removal efficiency of 86–88.2% was achieved in pH 7 CW as compared to 78–80% removal in pH 4 CW. These results clearly revealed that *T. pallida* plants can effectively enhance the Cr(VI) reduction and retention in the soil, thereby serving as an efficient system for treating Cr contaminated water. The high Cr removal efficiencies combined with the extremely low construction and operation cost of *T. pallida* based CW offer an attractive treatment method for Cr(VI) removal.

References

Barrera-Díaz, C.E., Lugo-Lugo, V., Bilyeu, B., 2012. A review of chemical, electro-chemical and biological methods for aqueous Cr(VI) reduction. J. Hazard. Mater. 223, 1–12.

Bolan, N., Kunhikrishnan, A., Gibbs, J., 2013. Rhizoreduction of arsenate and chromate in Australian native grass, shrub and tree vegetation. Plant soil 367 (1–2), 615–625.

Cainelli, G., Cardillo, G., 2012. Chromium Oxidations in Organic Chemistry, vol. 19. Springer Science & Business Media.

Chandra, R., Bharagava, R.N., Yadav, S., Mohan, D., 2009. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. J. Hazard. Mater. 162 (2), 1514–1521.

Chyan, J.M., Lin, C.J., Lin, Y.C., Chou, Y.A., 2016. Improving removal performance of pollutants by artificial aeration and flow rectification in free water surface constructed wetland. Int. Biodeterior. Biodegrad. 113, 146–154.

DalCorso, G., Manara, A., Furini, A., 2013. An overview of heavy metal challenge in plants: from roots to shoots. Metallomics 5 (9), 1117–1132.

Dhal, B., Thatoi, H.N., Das, N.N., Pandey, B.D., 2013. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J. Hazard. Mater. 250, 272–291.

Duarte, B., Silva, V., Caçador, I., 2012. Hexavalent chromium reduction, uptake and oxidative biomarkers in Halimione portulacoides. Ecotoxicol. Environ. Saf. 83, 1–7.

El Zahar Haichar, F., Santaella, C., Heulin, T., Achouak, W., 2014. Root exudates mediated interactions belowground. Soil Biol. Biochem. 77, 69–80.

Fathy, N.A., El-Wakeel, S.T., El-Latif, R.R.A., 2015. Biosorption and desorption studies on chromium (VI) by novel biosorbents of raw rutin and rutin resin. J. Environ. Chem. Eng. 3 (2), 1137–1145.

Fibbi, D., Doumett, S., Lepri, L., Checchini, L., Gonnelli, C., Coppini, E., Del Bubba, M., 2012. Distribution and mass balance of hexavalent and trivalent chromium in a subsurface, horizontal flow (SF-h) constructed wetland operating as post-treatment of textile wastewater for water reuse. J. Hazard. Mater. 199, 209–216.

Förstner, U., Wittmann, G.T., 1979. Metal Pollution in the Aquatic Environment. Springer Springer-Verlag, Berlin Heidelberg New York.

Ge, Z., Feng, C., Wang, X., Zhang, J., 2016. Seasonal applicability of three vegetation constructed floating treatment wetlands for nutrient removal and harvesting strategy in urban stormwater retention ponds. Int. Biodeterior. Biodegrad. 112, 80–87.

Gowd, S.S., Govil, P.K., 2008. Distribution of heavy metals in surface water of Ranipet industrial area in Tamil Nadu, India. Environ. Monit. Assess. 136 (1–3), 197–207.

Gu, H., Rapole, S.B., Sharma, J., Huang, Y., Cao, D., Colorado, H.A., Luo, Z., Haldolaarachchige, N., Young, D.P., Walters, B., Wei, S., Wei, S., 2012. Magnetic polyaniline nanocomposites toward toxic hexavalent chromium removal. RSC Adv. 2 (29), 11007–11018.

Gupta, S.S., Bhattacharyya, K.G., 2011. Kinetics of adsorption of metal ions on inorganic materials: a review. Adv. Colloid Interface Sci. 162 (1), 39–58.

Jin, W., Du, H., Zheng, S., Zhang, Y., 2016. Electrochemical processes for the environmental remediation of toxic Cr (VI): a review. Electrochimica Acta 191, 1044–1055.

Krishna, K.R., Philip, L., 2005. Bioremediation of Cr(VI) in contaminated soils. J. Hazard. Mater. 121 (1), 109–117.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzym. 148, 350–382.

Liu, J., Zhang, X.H., You, S.H., Wu, Q.X., Chen, S.M., Zhou, K.N., 2014. Cr(VI) removal and detoxification in constructed wetlands planted with *Leersia hexandra* Swartz. Ecol. Eng. 71, 36–40.

Lofrano, G., Meriç, S., Zengin, G.E., Orhon, D., 2013. Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: a review. Sci. Total Environ. 461, 265–281.

Narayani, M., Shetty, K.V., 2013. Chromium-resistant bacteria and their environmental condition for hexavalent chromium removal: a review. Crit. Rev. Environ. Sci. Technol. 43 (9), 955–1009.

Paiva, É.A.S., Isaias, R.M.D.S., Vale, F.H.A., Queiroz, C.G.D.S., 2003. The influence of light intensity on anatomical structure and pigment contents of *Tradescantia pallida* (Rose) Hunt. cv. purpurea Boom (Commelinaceae) leaves. Braz. Archives Biol. Technol. 46 (4), 617–624.

Prado, C., Rodríguez-Montelongo, L., González, J.A., Pagano, E.A., Hilal, M.,

Prado, F.E., 2010. Uptake of chromium by Salvinia minima: effect on plant growth, leaf respiration and carbohydrate metabolism. J. Hazard. Mater. 177 (1), 546—553.

Ranieri, E., Gikas, P., 2014. Effects of plants for reduction and removal of hexavalent chromium from a contaminated soil. Water, Air, & Soil Pollut. 225 (6), 1—9.

Rascio, N., Navari-Izzo, F., 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci. 180 (2), 169—181.

Shao, Y., Pei, H., Hu, W., Chanway, C.P., Meng, P., Ji, Y., Li, Z., 2014. Bioaugmentation in lab scale constructed wetland microcosms for treating polluted river water and domestic wastewater in northern China. Int. Biodeterior. Biodegrad. 95, 151—159.

Sharma, S., Adholeya, A., 2011. Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi. Int. Biodeterior. Biodegrad. 65 (2), 309—317.

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. Evaluation of Cr(VI) exposed and unexposed plant parts of *Tradescantia pallida* (rose) DR Hunt. For Cr removal from wastewater by biosorption. Int. J. phytoremediation 17 (12), 1204—1211.

Sultana, M.Y., Akratos, C.S., Pavlou, S., Vayenas, D.V., 2014. Chromium removal in constructed wetlands: a review. Int. Biodeterior. Biodegrad. 96, 181—190.

Sultana, M.Y., Chowdhury, A.K.M.M.B., Michailides, M.K., Akratos, C.S., Tekerlekopoulou, A.G., Vayenas, D.V., 2015. Integrated Cr(VI) removal using constructed wetlands and composting. J. Hazard. Mater. 281, 106—113.

UdDin, I., Bano, A., Masood, S., 2015. Chromium toxicity tolerance of Solanum nigrum L. and Parthenium hysterophorus L. plants with reference to ion pattern, antioxidation activity and root exudation. Ecotoxicol. Environ. Saf. 113, 271—278.

USEPA (United States Environmental Protection Agency), 1995. Method 3051: microwave assisted acid digestion of sediments, sludges, soils, and oils. Test Methods Eval. Solid Waste.

Vitale, R., Mussoline, G., Petura, J., James, B., 1993. A Method Evaluation Study of an Alkaline Digestion (Modified Method 3060) Followed by Colorimetric Determination (Method 7196) for the Analysis for Hexavalent Chromium in Solid Matrices. Environmental Standards, Inc., Valley Forge, PA, p. 19482.

WHO, 1993. Guidelines for Drinking-water Quality. World Health Organization, Geneva.

Wu, H., Fan, J., Zhang, J., Ngo, H.H., Guo, W., Hu, Z., Lv, J., 2016. Optimization of organics and nitrogen removal in intermittently aerated vertical flow constructed wetlands: Effects of aeration time and aeration rate. Int. Biodeterior. Biodegrad. 113, 139—145.