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Kinetics of Cr(III) and Cr(VI) removal from water by two floating macrophytes

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Abstract

The aim of this work was to compare Cr(III) and Cr(VI) removal kinetics from water by *Pistia stratiotes* and *Salvinia herzogii*. The accumulation in plant tissues and the effects of both Cr forms on plant growth were also evaluated. Plants were exposed to 2 and 6 mg L^{-1} of Cr(III) or Cr(VI) during 30 days. At the end of the experiment, Cr(VI) removal percentages were significantly lower than those obtained for Cr(III) for both macrophytes. Cr(III) removal kinetics involved a fast and a slow component. The fast component was primarily responsible for Cr(III) removal while Cr(VI) removal kinetics involved only a slow process. Cr accumulated principally in the roots. In the Cr(VI) treatments a higher translocation from roots to aerial parts than in Cr(III) treatments was observed. Both macrophytes demonstrated a high ability to remove Cr(III) but not Cr(VI). Cr(III) inhibited the growth at the highest studied concentration of both macrophytes while Cr(VI) caused senescence. These results have important implications in the use of constructed wetlands for secondary industrial wastewater treatment. Common primary treatments of effluents containing Cr(VI) consists in its reduction to Cr(III). Cr(III) concentrations in these effluents are normally below the highest studied concentrations in this work.

Keywords: metal, uptake efficiency, phytoremediation, wetlands

1. Introduction

Some trace metals, such as Cr, Cu, Ni and Zn, play an important role as micronutrients in organisms. However, they have toxic effects at high concentrations (Stumm and Morgan 1996; Kabata-Pendias 2011). Cr can occur in several oxidation states, although the most stable forms are the trivalent Cr(III) and hexavalent Cr(VI) species in surface waters (Fendorf, 1995). Cr(III) is a cation which forms colloidal hydrous oxides while Cr(VI) exists as chromate, a strong divalent anionic oxidant which is highly soluble in water. In most effluent

primary treatments, Cr(VI) is reduced totally or partially to Cr(III). Usually both Cr forms are present in industrial effluents that reach a secondary treatment, such as constructed wetland.

Cr(VI) is toxic for humans, plants and animals while Cr(III) is essential for humans and animals (Srivastava *et al.* 2002). However, researchers still debate whether or not Cr(III) is essential for plants (Sharma *et al.* 2003; Gardea-Torresday *et al.* 2005). Both Cr species are taken up by plants. It has been proposed that the processes performed by plants to take up metals are not necessarily the same for different plants and for different metals. Sorption by roots (including adsorption, chelation, ionic exchange and chemical precipitation), and biological processes (translocation to aerial parts, precipitation induced by root exudates,) are considered to be the responsible processes (Dushenkov *et al.* 1995; Maine *et al.* 2001; Chakraborty *et al.* 2014).

Cr (III) and Cr(VI) uptake was compared in terrestrial plants. Mishra *et al.* (1995) compared Cr(III) and Cr(VI) uptake by maize. Gardea-Torresday *et al.* (2005) compared differential uptake and transport of Cr(III) and Cr(VI) by *Salsola kali*, reporting that uptake was influenced by Cr speciation and concentration.

Regarding floating macrophytes, Hadad *et al.* (2009) compared the uptake kinetics of a metal and a nutrient, and reported that *Eichhornia crassipes* removed the metal faster than the nutrient, suggesting that adsorption to the cell walls of roots was probably the process responsible for the high bioaccumulation rate of the metal. Cr(III), Ni(II) and Zn(II) uptake kinetics by *E. crassipes* was also compared (Hadad *et al.* 2011). Maine *et al.* (2004) studied Cr(III) uptake sorption processes between *Pistia stratiotes* and *Salvinia herzogii.* Cr(III) and Cr(VI) uptake capacity from water by different macrophytes was studied (Delgado *et al.* 1993; Di Luca *et al.* 2014; Uysal and Ar 2007; Chakraborty *et al.* 2014), but the comparison between Cr(III) and Cr(VI) uptake process by living free floating macrophytes was not found in the literature.

S. herzogii and *P. stratiotes* are among the free floating aquatic plants of greatest dispersion and productivity that can be found in natural wetlands in Argentina and they have demonstrated to be efficient in metal uptake (Maine *et al.* 2001, 2004; Odjegba and Fasidi 2004; Hadad *et al.* 2007; Mishra and Tripathi 2008; Mufarrege *et al.* 2010; Di Luca *et al.* 2014). These species were used in wetlands constructed for industrial effluent and sewage treatment (Aoi and Hayashi 1996; Chen *et al.* 2006; Hadad *et al.* 2006; Lu *et al.* 2010).

The aims of this work were to compare: the kinetics of Cr(III) and Cr(VI) removal from water by *P. stratiotes* and *S. herzogii*; the Cr(III) and Cr(VI) accumulation in plant tissues, and the effects of both Cr forms on macrophyte growth.

Studies on the bioaccumulation process by macrophytes would allow us to determine their tolerance and provide basic information for their preservation in natural wetlands or related to the potential use in water depuration.

2. Material and methods

2.1. Experimental design

Water and healthy plants of *S. herzogii* and *P. stratiotes* were collected from an unpolluted pond from the Paraná River floodplain (Argentina). The chemical composition of the pond water used in the experiment was (mean ± standard deviation): conductivity = 124 ± 1 µS cm^{-1}; dissolved oxygen (DO) = 7.6 ± 0.10 mg L^{-1}; soluble reactive phosphorus (SRP) = 0.035 ± 0.002 mg L^{-1}; $N\text{-}NH_4^+$ = 0.550 ± 0.019 mg L^{-1}; $N\text{-}NO_3^-$ = 0.651 ± 0.005 mg L^{-1}; $N\text{-}NO_2^-$ = 0.008 ± 0.001 mg L^{-1}; Ca^{2+} = 10.3 ± 0.8 mg L^{-1}; Mg^{2+} = 3.8 ± 0.5 mg L^{-1}; Na^+ = 13.7 ± 1.0 mg L^{-1}; K^+ = 3.50 ± 0.5 mg L^{-1}; Cl^- = 10.6 ± 1.3 mg L^{-1}; SO_4^{2-} = 8.0 ± 1.8 mg L^{-1}; HCO_3^- = 51.7 ± 0.8 mg l^{-1}, Fe = 5 µg L^{-1}, Cr = non detected (Detection limit = 2 µg L^{-1}). The collected plants were washed and then grown outdoors in reactors containing pond water. After a suitable acclimation period, plants of a similar size and weight were selected for experimental purposes.

For the experiment, plastic reactors containing 7 L of pond water and 100 g of wet plant biomass were disposed. Cr solutions were added to reach 2 and 6 mg L^{-1} Cr(III) or Cr(VI). Cr(III) solutions were prepared using $Cr(NO_3)_3.9H_2O$ while $K_2Cr_2O_7$ was used for Cr(VI) solutions. The studied concentrations were chosen because they are in the range found in natural aquatic systems near industrial areas of our zone and in constructed wetlands for effluent treatments.

Chemical controls (with the addition of metal, without plants) and biological control (with plants, without the addition of metal) were performed simultaneously. The treatments were arranged in triplicated, according to Table 1. During the experimental period (spring), reactors were placed outdoors under a semi-transparent plastic roof receiving natural light. Mean temperature ranged from 24 to 28ºC.

In the Cr(III) treatment, water pH was maintained between 5.4-5.8 to avoid metal precipitation. In the Cr(VI) treatment, water pH was adjusted to 7.2 to obtain Cr as $CrO_4^=$. Water was added on a daily basis to compensate water losses through plant transpiration and evaporation. The experimental period was 31 days for Cr(III) treatments. Due to the fact that the studied macrophytes showed differences in tolerance in the Cr(VI) treatments, the experimental periods were reduced to 11 and 21 days for *P. stratiotes* and *S. herzogii* treatments, respectively.

Water was sampled initially and at 2, 8 and 24 h and at 2, 3, 7, 9, 11, 16, 21 and 31 days. Cr(III) or Cr(VI) concentrations were determined. Plants sampled at the end of the experiment were rinsed in distilled water, dried and separated into aerial parts and roots, and Cr concentration and dry weight at 105°C were determined (APHA, 1998). Relative growth rate (RGR) was calculated according to Hunt's equation (1978):

$RGR = \ln W_2 - \ln W_1 / T_2 - T_1$ (Eq. 1)

where RGR is the relative growth rate (g g^{-1} day^{-1}), W_1 and W_2 are the initial and final dry weight, respectively, and (T_2 - T_1) is the experimental period (days).

2.2. Analytical methods

Root specific surface of *P. stratiotes* and *S. herzogii* was determined by the BET method with liquid N_2. The physicochemical characterization of pond water used in the experiment was done according to APHA (1998). Dried plant tissues (aerial parts and roots) were ground and digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture (Maine *et al.* 2001). Total Cr concentrations in water samples and digests of plant tissues were determined by atomic absorption spectrometry (Perkin Elmer AA 200). Cr(VI) concentrations were determined colorimetrically and Cr(III) by difference (APHA, 1998). X-ray microanalysis of roots was performed with a Scanning electron microscope JEOL, JSM-35C, Si(Li), EDAX, model PV9100. Micrographies were obtained from images of secondary electrons at an accelerating voltage of 20 Kv. Images were obtained digitally applying the SemAfore system. X-ray spectra were obtained at the same accelerating voltage over a time interval of 300s of life for the longitudinal analysis and 360s for the transversal analysis, enough time for providing a good signal/noise ratio.

2.3. Statistical analysis

Dunett's test was used to compare the final Cr concentration in water in the reactors with macrophytes and chemical controls (Walpole and Myers 1992). Three-way analysis of variance was used to determine whether significant differences in Cr water removal and RGR existed (factors: Cr forms, Cr concentrations and macrophyte species). Besides, this analysis was performed in order to determine whether significant differences in Cr concentrations in tissues existed (factors: Cr forms, Cr concentrations and plant tissues). The normality of residuals was tested graphically, and the variance homoscedasticity was checked applying Bartlett's test. Duncan's test was used to differentiate means where appropriate. A level of $p<0.05$ was used in all comparisons.

2.4. QA/QC

Glassware was pre-cleaned and washed with 2N HNO_3 prior to each use. All reagents were of analytical grade. Certified standard solutions were used. Blank solutions were run. Replicate analyses (at least ten times) of

the samples showed a precision of typically less than 4% (coefficient of variation). The Cr detection limits were 2 µg L^{-1} and 5 µg g^{-1} for water and macrophyte tissues, respectively.

3. Results

3.1. Cr removal from water

In both macrophyte treatments, Cr(III) was efficiently removed from water and the highest removal was observed during the first 24 h of the experiment (68 and 80% and 58 and 78% for *S. herzogii* and *P. stratiotes*, respectively) (Fig. 1). The higher the initial concentration, the higher the removal rate during the first 24 h of contact. Cr(III) removal followed a non-linear kinetics. After 31 days of experiment, the final removal percentages were not significantly different, 91-93%, regardless of the initial concentration or species.

Cr(VI) removal from water was significantly lower than that obtained for Cr(III) for both macrophytes (Fig. 1). Along the experiment, the plants were examined for possible external phytotoxic signs. Plants could not tolerate Cr(VI) treatments and changes in colour and vigour were observed in 3 days eventually leading to senescence at 11 days for *P. stratiotes* and 21 days for *S. herzogii*. Therefore, the experimental periods were reduced to 11 and 21 days for *P. stratiotes* and *S. herzogii* treatments, respectively. At the end of the experiment, Cr(VI) removal from water by *S. herzogii* was 28% for the two concentrations studied. In *P. stratiotes* treatments, Cr(VI) removal percentages from water were significantly higher in the lowest concentration treatment than those obtained in the highest concentration treatment (22 and 10%, respectively). No significant decrease in Cr concentration was observed in the chemical controls at the Cr(III) and Cr(VI) concentrations studied.

3.2. Kinetics of Cr removal from water

Experimental data for Cr(III) and Cr(VI) concentrations in water over time (Fig. 1) were adjusted to the following equation proposed by Maine *et al.* (2004):

$$C_W - C_{0W} = A_W (1 - e^{-t/r}) + B_W (1 - e^{-t/s}) \qquad \text{Eq. (2)}$$

in which:

C_{0W}: initial concentration of metal in water.

C_W: concentration of metal in water at time t.

t: time.

The other parameters are empirical constants.

Representing both terms of Eq. (2) versus time for each species and each concentration in separate graphs, it can be seen that the sorption kinetics was significantly different for Cr(III) and Cr(VI) treatments (Figs. 2 and 3). The values of the parameters of Eq. (2) are shown in Table 2.

Cr(III) removal from water involved two stages or components: a fast one and a slow one. There were no significant differences between species for the fast stage which was responsible for a greater decrease of Cr(III) in water (Fig 2.). The slow component was responsible for a higher Cr(III) removal in *P. stratiotes* than in *S. herzogii* treatments at the two concentrations studied.

Cr(VI) removal from water showed only a slow component (Fig. 2), due to Aw = 0 in all cases (Table 2). This component was significantly higher in *S. herzogii* in comparison with *P. stratiotes* in the treatment of 6 mg L^{-1}.

3.3. Cr concentrations in tissues

Cr concentrations in plant tissues in the Cr(III) and Cr(VI) treatments are shown in Table 3. Both macrophytes showed a significant increase in Cr concentrations in tissues at the end of the experiment. Cr concentration in tissues was significantly higher in plants exposed to 6 mg L^{-1} than that exposed to 2 mg L^{-1}. In all treatments, Cr concentrations in roots were significantly higher than those measured in aerial parts.

For both macrophytes, Cr concentrations in roots were significantly higher in the Cr(III) treatments than those determined in the Cr(VI) treatments. However, aerial parts in both species showed higher Cr concentrations in the Cr(VI) treatments than in the Cr(III) treatments.

3.4. Plant study

P. stratiotes showed a greater surface area (4.6 m^2 g^{-1}) than *S. herzogii* (2.4 m^2 g^{-1}). At the end of the experiment, RGRs of both macrophytes were positive in Cr(III) treatments. However, RGRs were significantly lower than those obtained in the biological control at the highest studied concentration, demonstrating growth inhibition (Fig. 3). In the treatment of 6 mg L^{-1}, *P. stratiotes* showed a significantly lower RGR than that of *S. herzogii*. Neither macrophyte showed visible phytotoxic signs.

In the Cr(VI) treatments, chlorosis and senescence were observed in both macrophytes in the Cr(VI) treatments, causing a shortening of the experimental period. RGRs were negative, demonstrating a lower tolerance in comparison with Cr(III). The RGR of *P. stratiotes* was significantly lower than that of *S. herzogii,* demonstrating its low tolerance.

At the end of the experiment, micrographies of roots of *P. stratiotes* and *S. herzogii* were obtained with an electron microscope (Figs. 4a and 4b). Precipitates on the surface of roots of *P. stratiotes* were detected with

the electron microscope (Fig. 4c). One of them presented the main relative relationships of Si, typically as a grain of sand. The X-ray microanalysis of the other precipitate showed that the present elements were (in % w/w): Cr (41 %), Fe (26 %), K (16 %), Mn (10 %), Cl (4 %) and Al (3 %) (Fig. 4 c).

4. Discussion

Cr(III) was efficiently depleted from water after 31 days of experiment regardless of the initial concentration or species. In no case 100% removal was reached (Fig. 1), suggesting that the metal uptake is probably a competitive-consecutive mechanism with reversible reaction steps. The greatest decrease was observed during the first hours of exposure. These results are similar to previous works for Cr, Pb, Zn, Cd, etc. (Delgado *et al.* 1993; Maine *et al.* 2001; Hasan *et al.* 2007; Suñe *et al.* 2007).

Cr(VI) was not efficiently removed from water, in agreement with Mishra *et al.* (1995), who reported that the uptake of Cr(III) is higher than that of Cr(VI) in maize. This could be due to passive transport of Cr(III) in the plant, dissipating no metabolic energy in this process (Skeffington *et al.* 1976) whereas Cr(VI) is actively taken up by plants and thus forms a metabolically driven process (Aldrich *et al.* 2003; Diwan *et al.* 2008).

Cr(III) removal kinetics involved two processes or components: a fast one and a slow one. The fast component, virtually instantaneous, was produced during the first hours of contact and it was responsible for the greatest Cr(III) removal from water. The rapidity of the uptake would suggest that physical sorption or adsorption is an important removal mechanism. Cr(III) could be adsorbed and retained by the cation exchange sites of the cell wall (Gardea-Torresday *et al.* 2005). No significant differences in the fast component between species were observed at the two studied concentrations. Contrarily, the slow component presented differences between these species, being responsible for a greater Cr(III) removal in *P. stratiotes* than in *S. herzogii* treatments at both concentrations studied. The slow component of Cr(III) removal from water could be caused by root-mediated precipitation and biological processes as intracellular uptake (transported through the plasmalemma into the cells). The differences found between species could probably be due to the fact that the chemical precipitation induced by the roots is one of the slow mechanisms of Cr(III) removal for *P. stratiotes*. *P. stratiotes* presents higher root surface area than *S. herzogii*. Besides, the formation of precipitates on the surface of roots of *P. stratiotes* was observed with an electron microscope. X-ray microanalysis of the precipitates showed that not only Cr precipitation took place, but also the precipitation of its neighbour elements on the periodic table (Mn and Fe), which have similar chemical characteristics. Fe(III) can deposit onto the root surfaces of aquatic macrophytes (Weiss *et al.* 2003), forming plaques of a large capacity to adsorb metals

(Doyle and Otte 1997; Cambrolle *et al.* 2008). Hu *et al.* (2014) suggested that iron plaque could be a trap for immobilizing Cr in roots. Probably, the formation of iron plaque in the roots of *S. herzogii* was not favored due to its different root anatomy.

On the other hand, Cr(VI) removal from the solution showed only a slow component (Fig. 2). Probably, it was due to the lack of adsorption, which is the main responsible process in Cr(III) removal. Cr(VI) uptake is mediated through carriers used for the uptake of essential nutrients for plant metabolism. In barley plants, chromate influx shows Michaelis-Menten kinetics, and it is competitively inhibited by sulphate (Shewry and Peterson 1974; Chatterjee and Chatterjee 2000; Cervantes *et al.* 2001). Cr(VI) uptake is a metabolically-mediated process via the sulphate pathway (Skeffington *et al.* 1976; Smith *et al.* 1989; Kleiman and Cogliatti 1998). Evidence for independent uptake mechanisms for Cr(III) and Cr(VI) was observed in barley seedlings, indicating that Cr(VI) uptake depends on metabolic energy whereas Cr(III) uptake does not (Cervantes *et al.* 2001).

The slow component of Cr(VI) removal did not show differences at the lowest studied concentration while it was significantly higher in *S. herzogii* in comparison with *P. stratiotes* in the treatment of 6 mg L^{-1}. Cr(VI) is not only actively taken up by plants, Espinoza-Quiñones *et al.* (2009) studied Cr(VI) and Cr(III) uptake by *Salvinia auriculata*, *P. stratiotes* and *E. crassipes*, using high resolution XRF technique. These authors concluded that Cr(VI) reduction to less toxic Cr(III) process occurred during the metal uptake by these plants. Lytle *et al.* (1998) proposed that the reduction of Cr(VI) to Cr(III) appeared to occur in the fine lateral roots, then Cr(III) was translocated to leaf tissues. Probably, *P. stratiotes* could not reduce Cr(VI) to Cr(III). In consequence, Cr(VI) was transported through carriers used for the uptake of essential nutrients for plant metabolism being nutrient uptake competitively inhibited by this metal. For this reason, *P. stratiotes* showed early senescence and it did not tolerate the treatment of 6 mg L^{-1}.

As expected, a higher Cr concentration in roots than in aerial parts was observed for all treatments in agreement with literature (Shanker *et al.* 2005; Barbosa *et al.* 2007; Vernay *et al.* 2007; Prado *et al.* 2010). The exclusion of metals from aerial part tissues is a metal tolerance strategy (Taylor and Crowder 1983; Kabata-Pendias 2011). Despite the fact that higher Cr(III) than Cr(VI) accumulation in roots was observed for both macrophytes, a higher translocation from roots to aerial parts was observed in the Cr(VI) treatment. Similar results were reported by Gardea-Torresday *et al.* (2005) for *S. kali*. Probably, the higher toxicity of Cr(VI) than Cr(III) produced that tolerance strategy of metal accumulation in roots decreased, and Cr is easily transported to

the aerial parts. Meanwhile, Cr(III) could be adsorbed and retained by the cation exchange sites of the root cell wall.

Comparing with the biological control, RGRs of both macrophytes measured in Cr(III) treatments did not show significant differences at 2 mg L^{-1} but they were significantly lower at the highest studied concentration. However, they were positive, while the Cr(VI) treatments were toxic to the plants showing negative RGRs. Growth inhibition by Cr(III) exposure observed in the present study has previously been reported and represents a sensitive indicator of Cr toxicity (Maine *et al.* 2004; Shanker *et al.* 2005; Hadad *et al.* 2007). Delgado *et al.* (1993) reported that *E. crassipes* did not show weight reduction when exposed to concentrations up to 2 mg L^{-1} Cr(III). Di Luca *et al.* (2014) reported reductions in *P. stratiotes* RGR due to 5 mg L^{-1} Cr(III) exposure, which were attenuated by nutrient enrichment.

As it can be seen, both macrophytes exhibited a better adaptation to Cr(III) than Cr(VI) perturbation. Cr(III) inhibited growth of both macrophytes at the highest studied concentration while Cr(VI) caused senescence. Higher toxicity of Cr(VI) compared with that of Cr(III) has been explained by various hypotheses. The toxic action of Cr(VI) is due to the negatively charged Cr(VI) complexes, which can easily cross cellular membranes by means of sulfate ionic channels, penetrate the cytoplasm and react with the intracellular material leading to the formation of various reactive intermediates (Gikas and Romanos 2006). Also, it has been proposed that at natural pH levels, Cr(VI) being water soluble and of a smaller size than the hydrated Cr(III) ion, readily penetrates cell walls and exhibits its toxic behavior (Mishra *et al.* 1995). The hydrated Cr(III) cation does not pass through the cell membrane, even at low pH (Cary *et al.* 1977). The more toxic nature of Cr(VI) may also be explained by its ability, being a strong oxidizer, to cause oxidative damage to the cells. This may cause malfunctions in the uptake of mineral nutrients and water, leading to chlorosis and eventually death (Vazquez *et al.* 1987), as it was observed in this work.

Both macrophytes demonstrated a high ability to remove Cr(III) but not Cr(VI). Cr(III) inhibited the growth of both macrophytes while Cr(VI) caused senescence. However, both macrophytes could be used in constructed wetlands for the final treatment of industrial effluents containing Cr. Common primary treatments of effluents containing Cr(VI) consists in its reduction to Cr(III). In consequence, when effluents reach constructed wetlands after the primary treatment, Cr is mainly as Cr(III) form and both species can tolerate and uptake it. Besides, Cr(III) concentrations in these effluents are normally below the highest studied concentrations in this work. (Maine et al., 2009; 2013).

5. Conclusions

Independent uptake mechanisms for Cr(III) and Cr(VI) was observed. Cr(III) removal from water was significantly higher than Cr(VI) removal. Cr(III) removal kinetics involved a fast and a slow component. The fast component, produced mainly by an adsorption process, was the main responsible for Cr(III) removal for both macrophytes. The slow component was responsible for a higher Cr(III) removal in *P. stratiotes* than in *S. herzogii* treatments. Cr(VI) removal kinetics involved only a slow process, indicating lack of adsorption.

Cr concentrations in roots in both macrophytes were significantly higher in the Cr(III) than in Cr(VI) treatments. However, Cr(VI) was translocated to the aerial parts in a higher proportion in comparison with Cr(III). The anionic Cr(VI) form is easily transported to aerial parts due to the lack of adsorption on the root cell walls. Meanwhile, the cation Cr(III) is adsorbed by the cell wall, being retained in roots.

Both macrophytes demonstrated a higher capacity to remove Cr(III) from water than Cr(VI). Cr(III) inhibited the growth at the highest concentration of both macrophytes while Cr(VI) caused senescence.

These results have important implications in the use of constructed wetlands for secondary industrial wastewater treatment since effluents after a primary treatment contain Cr as Cr(III) form. Cr(III) concentrations in these effluents are normally below the highest studied concentrations in this work.

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Table 1. Arrangement of the treatments studied in the Cr(III) and Cr(VI) treatments.

Macrophyte	Cr(III) treatment (mg L^{-1} Cr)		Cr(VI) treatment (mg L^{-1} Cr)	
S. herzogii	2	6	2	6
P. stratiotes	2	6	2	6
Chemical Controls	2	6	2	6
Biological Control *S. herzogii*	Without Cr(III) or Cr(VI)additions			
Biological Control *P. stratiotes*				

Table 2. Empirical constants obtained in Eq. (2) for the two studied macrophytes and the different Cr concentrations in water (C_{0w}) in the Cr(III) and Cr(VI) treatments.

	Macrophytes	C_{0w}	A_w	B_w	r	s	r^2
Cr(III) treatment	*S. herzogii*	2	-1.2989	-0.3633	0.06854	14.9978	0.9989
		6	-4.2538	-1.1521	0.03323	32.4625	0.9990
	P. stratiotes	2	-1.0714	-0.8344	0.03124	5.99491	0.9966
		6	-4.6995	-1.8904	0.02299	22.0067	0.9994
Cr(VI) treatment	*S. herzogii*	2	0.0042	-0.6086	1.0035	7.9989	0.9965
		6	0.0046	-1.75521	1.0032	6.3114	0.9990
	P. stratiotes	2	0.0049	-0.58423	1.0041	3.0644	0.9939
		6	0.0053	-0.38862	1.0023	6.3931	0.9992

Table 3. Final Cr concentrations in plant tissues (mg g^{-1} dry weight) obtained in the Cr(III) and Cr(VI) treatments and in the biological control. Each value is the mean value from five replicates ± standard deviation.

Macrophyte	Biological Control	2 mg L^{-1}		6 mg L^{-1}	
		Cr(III) treatment	Cr(VI) treatment	Cr(III) treatment	Cr(VI) treatment
S. herzogii					
aerial parts	0.016 ±0.004	0.209 ± 0.016	0.332 ± 0.021	0.342 ± 0.017	0.880 ± 0.023
roots	0.031 ± 0.010	1.73 ± 0.19	1.091 ± 0.19	5.03 ± 0.19	2.57 ± 0.26
P. stratiotes					
aerial parts	0.011 ± 0.002	0.168 ± 0.019	0.249 ± 0.018	0.299 ± 0.026	0.527 ± 0.022
roots	0.021 ± 0.002	1.52 ± 0.102	0.939 ± 0.15	4.58 ± 0.21	1.86 ± 0.19

Figure captions

Fig. 1 Cr(III) and Cr(VI) removal from water (%) obtained along time for the two macrophytes and metal concentrations studied

Fig. 2 Cr(III) and Cr(VI) concentrations in water obtained along time for the two macrophytes and metal concentrations studied, according to Eq. (2)

Fig. 3 Relative growth rates (RGR) obtained at the end of the experiment in Cr(III) and Cr(VI) treatments compared with the control for the two macrophytes and metal concentrations studied. Bars represent standard deviations

Fig. 4 Micrographies of *P. stratiotes* (a) and *S. herzogii* (b) roots exposed to Cr(III), and precipitates on the surface of roots of *P. stratiotes* (c) where it was determined main relative relationships of Si an Cr.

Fig. 1.

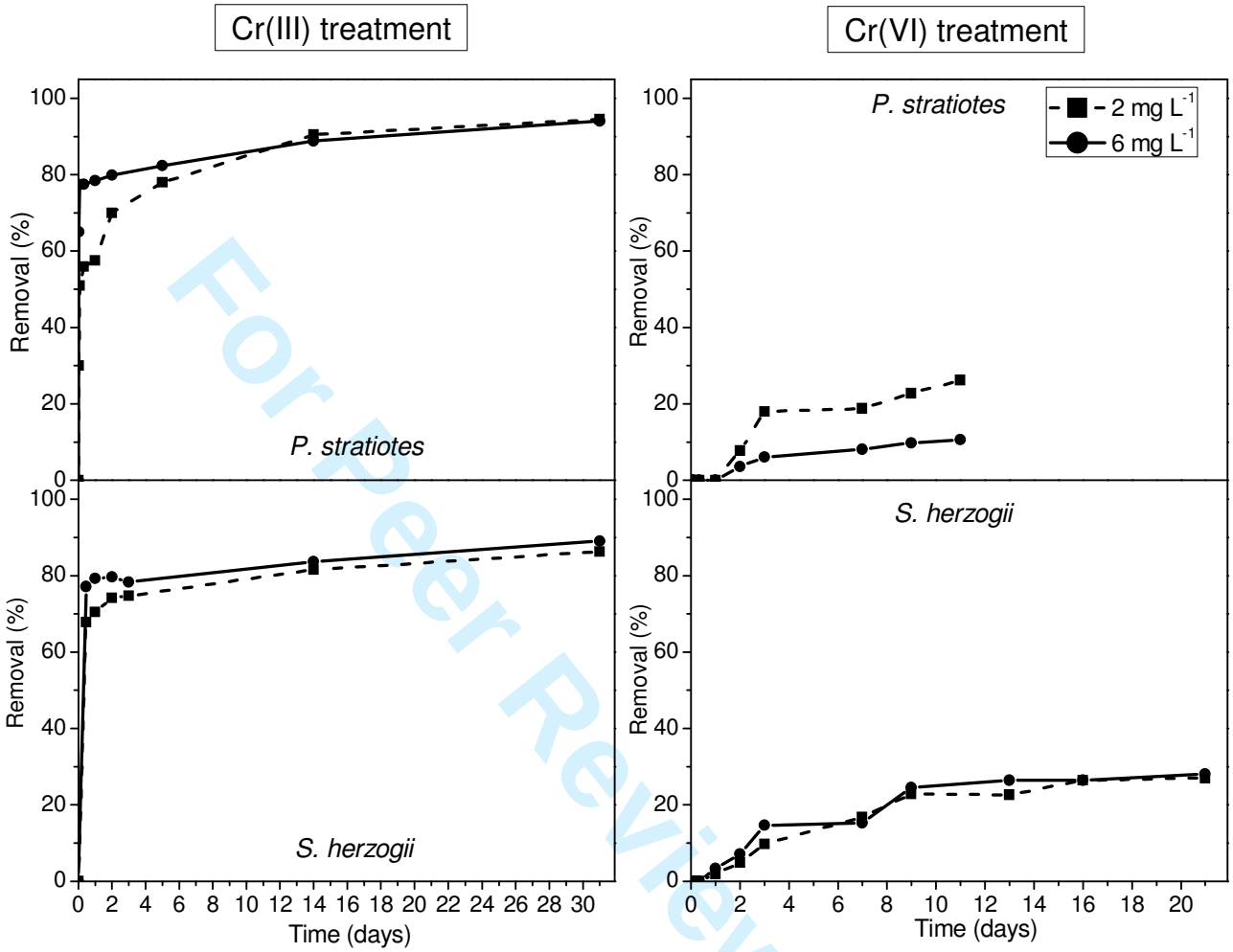


Fig. 2.

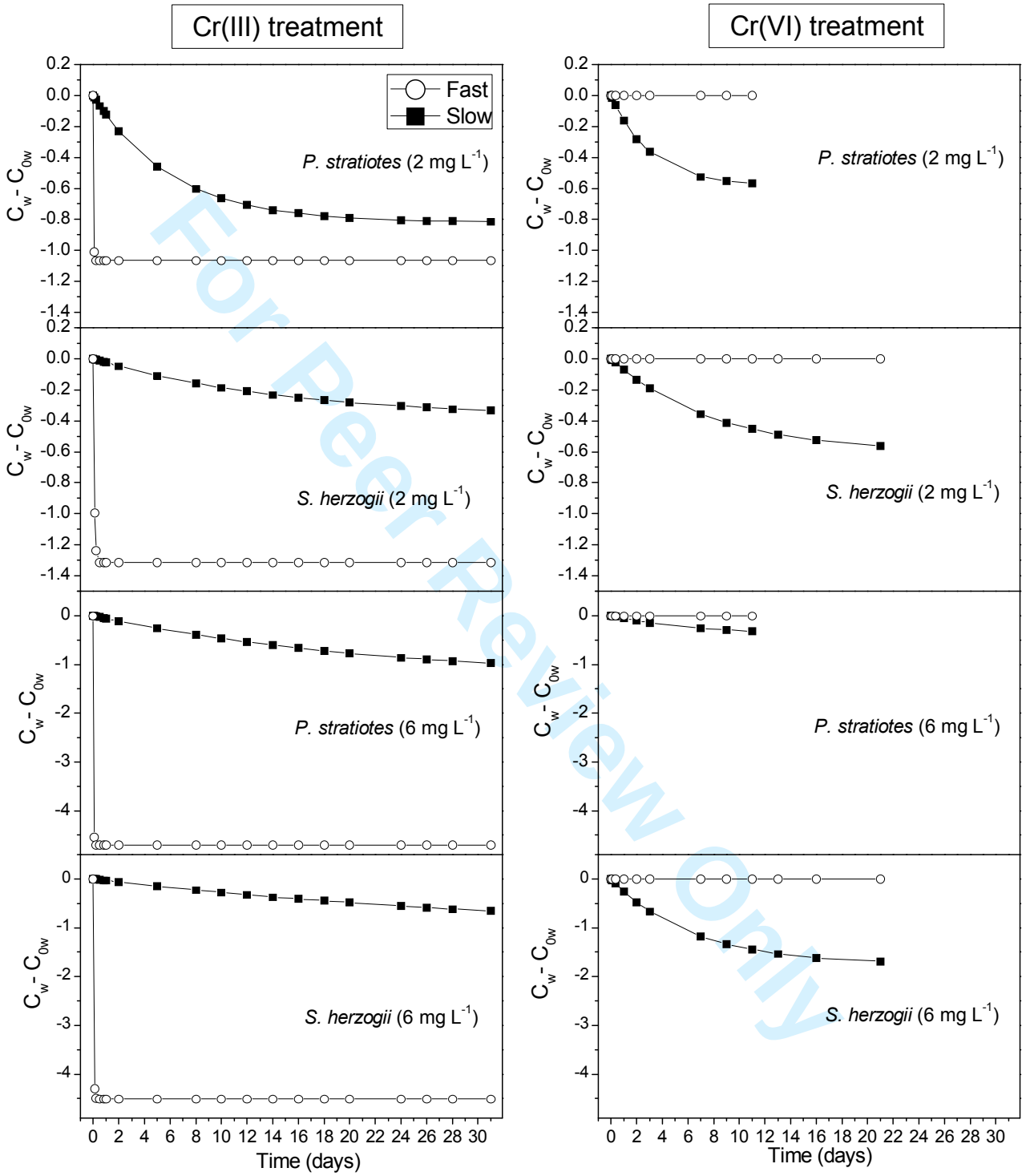
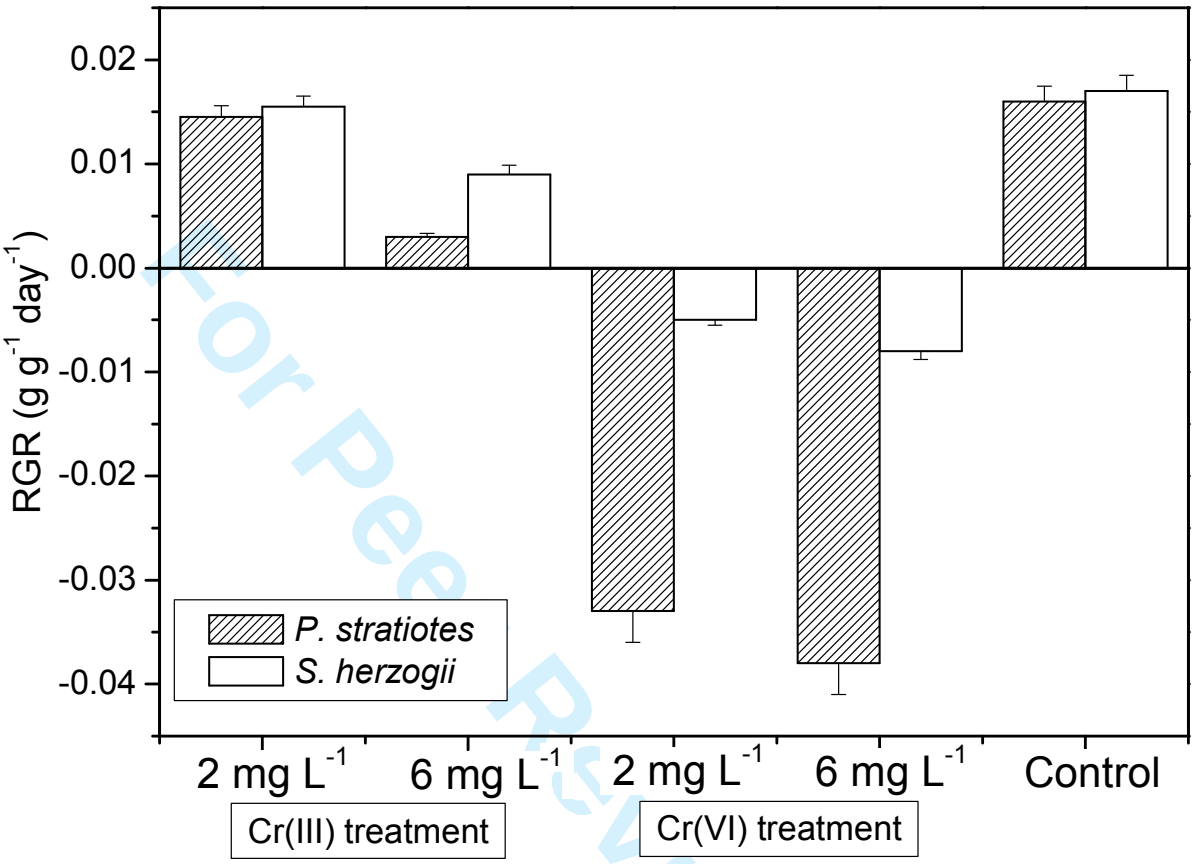


Fig. 3.



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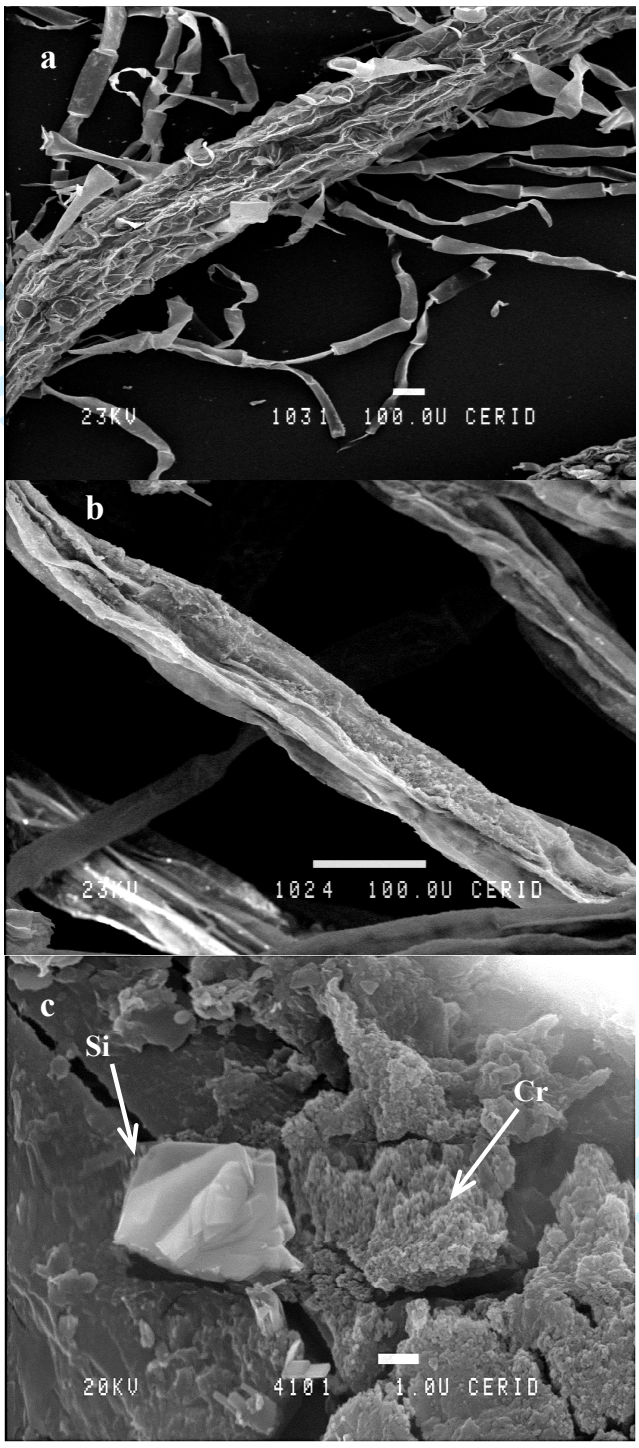
a
23KV 1031 100.0U CERID
b
23KV 1024 100.0U CERID
c
Si
Cr
20KV 4101 1.0U CERID