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ORIGINAL PAPER

Hexavalent chromium accumulation kinetics and physiological responses exhibited by *Eichhornia* sp. and *Pistia* sp.

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Abstract
In the present study, hexavalent chromium (5, 10 and 30 mg/L) phytoaccumulation by two free floating macrophytes, *Eichhornia* sp. and *Pistia* sp., was investigated in a greenhouse. The results revealed higher accumulation of chromium by *Eichhornia* sp. at 30 mg/L Cr solution. However, *Pistia* sp. showed highest accumulation at intermediate chromium solution of 10 mg/L. Pigment data indicated higher reduction of chlorophyll for *Pistia* sp. compared to *Eichhornia* sp. Both the tested species showed gradual reduction of both chlorophyll-a and chlorophyll-b significantly with increasing metal concentration from 5 to 30 mg/L. However, chlorophyll stability index data indicated higher chlorophyll stability index at higher Cr concentrations in case of both the macrophytes. On the other hand, lipid peroxidation in the form of malondialdehyde concentration was observed to increase with increase in chromium load for both the tested species. Almost similar results were recorded in the enzyme analysis data. Study results revealed that all the studied enzymes are highly sensitive toward chromium. However, catalase activity showed the highest sensitivity. Chromium bioaccumulation kinetics study revealed that only *Pistia* sp. is more suited with pseudo-first-order (0.910) and pseudo-second-order (0.665) kinetics equation compared to *Eichhornia* sp. New root development was observed only for *Eichhornia* sp. during the third day of incubation. The wet biomass of both the macrophytes showed gradual reduction in chromium solutions of increasing concentrations. Therefore, it may be concluded that *Eichhornia* sp. and *Pistia* sp. may be effectively used in remediation of Cr(VI) contaminated aquatic bodies.

Keywords Chromium · Phytoremediation · Macrophytes · Antioxidant enzyme · Accumulation kinetics

Introduction

Heavy metals have density equal to or higher than 5 g/cm^3 (Nies 1999). Different heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr) are constantly discharged into the environment through industrial and anthropogenic activities (RoyChowdhury et al. 2017). All the heavy metals at higher concentration levels have negative impact on the plant community (Fan et al. 2017). Among the various heavy metals, chromium has been specially studied by toxicologists (Medda and Mondal 2017). Chromium enters the environment through two main paths, as natural ferrochromite ($Fe_2Cr_2O_4$) and in the form of other minerals in the earth's crust. It mainly exists in two stable oxidation states: Cr(III) and Cr(VI) (Gil-Cardeza et al. 2014). Cr(VI), mainly originated from anthropogenic sources, is found in water as $HCrO_4^-$, $Cr_2O_7^{2-}$ and CrO_4^{2-}, which are highly mobile anions. The dominant forms of Cr(III) are mainly cationic $CrOH^{2+}$aq, $Cr(OH)^{2-}$aq or neutral $Cr(OH)_3$. Previous data suggest that Cr(III) is less toxic and less absorbed by the plants than Cr(VI) (Dhal et al. 2013). On the other hand, the hexavalent chromium is considered as a potent mutagenic and teratogenic metal due to its high penetration power in the cell membranes and subsequent interaction with protein and nucleic acid (Mishra and Bharagava 2016). Also, hexavalent chromium has strong oxidizing capacity at lower pH (Kota and Stasicka 2000).

Heavy metal stress always leads to the generation of reactive oxygen species (ROS) which are detrimental to all plants (Singh et al. 2017). Lipid peroxidation is another major consequence of heavy metal stress in plants (Goswami and Das 2016). Every plant system has auto scavenging

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of ROS which protects the plants from oxidative damage (Singh et al. 2017). The antioxidant enzymes are catalase (CAT), peroxidase (POD) and ascorbic acid oxidase (AAO), which play an important role in scavenging reactive oxygen species (Rusina et al. 2004). Every antioxidant has a distinct function toward detoxification of ROS such as CAT and POD which can reduce H_2O_2 to water and oxygen (Sharma and Dietz 2009). Similarly ascorbic acid oxidase enzyme catalyzes the oxidation of ascorbic acid to dehydroascorbic acid (Shimada and Ko 2008).

Therefore, it is of utmost importance to control and check the introduction of Cr(VI) into the environment. The only possible way is through the development of remediation strategies (Gil-Cardeza et al. 2014). The previous literature highlighted one common strategy for reduction of toxicity through conversion of Cr(VI) to Cr(III) (Sagar et al. 2012). Amin et al. (2015) and Sheoran and Sheoran (2006) suggested that some specific mechanisms that restrict the mobility of element like Cr in plants body and subsequently protect the plants from toxicity. It is also documented that some aquatic plants can hyperaccumulate the metals in their roots in comparison with other parts (Yabanli et al. 2014). However, traditional management techniques are also available such as chemical precipitation, reverse osmosis, coagulation, adsorption, reverse osmosis. But, all these methods have serious limitations and may also not be economically viable or environment friendly. To overcome these difficulties, a very popular method called phytoremediation has been introduced (Rahman and Hasegawa 2011). Phytoremediation is a green technology which is absolutely plant based (Torok and Dransfield 2017; Thijs et al. 2017). The different types of phytoremediation are phytoextraction, phytostabilization, phytovolatilization, phyto-transformation and rhizofiltration (Vamerali et al. 2010). Among these, phytoextraction is a technique in which plants take up pollutants from the soil or aquatic bodies and store them in their body parts including vacuoles with the help of phytochelators (Kontoghiorghe et al. 2015).

Macrophytes are vegetative and photosynthetic organisms and can comfortably grow in the water bodies. They have an important role in the aquatic ecosystem owing to their capability to act as phytoremediating agents through accumulation of both inorganic and organic pollutants (Lin et al. 2018; Wang et al. 2018).They are found throughout the world and constitute an important constituent of wetlands. Very recently Fariasa et al. (2018) highlighted that macrophytes can acts as bioindicators of heavy metal pollution. Different varieties of macrophytes are available in our locality such as *Eichhornia crassipes*, *Lemna minor* L., *Pistia stratiotes* L. Among them, some are emergent, floating-leaved, and some submerged, and free floating. Out of the different varieties of macrophytes, water hyacinth (*Eichhornia crassipes*) is the most noxious aquatic vegetative organism because of its very fast growth rate, high pollution resistance and enormous nutrient absorption capacity (Swarnalatha and Radhakrishnan 2015). *Pistia stratiotes* is another abundant macrophyte, also known as water cabbage or water lettuce, etc. This macrophyte has enormous capability toward absorption of heavy metals from contaminated aquatic bodies (Vesely et al. 2011).

The present study has highlighted the potentiality of *Eichhornia* sp. and *Pistia* sp. toward phytoremediation of hexavalent chromium with variation of incubation time. Additionally, kinetics of Cr(VI) accumulation, growth parameters and biochemical analysis including enzyme studies have been undertaken. The present study was conducted at the Department of Environmental Science (both inside (laboratory) and outside (at the greenhouse) during the period from November to February 2017.

Materials and methods

Plant material and experimental design

The aquatic macrophytes used for the removal of Chromium from synthetic medium were *Eichhornia* sp. and *Pistia* sp. The aquatic macrophytes, with an average weight of 20 g, were collected from a stagnant water body in Golapbag, Burdwan University Campus (87°50′ 53.71″ E and 23°15′ 19.68″ N), and washed thoroughly with distilled water to remove the particles adhering to the plants. All the macrophytes were sterilized according to Tiwari et al. (2012) and maintained as stock cultures on Pirson–Seidel nutrient medium (Pirson and Seidel 1950). Each treatment was run in triplicate. pH of all the treatment sets was adjusted to 6.5 ± 0.1 throughout the entire incubation period. All the treatment sets were incubated under a 15-h light/9-h dark photoperiod (1500LX) using light-emitting diodes (LEDs) (2 red and 1 blue).

Stock chromium solution

Stock of chromium solution was prepared by dissolving 0.283 g anhydrous potassium dichromate ($K_2Cr_2O_7$) in distilled water and diluted to 1000 mL. The intermediate concentration was prepared by proper dilution method. The pH of the medium was adjusted by using 1(N) HCl and 1(N) NaOH.

Standard chromium solution

From stock chromium solution, 100 mL was taken and diluted to 1000 mL with distilled water.

Growth parameters

According to the ISO 20079 test protocol (2004), the growth of *Eichhornia* sp. and *Pistia* sp's was determined measuring new root growth, root length, fresh weight (FW) and dry weight (DW). Plants were surface-dried between layers of paper towels and then dried at 80 °C (Cedergreen et al. 2007) up to constant weight. Relative growth rate (RGR) was calculated from the following equation with the measured parameter × (FW) and the start of the test (t_0) for each replicate separately: $\mathrm{RGR} = \frac{(\ln x_{t_1} - \ln x_{t_o})}{t_1 - t_o}$.

Dry-to-fresh weight ratio (DW/FW) was determined according to calculation: dry weight (g)/fresh weight (g).

Biochemical parameters

Photosynthetic pigment

Estimation of total chlorophyll and carotenoids content from plant material (Arnon 1949). For the estimation of chlorophyll content, Arnon's (1949) method was employed. Chlorophyll is extracted in 80% acetone, and the absorption at 645, 652 and 663 nm is read in spectrophotometer (PerkinElmer Lambda 35).

Using the absorption coefficient, the amount of chlorophyll is calculated.

Chlorophyll Stability Index (CSI)

The CSI was calculated in stressed and control plants according to the following formula (Sairam et al. 2008).

$$\mathrm{CSI}(\%) = \frac{\text{Total Chl}' \text{ in metal stressed plant}}{\text{Total Chl}' \text{ in control plant}} \times 100$$

Enzyme antioxidants and protein content

After 10 days of incubation, three antioxidant enzymes including catalase (CAT) (EC 1.11.1.6), peroxidase (POD) (EC 1.11.1.7) and ascorbic acid oxidase (AAO) (EC 1.10.3.3) and total protein in leaves were evaluated by using spectrophotometer (PerkinElmer Lambda 35). The fresh leaves along with 0.05 M phosphate buffer were ground in a mortar and pestle and filtered through four layers of muslin cloth followed by centrifugation at 12,000 rpm for 10 min in cold centrifuge (REMI C24). Finally, this extract was used for estimation of CAT, POD and AAO according to Zhang (1992). Estimation of total protein was done by following the method of Lowry et al. (1951) using bovine serum albumin as the standard protein, and the results were expressed as mg g^{-1} F.W.

Lipid Peroxidation content

The level of lipid peroxidation was measured on the basis of malondialdehyde (MDA) formation as demonstrated by Heath and Packer (1968). Plant material (0.5 g) was ground with 5 mL (0.1%) trichloroacetic acid (TCA) and centrifuged at 11,000 rpm for 5 min. After centrifugation, 1 mL of supernatant liquid was mixed with 4 mL (0.5%) thiobarbituric acid (TBA) which was prepared with 20% TCA. The entire mixture was then heated at 95 °C for 30 min and quickly frozen by placing the mixture container in ice bath followed by centrifugation (Remi R24) at 11,000 rpm for 15 min. The absorbance at 532 and 600 nm was measured using UV–Vis spectrophotometer (PerkinElmer Lambda 35).

Percentage of metal accumulation and accumulation capacity

The culture mediums were centrifuged at 5000 rpm for 10 min at room temperature at different time intervals (1–10 days) followed by filtration through cellulose acetate filter (0.45 µm), and the filtrate was used for estimation of total Cr(VI) by atomic absorption spectrometry (AAS, Agilent). The percentage of Cr(VI) removal was calculated by the following formula (Zhou et al. 2012):

$$\mathrm{Removal}(\%) = \frac{(C_0 - C_f)}{C_0} \times 100$$

where C_0 and C_f are initial and final Cr(VI) concentration (mg/L), respectively. The uptake capacities of the two macrophytes were also measured by applying the following equation (Yang et al. 2015):

$$q(\mathrm{mg/g}) = \frac{(C_0 - C_f)}{M} \times V$$

where V is volume of the experimental solution and M is the weight (g) of macrophyte at the end of the experiment.

Modeling

The modeling procedure of chromium ion bioaccumulation data was based on the chromium mass balance where the relation between the biomass growth and the metal concentration reduction from the hydroponic media against time was quantified. Chromium ion bioaccumulation from hydroponic media by using living aquatic macrophytes *Eichhornia* sp. and *Pistia* sp. has been described by applying four non-structural kinetic models (Mondal et al. 2014), and the accumulated metal, maximum capacity and rate constants of bioaccumulation process were estimated. These adsorption models resemble the Langmuir-type irreversible, pseudo-first and second

interaction models, respectively. It is a great challenge from a mathematical point of view to consider the mass transfer mechanisms involved in the heavy metal removal process by living macrophytes. The mechanistically classical models of enzymatic and adsorption kinetics have been used. Hence, we have followed the non-living biosorbent action-based adsorption kinetic classical modeling.

$$r(t) = k_1\left[q_{\max} - q(t)\right]C(t) \quad (1)$$

$$r(t) = k_2\left[q_{\max} - q(t)\right] \quad (2)$$

$$r(t) = k_3\left[q_{\max} - q(t)^2\right] \quad (3)$$

where C (in mg Cr L^{-1}) is the chromium concentration in the liquid phase in time; r (in mg Cr g^{-1} day^{-1}) is the chromium bioaccumulation action rate by aquatic plant; q (in mg Cr g^{-1}) is the macrophyte-accumulated chromium concentration; q_{max} (in mg Cr g^{-1}) is the macrophyte-accumulated maximum chromium content constant; k_1 (in l mg^{-1} Cr day^{-1}), k_2 (in day^{-1}) and k_3 (in g mg^{-1} Cr day^{-1}) are the bioaccumulation rate constants; and k_b (in day^{-1}) is the chromium desorption rate constant only for irreversible kinetic model.

Quality control study

Both precision and accuracy of analysis can be achieved through repeated measurement by following the standard method (SRM 1570) for heavy metals. These results were recorded ±2% of the certified value. The instrument was calibrated after fine determination/readings. Overall quality control measures were taken to assess the contamination level and reliability of data.

Statistical Analysis

All the replicated data were analyzed statistically by one-way ANOVA and Duncan's multiple range test (DMRT). For statistical interpretation of the observed tabulated data, Panse and Sukhatme (1967), together with Gomez and Gomez (1984), were consulted.

Results and discussion

Cr uptake modeling

The Cr(VI) accumulation data clearly indicate that gradual reduction of accumulation with increasing incubation time for *Eichhornia* sp. (Fig. 1a). However, almost an opposite trend was recorded for *Pistia* sp. especially with higher incubation time (Fig. 1b). The chromium bioaccumulation from a nutrient medium by living aquatic macrophytes *Eichhornia* sp. and *Pistia* sp. has been described by three non-structural kinetics model, but instead of the adsorb rate determination, the accumulated metal maximum capacity and rate constant of bioaccumulation were estimated. These models resemble the Langmuir-type irreversible, pseudo-first-order and pseudo-second-order models, respectively. Indeed, there are several mass transfer mechanisms involved in the heavy metal removal process by living microorganisms, which cause great difficulties for the modeling of the whole process (Mishra and Tripathi 2009).

A mechanistically classical model of enzymatic and adsorption kinetics has been used, in order to represent the heavy metal bioaccumulation kinetic for living organism. In this work, the non-living biosorbent action-based adsorption kinetic classical modeling was followed (Eqs. 1–3).

The entire rate constants and goodness of fit value are presented in Table 1. From Table 1 it is clear that only *Pistia* sp. showed very good fitness with both pseudo-first-order ($p < 0.01$) and pseudo-second-order ($p < 0.05$) kinetic equation with very high R^2 values at lower (5 mg/L) and

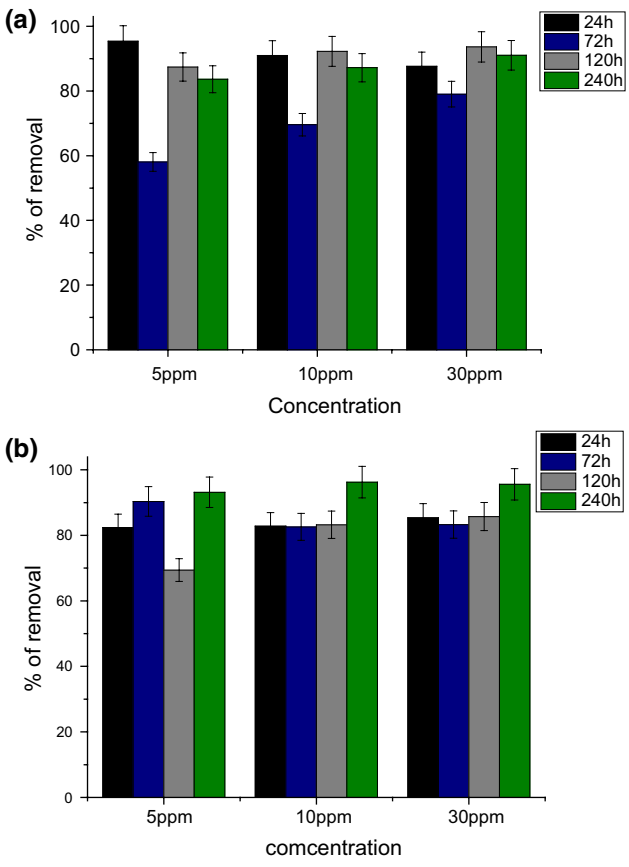

Fig. 1 Percent removal of Cr by **a** *Eichhornia* sp. and **b** *Pistia* sp. under different concentration of Cr

higher (30 mg/L) concentration of Cr(VI), respectively. On the other hand, none of the macrophyte showed good fitness with irreversible kinetic equation. Almost similar lead (Pb) accumulation by living aquatic macrophyte, *Salvinia auriculata,* was reported by Espinoza-Quiñones et al. (2009). They also stated that the non-structural kinetic models have shown good agreement with the lead uptake experimental data in all the investigated cases. The aquatic macrophytes have exceptional potentiality toward accumulation of heavy metals in their body parts (Reznia et al. 2016). It was reported that they can accumulate up to 1,00,000 times greater than the amount of associated aqueous substance (Muthusaravanan et al. 2018). Aquatic macrophyte can exhibit their metal accumulation mechanism through phytoaccumulation (Kamal et al. 2004), phytostabilization (Berti and Cunningham 2000), phytodegradation (Newman and Reynolds 2004), phytovolatilization (Zayed et al. 2000).

Growth and development

Biomass of plant is a valuable tool for characterizing the growth performance of heavy metal stressed plants (Malik et al. 2019). Growth patterns of the two studied macrophytes were understood from the wet biomass of the macrophytes. Study results revealed that wet biomass of *Eichhornia sp.* decreased with increase in number of days of incubation with 5 mg/L chromium solution. However, at 10 mg/L, wet biomass increased linearly up to 5 days of incubation (Table 2). Similar incremental picture of biomass was recorded with 30 mg/L chromium solution, but after third day of incubation, wet biomass showed almost constant value (Table 2). The shunted growth perhaps due to chromium interference in aquaporins or by altering the membrane permeability (Malar et al. 2015) or due to the impairment in cell division (Vecchia et al. 2005). On the other hand, *Pistia* sp. does not show any biomass growth in all the studied solution concentrations (Table 3). Several polynomial type functions and segment curves in different growing phase were recorded (figure not provided). Therefore, from this phytoremediation study, it may be easily concluded that *Eichhornia* sp. is a better for chromium accumulator than *Pistia* sp. Again from root growth data, it is clear that none of the concentration of chromium is suitable for the root growth of *Eichhornia* sp. except concentration 5.0 mg/L. Almost similar results were recorded for *Pistia* sp. also. Heavy metals have remarkable effects on root length of plants and it is well documented by the earlier researchers (Dey and Mondal 2016; Tang et al. 2001). Prasad et al. (2001) highlighted in their research that 88.78% reduction in biomass was achieved at 5 mg/L Cr solution for *Eichhornia* sp. Similar reduction in biomass was also reported by Chen et al. (2001), and they demonstrated that root weight

Table 1 Kinetics of Cr(VI) uptake by *Eichhornia* sp. and *Pistia* sp

Species	Kinetics order	Concentration (mg/L)	Rate constant	Regression	R^2	Level of significance
Eichhornia sp.	Irreversible	5	K_1	$Y=0.4013x+3.9748$	0.018	
		10		$Y=-0.108x+4.969$	0.001	
		30		$Y=1.854x+0.819$	0.466	
Pistia sp.		5		$Y=5.0533x-7.5398$	0.4372	
		10		$Y=1.5481+0.1135$	0.2652	
		30		$Y=-3.328+13.487$	0.0993	
Eichhornia sp.	Pseudo-first order	5	K_2	$Y=17.668x-22.763$	0.005	
		10		$Y=5.6476-x4.306$	0.4177	
		30		$Y=3500.0x-6356.5$	0.2737	
Pistia sp.		5		$Y=2.1812x-1.2862$	**0.910**	$p<0.01$
		10		$Y=5.261x-2.133$	0.658	
		30		$Y=-0.884x-6.228$	0.001	
Eichhornia sp.	Pseudo-second order	5	K_3	$Y=-6.3095x+6.6192$	0.0449	
		10		$Y=-40.047x+9.5657$	0.0302	
		30		$Y=-614.46x+28.253$	0.1751	
Pistia sp.		5		$Y=-64.602x+21.191$	0.2635	
		10		$Y=-168.59x+25.318$	0.4408	
		30		$Y=-1323.5x+55.706$	**0.6655**	$p<0.05$

Bold values indicate that the values are significant

Table 2 pH, fresh and dry biomass (±SD) of *Eichhornia* sp. after each collection time

Time (h)	pH			Wet biomass(g)			Dry biomass(g)		
	5 (mg/L)	10 (mg/L)	30 (mg/L)	5 (mg/L)	10 (mg/L)	30 (mg/L)	5 (mg/L)	10 (mg/L)	30 (mg/L)
24	$6.53^{d} \pm 0.24$	$6.50^{d} \pm 0.11$	$6.44^{c} \pm 0.22$	$25.1^{a} \pm 1.02$	$19.5^{d} \pm 0.04$	$16.1^{d} \pm 0.13$	$0.23^{d} \pm 0.01$	$0.90^{c} \pm 0.02$	$3.71^{b} \pm 0.01$
72	$6.97^{c} \pm 0.23$	$6.74^{c} \pm 0.34$	$6.32^{d} \pm 0.18$	$18.2^{c} \pm 1.2$	$21.2^{c} \pm 0.22$	$21.2^{a} \pm 0.24$	$2.1^{a} \pm 0.03$	$3.04^{a} \pm 0.11$	$6.29^{a} \pm 0.11$
120	$7.60^{ab} \pm 0.102$	$7.45^{b} \pm 0.05$	$7.47^{b} \pm 0.01$	$22.23^{b} \pm 0.65$	$28.6^{a} \pm 0.31$	$20.1^{b} \pm 0.03$	$0.65^{c} \pm 0.04$	$0.78^{d} \pm 0.04$	$1.91^{d} \pm 0.03$
240	$7.82^{a} \pm 0.33$	$7.86^{a} \pm 0.14$	$7.81^{a} \pm 0.13$	$16.5^{d} \pm 0.09$	$23.9^{b} \pm 0.25$	$19.2^{c} \pm 0.05$	$0.82^{b} \pm 0.01$	$1.28^{b} \pm 0.16$	$2.69^{c} \pm 0.22$

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

Table 3 pH, fresh and dry biomass (±SD) of *Pistia* sp. after each collection time

Time (h)	pH			Wet biomass(g)			Dry biomass(g)		
	5 (mg/L)	10 (mg/L)	30 (mg/L)	5 (mg/L)	10 (mg/L)	30 (mg/L)	5 (mg/L)	10 (mg/L)	30 (mg/L)
24	$3.00^{d} \pm 0.02$	$3.66^{d} \pm 0.22$	$4.73^{d} \pm 0.03$	$3.5^{a} \pm 0.03$	$1.94^{a} \pm 0.22$	$3.5^{ab} \pm 1.01$	$0.98^{b} \pm 0.05$	$1.72^{ab} \pm 0.07$	$4.38^{b} \pm 0.02$
72	$8.1^{ab} \pm 0.44$	$7.9^{ab} \pm 0.25$	$6.64^{b} \pm 0.11$	$2.41^{bc} \pm 0.09$	$1.43^{b} \pm 0.21$	$3.54^{a} \pm 1.1$	$0.48^{c} \pm 0.01$	$1.74^{a} \pm 0.11$	$5.01^{a} \pm 1.33$
120	$7.15^{c} \pm 0.31$	$6.99^{c} \pm 1.02$	$6.48^{bc} \pm 0.25$	$2.52^{b} \pm 0.01$	$1.24^{c} \pm 0.04$	$2.5^{c} \pm 0.05$	$1.53^{a} \pm 0.22$	$1.68^{c} \pm 0.06$	$4.28^{bc} \pm 0.09$
240	$8.47^{a} \pm 0.33$	$8.01^{a} \pm 1.11$	$7.23^{a} \pm 1.34$	$0.42^{d} \pm 0.01$	$0.33^{d} \pm 0.08$	$2.05^{cd} \pm 0.06$	$0.34^{d} \pm 0.04$	$0.39^{d} \pm 0.01$	$1.32^{d} \pm 0.11$

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

and root length of wheat were affected by a concentration of 20 mg Cr (VI) kg^{-1} soil as $K_2Cr_2O_7$. Currently, Malik et al. (2019) also supported that heavy metal can prevent the overall growth and development of plants through cellular damage.

Observation of medium pH

Throughout the incubation period of 1–10 days, the pH of the hydroponic medium was measured and the results are depicted in Tables 2 and 3. From Table 2, it is found that for *Eichhornia* sp. at 5 mg/L Cr solution, pH ranges from 6.53 to 7.82. Almost similar incremental patterns were recorded for other treatments also with slight deviation at 30 mg/L. On the other hand, *Pistia* sp. showed much lower pH during first day of incubation. However, after third day of incubation, there is remarkable change in pH (Table 3).

Enzyme study

The main toxicity of heavy metal is due to the production of reactive oxygen species which may causes oxidative stress in plants and also hamper the grain yield (Shahid et al. 2014). To overcome such stress conditions, plants inherently developed innate enzymatic defense mechanism (Hassanein et al. 2012) through generation of enzymes and subsequently control the toxic effects of free radicals (Chen et al. 2015). In the present study, it has been found that the activity of antioxidant enzymes of peroxidase, catalase and ascorbate increases with increase in incubation time period for all the studied concentration of *Eichhornia* sp. However, enzymatic activity of *Pistia* sp. initially decreased from first to third day of incubation, but after that increased at 5 days of inoculation and again decreased at 10 days of incubation (Table 4). The variations of peroxidase (EC 1.11.1.7) activity showed almost similar trends as catalase (EC 1.11.1.6) for *Eichhornia* sp., but *Pistia* sp. showed initial increment up to 5 days of incubation followed by decrease at 10 days of incubation except for 5 mg/L or solution (Table 4). On the other hand, ascorbate (EC 1.11.1.4) concentration did not show any such decremental trend with metal concentrations and days of incubation. For both *Eichhornia* sp. and *Pistia* sp., the activity of antioxidant (peroxidase and catalase) remarkably varied in all concentration of Cr(VI) salt, which is probably due to generation of ROS inside the plant (Tauqeer et al. 2016). This over expression of antioxidant enzyme will act as a valuable indicator for the survival of heavy metal accumulator plants (Hibiba et al. 2015).

Therefore, this enzymatic variation of plants under the influence of heavy metal stress may be a good marker of metal pollution in aquatic bodies (Hibiba et al. 2015). The fluctuation is the result of changes in lipid peroxidation (Sarwar et al. 2017). MDA content increases with increasing concentration of Cr VI salt solution at 5 mg/L. But with increasing incubation time, no such trend was recorded for

Table 4 Variation of lipid peroxidation and enzyme (catalase, peroxidation and ascorbate oxidase) of Eichhornia sp. and Pistia sp. under Cr stress

Species	Conc (mg/L)	MDA (μg g^{-1} f.w.)				CAT activity (U g^{-1} min^{-1})			
		24 h	72 h	120 h	240 h	24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	10.322×10^{-5d}	28.933×10^{-5b}	35.80×10^{-5c}	2.580×10^{-5c}	25.80×10^{-4d}	3.08×10^{-4c}	2.84×10^{-4d}	8.02×10^{-4b}
	10 mg/L	16.12×10^{-5b}	48.70×10^{-5c}	43.87×10^{-5d}	5.548×10^{-5a}	55.48×10^{-4b}	3.42×10^{-4b}	4.18×10^{-4c}	7.49×10^{-4d}
	30 mg/L	96.77×10^{-5a}	51.29×10^{-5b}	59.03×10^{-5b}	3.741×10^{-5b}	73.42×10^{-4c}	2.80×10^{-4d}	4.40×10^{-4b}	7.74×10^{-4c}
	Control	14.19×10^{-5c}	25.066×10^{-5a}	30.35×10^{-5a}	1.461×10^{-4d}	14.64^{a}	3408.5^{a}	3260.4^{a}	$16{,}068.0^{a}$
Pistia sp.	5 mg/L	31.42×10^{-5b}	25.419×10^{-5a}	30.671×10^{-5d}	47.74×10^{-4a}	2.58×10^{-4c}	2.08×10^{-4c}	7.49×10^{-4b}	5.11×10^{-4b}
	10 mg/L	55.48×10^{-5a}	48.38×10^{-5b}	52.90×10^{-5b}	55.54×10^{-5d}	3.70×10^{-4b}	3.21×10^{-4b}	5.48×10^{-4c}	4.16×10^{-4d}
	30 mg/L	67.09×10^{-5c}	53.354×10^{-5c}	90.32×10^{-5a}	59.35×10^{-5c}	2.61×10^{-4d}	1.43×10^{-4d}	3.30×10^{-4d}	4.89×10^{-4c}
	Control	15.54×10^{-5a}	20.399×10^{-5c}	25.71×10^{-5c}	37.22×10^{-5b}	1785.4^{a}	1562.2^{a}	5356.2^{a}	1654.1^{a}
Species	Conc (mg/L)	POD activity (U g^{-1} min^{-1})				Ascorbate (U/100 g)			
		24 h	72 h	120 h	240 h	24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	45.31^{b}	127.24^{a}	41.35^{c}	76.93^{b}	3.30^{c}	3.80^{a}	5.86^{a}	2.99^{c}
	10 mg/L	35.38^{d}	80.69^{b}	43.65^{b}	61.26^{c}	4.40^{b}	3.40^{b}	2.62^{c}	3.44^{b}
	30 mg/L	41.87^{c}	61.26^{c}	27.56^{d}	50.89^{d}	2.31^{d}	3.570^{a}	2.72^{c}	2.27^{d}
	Control	45.94^{a}	50.12^{d}	61.26^{a}	236.29^{a}	2.99^{a}	2.69^{d}	3.04^{b}	9.39^{a}
Pistia sp.	5 mg/L	34.28^{d}	35.57^{b}	26.67^{c}	62.42^{a}	9.45^{a}	3.55^{c}	4.62^{b}	3.65^{a}
	10 mg/L	40.84^{c}	62.42^{a}	80.69^{a}	47.94^{b}	4.20^{c}	5.43^{a}	4.10^{d}	2.47^{b}
	30 mg/L	80.69^{b}	61.26^{a}	61.26^{b}	27.11^{d}	6.11^{b}	4.60^{d}	4.33^{c}	3.52^{a}
	Control	157.53^{a}	30.91^{d}	22.50^{d}	40.84^{c}	4.36^{c}	5.51^{b}	5.48^{a}	1.23^{d}

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

Eichhornia sp. Almost similar enzymatic study was reported by Singh et al. (2016) for weed plants under metal stress condition. Very recently, Dalo et al. (2019) recorded the enhanced level of MDA under Ni-Fe stress in plants (*Phragmites australis*).

Root length and number of new roots

In phytoremediation study, the study of number of roots and root length is indispensable (Shanker et al. 2005). The present study results clearly revealed that root length of both *Eichhornia* sp. and *Pistia* sp. decreased with increasing concentration of chromium (Fig. 2a and b). Decrease in root growth is a well-documented phenomenon under metal stress condition (Mondal et al. 2013, 2015). However, toxicity of different heavy metals is different. Prasad et al. (2001) evaluated the toxicity of different heavy metals as Cd > Cr > Pb. They also highlighted that root length was more affected by Cr than by other heavy metals studied. The present study also highlighted that variation of root length is also concentration dependent with respect to control (Fig. 2a and b). This is possible due to destruction of root cells under chromium stress (Saddiqe et al. 2015). Results also demonstrated that at 5 mg/L Cr solution, *Eichhornia* sp. showed new root growth (figure not supplied). However, at higher concentrations (> 5 mg/L) no new root growth was recorded for both *Eichhornia* sp. and *Pistia* sp. Our study is in accordance with Panda and Patra (2000), and they demonstrated that 1 μM of Cr solution increases the root length in seedling growing under nitrogen nutrition levels. Another interesting observation was recorded during the growth period that the population of root hair steadily decreased with increasing Cr concentration (figure not provided) and growth of root hair almost stopped at 5 mg/L. Almost, identical result was reported by Suseela et al. (2002) through scanning electron microscopic study of root affected by Cr. They also examined the pitch and cortical tissue layer and root hair through microscopic study.

Pigment level

Both *Eichhornia* sp. and *Pistia* sp. showed gradual decrease in pigment concentration in the form of Chl-a, Chl-b and total Chl (Table 5). However, carotenoids showed initial increment when Cr concentration changed from 5 to 10 mg/L, but after that, pigment concentration decreased at 30 mg/L (Table 3). Chromium is such a heavy metal which can directly interfere in photosynthesis, activation of various functional enzymes, inadequate supply of sunlight energy required for conversion of ADP to ATP and electron transport (Clijsters and Van Assche 1985). Previous researches highlighted the negative impact of chromium on small

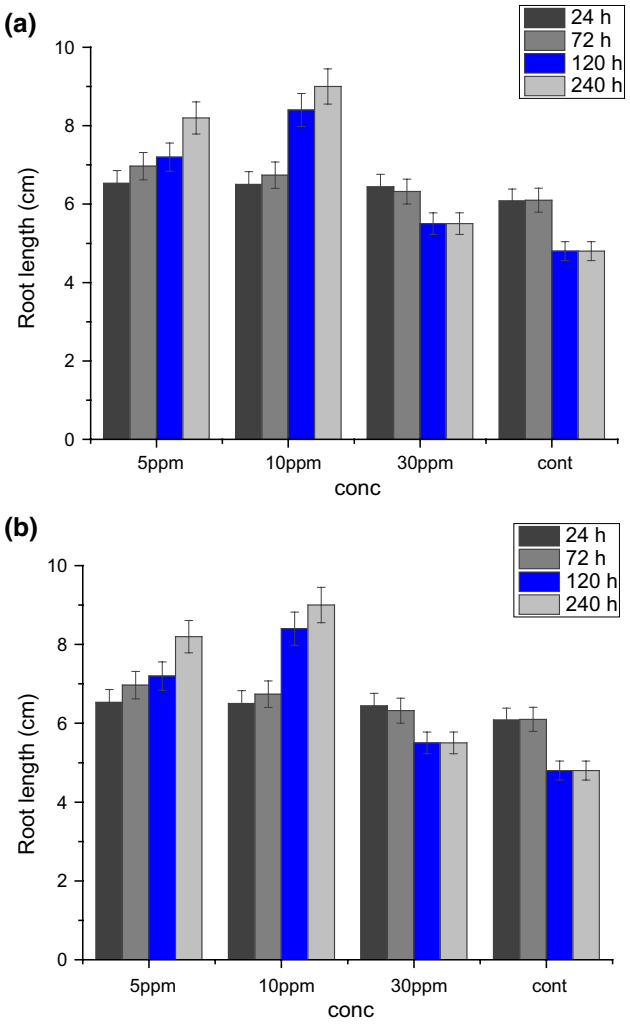

Fig. 2 Change of root length of **a** *Eichhornia* sp. and **b** *Pistia* sp. under different concentration of Cr

plants, but very limited information is available on higher plants (Medda and Mondal 2017). Chromium can inhibit photosynthesis which is probably due to disorganization of chloroplast ultra-structure (Mondal et al. 2015) and disruption in electron transport through photo-phosphorylation process (Bishnoi et al. 1993). Zeid (2001) observed that in peas, Cr at higher concentrations decreased photosynthesis drastically. The previous study also highlighted that not only Cr has negative effect on green plants, but also other metals such as Cd on wheat (Rizwan et al. 2015) and Cu on wheat (Keller et al. 2015), Cd on *Brassica napus* (Ehsan et al. 2014). Jarvis and Bielmyer-Fraser (2015) demonstrated that at higher metal concentration, higher metal accumulation occurred which subsequently causes the degradation of green pigment in leaves.

Results also revealed that Chl-a/b ratio increased with increase in the incubation period for both *Eichhornia* sp. and *Pistia* sp. (Table 5). However, Chl-a-to-Chl-b ratio is absolutely random at different concentration of Cr (Table 5). The decrease in the Chl-a/b ratio (Shanker 2003) under Cr stress is possibly due to reduction of size of the peripheral part of the antenna complex. The level of carotenoid under different concentrations of Cr(VI) solution is presented in Table 5.

Chlorophyll Stability Index (CSI)

Chlorophyll stability index (CSI) value gradually decreases with increase in the incubation period and strength of chromium solution for both the macrophytes (Table 6). However, degree of decomposition is different for different macrophytes. After 24 h, the reduction of CSI for *Eichhornia* sp. is 7.6%, 16.5% and 25.63% at 5, 10, 30 mg/L Cr(VI) solution, respectively (Table 5). But, during the same time interval *Pistia sp.* exhibited much higher level of CSI reduction as 18.6%, 28.1% and 34.2% at 5, 10, 30 mg/L Cr(VI) solutions, respectively (Table 6). The degradation of chlorophyll under the influence of heavy metal was well documented by the previous researchers (Hashem et al. 2015; Dey and Mondal 2016). Heavy metals such as Cd, Cr, Pb directly interfere with the chlorophyll synthesis either through direct inhibition of enzymatic synthesis or by causing deficiency of an essential nutrient (Meers et al. 2010). In another study conducted by Bajpai and Preti (2012), it was reported that CSI could be a viable method toward understanding of metal stress. Similarly, Begum et al. (2012) reported that CSI should be a good indicator for understanding the status of chlorophyll content under stress condition.

Protein level

Protein is a good indicator of oxidative heavy metal stress in plants (Plata et al. 2009). Protein level under the influence of different concentration of Cr(VI) of both *Eichhornia* sp. and *Pistia* sp. is presented in Table 7. From Table 7, it is clear that both the macrophytes exhibit a declining trend of protein level with increase in the concentration of Cr(VI) and different periods of incubation. In comparison, *Eichhornia* sp. showed much higher level of protein reduction than *Pistia* sp. This is probably due to the fact that heavy metals always cause stress which generate reactive oxygen species (ROS) and these ROS lead to membrane damage and cell death (Shahid et al. 2014). Almost similar results were reported by Mondal et al. (2015) while recording interference of Hg in mungbean. On the other hand, Tauqeer et al. 2016 and Aldoobie and Beltagi (2013) reported exactly opposite results; that is, protein content increases under lower level of metal stress. However, at higher level of metal stress, protein level decreases which suggest that plant can tolerate up to a certain level of metal.

Table 5 The level of 'Chl-a', 'Chl-b' and total Chl of *Eichhornia* sp. and *Pistia* sp. under Cr stress

Species	Conc (mg/L)	Chlorophyll 'a' (mg g^{-1} f.w.)				Chlorophyll 'b' (mg g^{-1} f.w.)			
		24 h	72 h	120 h	240 h	24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	$1.088^{c} \pm 0.010$	$1.054^{b} \pm 0.004$	$1.06^{b} \pm 0.050$	$0.111^{c} \pm 0.058$	$0.453^{c} \pm 0.003$	$0.442^{b} \pm 0.045$	$0.473^{a} \pm 0.005$	$0.058^{d} \pm 0.006$
	10 mg/L	$1.133^{ab} \pm 0.004$	$0.626^{d} \pm 0.003$	$0.987^{d} \pm 0.007$	$7 \times 10^{-3d} \pm 0.003$	$1.792^{a} \pm 0.004$	$0.278^{d} \pm 0.008$	$0.435^{b} \pm 0.008$	$0.263^{c} \pm 0.008$
	30 mg/L	$1.208^{a} \pm 0.008$	$0.838^{c} \pm 0.008$	$0.927^{c} \pm 0.006$	$0.488^{b} \pm 0.008$	$0.503^{b} \pm 0.055$	$0.312^{c} \pm 0.001$	$0.428^{bc} \pm 0.007$	$0.322^{a} \pm 0.004$
	Control	$1.073^{cd} \pm 0.004$	$1.232^{a} \pm 0.004$	$1.222^{a} \pm 0.004$	$0.559^{a} \pm 0.004$	$0.453^{c} \pm 0.005$	$0.417^{a} \pm 0.005$	$0.417^{d} \pm 0.006$	$0.309^{b} \pm 0.004$
Pistia sp.	5 mg/L	$0.82^{b} \pm 0.044$	$0.283^{d} \pm 0.006$	$0.541^{a} \pm 0.006$	$1.56^{b} \pm 0.040$	$0.366^{b} \pm 0.004$	$0.139^{d} \pm 0.005$	$0.241^{a} \pm 0.003$	$0.805^{b} \pm 0.005$
	10 mg/L	$1.285^{a} \pm 0.060$	$0.353^{c} \pm 0.006$	$0.418^{b} \pm 0.006$	$1.272^{c} \pm 0.004$	$5.867^{a} \pm 0.005$	$0.2^{c} \pm 0.088$	$0.173^{nc} \pm 0.005$	$0.611^{c} \pm 0.001$
	30 mg/L	$0.785^{c} \pm 0.005$	$0.577^{ab} \pm 0.005$	$0.412^{bc} \pm 0.004$	$0.855^{d} \pm 0.005$	$0.346^{bc} \pm 0.004$	$0.265^{b} \pm 0.005$	$0.175^{b} \pm 0.005$	$0.441^{d} \pm 0.006$
	Control	$0.515^{d} \pm 0.005$	$0.716^{a} \pm 0.005$	$0.256^{d} \pm 0.006$	$1.626^{a} \pm 0.572$	$0.231^{d} \pm 0.006$	$0.323^{a} \pm 0.01$	$0.104^{d} \pm 0.006$	$0.908^{a} \pm 0.006$
Species	Conc (mg/L)	Total chlorophyll (mg g^{-1} f.w.)				Carotenoids (mg g^{-1} f.w.)			
		24 h	72 h	120 h	240 h	24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	$1.652^{cd} \pm 0.004$	$1.59^{a} \pm 0.070$	$1.64^{a} \pm 0.560$	$0.2^{d} \pm 0.040$	$4.634^{c} \times 10^{-3} \pm 0.052$	$2.679^{b} \times 10^{-3} \pm 0.052$	$4.208^{b} \times 10^{-3} \pm 0.00$	$8.25^{a} \times 10^{-4} \pm 0.004$
	10 mg/L	$1.71^{b} \pm 0.050$	$0.97^{d} \pm 0.050$	$1.52^{c} \pm 0.040$	$0.889^{b} \pm 0.007$	$4.91^{a} \times 10^{-3} \pm 0.002$	$2.551^{c} \times 10^{-3} \pm 0.002$	$3.862^{d} \times 10^{-3} \pm 0.00$	$3.338^{c} \times 10^{-3} \pm 0.00$
	30 mg/L	$1.828^{a} \pm 0.010$	$1.298^{c} \pm 0.006$	$1.298^{d} \pm 0.006$	$0.884^{bc} \pm 0.004$	$4.887^{ab} \times 10^{-35} \pm 0.00$	$2.434^{d} \times 10^{-3} \pm 0.00$	$3.786^{c} \times 10^{-3} \pm 0.00$	$3.088^{d} \times 10^{-3} \pm 0.00$
	Control	$1.64^{c} \pm 0.040$	$1.585^{ab} \pm 0.005$	$1.585^{ab} \pm 0.005$	$0.924^{a} \pm 0.004$	$4.585^{cd} \times 10^{-5} \pm 0.00$	$3.737^{a} \times 10^{-3} \pm 0.02$	$8.5^{a} \times 10^{-5} \pm 0.001$	$4.698^{b} \times 10^{-3} \pm 0.004$
Pistia sp.	5 mg/L	$1.240^{b} \pm 0.005$	$0.443^{d} \pm 0.004$	$0.823^{a} \pm 0.004$	$2.533^{b} \pm 0.004$	$3.266 \times 10^{-3} \pm 0.00$	$1.326^{d} \times 10^{-3} \pm 0.00$	$2.764^{a} \times 10^{-3} \pm 0.00$	$5.795^{b} \times 10^{-3} \pm 0.00$
	10 mg/L	$1.98^{a} \pm 0.060$	$0.605^{c} \pm 0.004$	$0.617^{bc} \pm 0.005$	$1.997^{c} \pm 0.006$	$5.128 \times 10^{-3} \pm 0.00$	$1.786^{c} \times 10^{-3} \pm 0.00$	$1.449^{c} \times 10^{-3} \pm 0.0001$	$5.154^{c} \times 10^{-3} \pm 0.00$
	30 mg/L	$1.22^{bc} \pm 0.005$	$0.884^{b} \pm 0.005$	$0.620^{b} \pm 0.005$	$1.394^{d} \pm 0.005$	$3.082 \times 10^{-3} \pm 0.0002$	$2.302^{b} \times 10^{-3} \pm 0.00$	$1.909^{b} \times 10^{-3} \pm 0.0002$	$3.418^{d} \times 10^{-3} \pm 0.00$
	Control	$0.785^{d} \pm 0.016$	$1.09^{a} \pm 0.001$	$0.359^{d} \pm 0.021$	$2.678^{a} \pm 0.034$	$2.196 \times 10^{-3} \pm 0.004$	$2.853^{a} \times 10^{-3} \pm 0.001$	$1.053^{d} \times 10^{-3} \pm 0.002$	$6.494^{a} \times 10^{-3} \pm 0.003$

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

Table 6 Carotinoid and CSI of *Eichhornia* sp. and *Pistia* sp. under Cr stress

Species	Conc (mg/L)	Carotenoids (mg.g^{-1}.f.w.)				CSI (%)			
		24 h	72 h	120 h	240 h	24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	$4.634^{c}\times10^{-3}$	2.679×10^{-3}	2.208×10^{-3}	2.21×10^{-4}	$87.2^{b}\pm1.22$	$82.6^{b}\pm1.11$	$80.1^{b}\pm1.36$	$78.8^{b}\pm4.01$
	10 mg/L	$4.91^{b}\times10^{-3}$	2.551×10^{-3}	3.862×10^{-3}	3.338×10^{-3}	$78.8^{c}\pm2.05$	$77.3^{c}\pm2.14$	$76.5^{c}\pm2.05$	$75.4^{bc}\pm2.03$
	30 mg/L	$4.887^{d}\times10^{-3}$	2.434×10^{-3}	3.786×10^{-3}	3.088×10^{-3}	$70.2^{d}\pm1.66$	$69.8^{d}\pm3.02$	$68.8^{d}\pm1.96$	$63.6^{d}\pm2.22$
	Control	$4.937^{a}\times10^{-3}$	4.12×10^{-3}	3.877×10^{-3}	3.311×10^{-3}	$94.4^{a}\pm0.066$	$93.7^{a}\pm0.39$	$92.8^{a}\pm2.054$	$91.6^{a}\pm1.97$
Pistia sp.	5 mg/L	$3.266^{b}\times10^{-3}$	1.326×10^{-3}	2.764×10^{-3}	1.795×10^{-3}	$78.3^{b}\pm1.08$	$76.0^{b}\pm0.08$	$72.5^{b}\pm2.00$	$70.6^{b}\pm0.28$
	10 mg/L	$3.128^{bc}\times10^{-3}$	1.786×10^{-3}	1.449×10^{-3}	1.154×10^{-3}	$69.2^{c}\pm2.31$	$68.3^{c}\pm1.01$	$67.9^{c}\pm1.09$	$61.4^{c}\pm2.7$
	30 mg/L	$3.082^{d}\times10^{-3}$	2.302×10^{-3}	1.909×10^{-3}	1.418×10^{-3}	$53.4^{d}\pm0.28$	$65.2^{c}\pm0.33$	$63.1^{d}\pm0.22$	$59.6^{cd}\pm1.66$
	Control	$4.160^{a}\times10^{-3}$	3.986×10^{-3}	2.967×10^{-3}	2.448×10^{-3}	$96.3^{a}\pm1.96$	$95.6^{a}\pm2.8$	$90.2^{a}\pm0.98$	$88.75^{a}\pm4.33$

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

Table 7 Level of protein of *Eichhornia* sp. And *Pistia* sp. under Cr stress

Species	Conc (mg/L)	Protein (mg g^{-1})			
		24 h	72 h	120 h	240 h
Eichhornia sp.	5 mg/L	$1.27^{b}\pm0.11$	$1.33^{c}\pm0.01$	$1.11^{b}\pm0.01$	$0.95^{c}\pm0.14$
	10 mg/L	$1.11^{c}\pm0.22$	$1.36^{c}\pm0.14$	$0.66^{cd}\pm0.11$	$1.46^{a}\pm0.44$
	30 mg/L	$0.89^{c}\pm0.03$	$2.76^{a}\pm0.22$	$0.70^{c}\pm0.04$	$0.42^{d}\pm0.01$
	Control	$1.43^{a}\pm0.14$	$1.40^{b}\pm0.01$	$1.32^{a}\pm0.05$	$1.25^{b}\pm0.36$
Pistia sp.	5 mg/L	$0.09^{d}\pm0.001$	$1.68^{a}\pm0.34$	$1.46^{a}\pm0.44$	$0.99^{c}\pm0.01$
	10 mg/L	$0.89^{b}\pm0.021$	$1.14^{d}\pm0.16$	$0.89^{d}\pm0.01$	$1.08^{a}\pm0.11$
	30 mg/L	$0.79^{c}\pm0.11$	$1.40^{b}\pm0.06$	$1.36^{b}\pm0.12$	$0.87^{c}\pm0.33$
	Control	$1.33^{a}\pm0.12$	$1.30^{bc}\pm0.11$	$1.11^{c}\pm0.33$	$1.00^{b}\pm0.007$

Means followed by the same letter within a treatment are not significantly different at 5% level using Duncan's multiple range test (DMRT), and means of three replicates are taken

Conclusion

Plant-mediated remediation of the contaminated water seems to be a good alternative in the long term. Many aquatic plant species have been tested for their potentiality toward bioaccumulation of toxic heavy metals. However, very few macrophytes have shown the ability to accumulate chromium from aqueous medium. The present study shows that both *Eichhornia* sp. and *Pistia* sp. effectively accumulate chromium from aquatic medium, but *Eichhornia* sp. showed higher chromium accumulation at higher concentrations than *Pistia* sp. Biochemical analysis suggests that both pigment and CSI decrease with increasing chromium concentration, but malondialdehyde concentration increases with increasing chromium concentration for both the tested species. Among the studied enzymes, catalase showed the highest sensitivity toward chromium. On the other hand, chromium bioaccumulation kinetic study revealed that both pseudo-first-order and pseudo-second-order kinetics are better fitted for *Pistia* sp. than *Eichhornia* sp. Based on this study, it may be concluded that both *Eichhornia* sp. and *Pistia* sp. can be good candidates for phytoremediation of Cr(VI) from contaminated water. Finally, we have to take special care during disposal of used plants. This time we recommend that the used plants should be used for generation of energy and the ash may be utilized in the cement or bricks industry.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Abbreviations

CSI: Chlorophyll stability index; Chl: Chlorophyll; ROS: Reactive oxygen species; CAT: Catalase; POD: Peroxidase; AAO: Ascorbic acid oxidase; MDA: Malondialdehyde; TCA: Trichloroacetic acid; ROS: Reactive oxygen species; LED: Light emitting diodes; TBA: Thiobarbituric acid; AAS: Atomic absorption spectrometry; DW: Dry weight; FW: Fresh weight; RGR: Relative growth rate; EC: Enzyme commission; ANOVA: Analysis of variance; DMRT: Duncan's multiple range test

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