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Effect of solution pH on the dynamic of biosorption of Cr(VI) by living plants of *Salvinia minima*

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ABSTRACT

Salvinia minima is a widely distributed floating fern that bioaccumulates high concentrations of heavy metals and is a promising species for Cr(VI) removal from polluted waters. Metal bioaccumulation efficiency of aquatic plants can be affected by different factors such as surrounding pH and metal concentration. In this study we investigated the effect of solution pH on the biosorption and reduction of Cr(VI) in living plants of *S. minima* under both pH-stat (buffered) and pH-shift (unbuffered) conditions. Plants were exposed to 0, 5 and 20 mg L^{-1} Cr(VI) concentrations for 7 days under controlled conditions at pH 4.0, 6.0 and 7.6. Cr(VI) was determined using 1,5-diphenylcarbazide in presence and absence of $KMnO_4$. Cr(III) concentration was estimated as the difference between Cr_{total} [Cr(VI) + Cr(III)] (with $KMnO_4$) and Cr(VI) (without $KMnO_4$) concentrations. Metal biosorption was significantly higher in acid buffered solution than in unbuffered solution, but the biosorption pattern was different indicating that solution pH could be a key factor controlling the removal of Cr(VI) by *S. minima*. The pH of acid unbuffered Cr(VI)-containing solutions was significantly increased during *Salvinia* growth. Contrarily no significant changes of pH were observed in unbuffered solutions without Cr(VI). Reduction of Cr(VI) to Cr(III) was significantly higher at lower pH. Visual symptoms of Cr(VI)-induced damage were less evident at lower pH. Results demonstrated that *S. minima* can survive and remove Cr(VI) at low pH values. This work indicates that selection of suitable aquatic macrophytes for potential application in heavy metal phytoremediation requires an additional focus regarding interactions among metal, solution pH, and plant performance.

1. Introduction

Increasing industrialization and technological development during the last century has resulted in severe metal pollution of the environment. Heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), and mercury (Hg) are nowadays among the most important pollutants in aquatic environments (Kabata-Pendias, 2011). Cr is one of the most toxic pollutants commonly occurring in aquatic systems (Barrera-Díaz et al., 2012). Anthropogenic release of Cr from industries as leather tanning, electroplating, textile dyes, ceramic glazes, refractory bricks, chromic acid production, wood preserving, and refractory steel, constitute the main sources of environmental Cr pollution (Oliveira, 2012). Cr is not destroyed by degradation and for long time accumulates in the environment giving an increased contamination of soils and waters, which has raised severely the menace of this toxic element in the last few decades (Madhavi et al., 2013). In the environment Cr is found primarily in two oxidation states i.e., hexavalent form [Cr(VI)] and trivalent form [Cr (III)] (Zayed and Terry, 2003). Cr(VI) is very toxic to living organisms, being its toxicity due to its high redox potential, mobility, and ability to penetrate biological membranes. Cr(III) is less toxic and acts as essential microelement in mammalians, but in plants its role has not been elucidated yet (Das and Mishra, 2008). Increasing accumulation of Cr(VI) in the environment and its possible repercussion on population health has lead to develop different technologies to overcome Cr(VI) contamination. Conventional physicochemical methods used in Cr(VI) removal from contaminated wastewaters include both ion exchange resins and electro kinetic procedures. These procedures have high cost and lead to formation of harmful by-products (Madhavi et al., 2013). Thus, biosorption processes appear as a more attractive technology

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for Cr(VI) removal. They are characterized by their low cost and high capacity of metal removal, especially from solutions with low metal concentrations (Saha and Orvig, 2010). Non-living and living biological materials have been used to Cr(VI) biosorption, but a literature survey revealed that most of biosorption procedures have been performed using non-living biomass from bacteria, fungi, seaweed, micro-algae and plants (Park et al., 2006). Among living biomass, aquatic macrophytes (floating, emergent and submerged) appear as suitable candidates to remove Cr(VI) from polluted waters (Krems et al., 2013).

Both biosorption and bioaccumulation occur in metal-absorbing plants, but they are different processes. In the first one, metal is accumulated on the plant surface whereas in the second one it is accumulated either inside (intracellular accumulation) or outside (apoplastic accumulation) of cells (Chojnacka, 2010). Biosorption is a passive, non-energy-dependent, fast and reversible mechanism that takes place in both living and non-living plants. It is produced by physical adsorption, ion exchange, precipitation or complexation with ligands occurring on the biosorbent surface. By contrast, bioaccumulation is an active, energy-dependent and slow process that only occurs in living plants. Together, biosorption and bioaccumulation constitute the bioremoval capacity, i.e., plant ability to remove heavy metals. In fact, both processes are a component part of all currently used bioremediation technologies (Oporto et al., 2008). Biosorption is based on an ion exchange mechanism in which protons compete with metal cations by binding sites on the biosorbent surface. In non-living plants depending on the solution pH, functional groups on the cell wall can bond or release protons giving a positively or negatively charged cell surface (Elangovan et al., 2008; Miretzky and Fernandez Cirelli, 2010). By contrast in living plants the availability of functional groups to bond or release protons is mainly controlled by cellular metabolism (Krzesłowska, 2011). Notwithstanding the cellular metabolism can be affected by solution pH, which influences biochemical reactions involved in the protonation/deprotonation of cell wall functional groups (Bhatia and Ashwath, 2005). To avoid metabolic alterations induced by solution pH, the intracellular pH of plant cells is maintained within a narrow range (7.2–7.5) that is compatible with all metabolic functions (Felle, 2001). In fact the regulation of intracellular pH, especially of the cytoplasm, is the most basic homeostatic process that takes place in living cells, even following various environmental disturbances (Pittman, 2012). In addition the solution pH also affects the mobility and availability of metal ions with cationic species being more soluble at pH below 7.0 and anionic forms more soluble at pH above 7.0 (Chojnacka, 2010). Therefore, the pH of polluted solution becomes a crucial factor of the metal biosorption process in both non-living and living plants.

Cr(VI) is known to have phytotoxic, genotoxic and cytotoxic effects on both terrestrial an aquatic plants (Rodríguez et al., 2011; Oliveira, 2012; Eleftheriou et al., 2013). Toxic effects of Cr(VI) are generally attributed to its propensity to induce oxidative stress, and also generate reactive oxygen species (ROS) in cellular systems (Shanker et al., 2005; Prado et al., 2010a; Patnaik et al., 2013). ROS-induced toxic effects of Cr(VI) have been extensively studied in many plant species at both cellular and molecular levels (Zayed and Terry, 2003; Chandra and Kulshreshtha, 2004; Shanker et al., 2005; Oliveira, 2012; Santos and Rodriguez, 2012). In contrast visual symptoms of Cr(VI) damage occurring in plant leaves have been reported only for a few species (Sharma et al., 2003; Paiva et al., 2009; Gopal and Sharma, 2014). Depending on metal concentration visual symptoms of Cr(VI) damage can vary from chlorotic leaves [5–10 mg L^{-1} Cr(VI)] to brownish-red colored leaves with necrotic areas and even wilting [25–50 mg L^{-1} Cr(VI)] (Zayed and Terry, 2003). In polluted aquatic systems changes of pH and richness of Cr species frequently occur (Javed and Greger, 2011), so to get a better phytoremediation performance of metal-accumulating plants will be necessary to investigate how changes of surrounding solution pH affect both metal biosorption and plant health. Thus, the aim of the present work was to study the effect of solution pH on biosorption and reduction of Cr(VI) occurring in living plants of *S. minima* cultivated in buffered and unbuffered solutions. Desorption of Cr species and visual symptoms of Cr(VI) damage were also analyzed.

2. Materials and methods

2.1. Plant material and growth conditions

Healthy plants of *S. minima* with similar size and weight were collected from a heavy metal non-polluted water pond located at 500 m a.s.l (Tucumán, Argentina, 26° 50′S, 65° 12′W). Collected plants were thoroughly washed with running tap water to eliminate sediment particles and microalgae. After that, plants were transferred to 140-L clean plastic tank filled with tap water (130 L) for 3 days under outdoor conditions. This treatment was performed to recovery stressed plants. Uniform plants with fully expanded leaves were transferred to plastic trays containing 150 mL of different Cr(VI) solutions. Cr(VI) solutions were obtained from a stock solution (500 mg L^{-1}) prepared with analytical grade potassium dichromate ($K_2Cr_2O_7$). All metal solutions were prepared using tap water in order to get an approximately similar aquatic medium to that of collected plants. Similarly, plants were not cultivated in Hoagland's solution to avoid chelation and/or ion competition between Hoagland ions and dichromate anion ($Cr_2O_7^{2-}$) for cell wall binding sites (Prado et al., 2010b). For each tray 15 plants with similar size and shape (~35 g FW) were transferred from acclimation tank. Plants were exposed to 0 (control), 5 and 20 mg L^{-1} Cr(VI) concentrations during 7 days under controlled conditions: 200 μmol m^{-2} s^{-1} light intensity, 12 h dark/light cycle, 80% relative humidity and 25/20 ± 1 °C day/night temperature.

2.2. Determination of Cr(VI) and Cr(III)

Cr(VI) and Cr(III) were determined using 1,5-diphenylcarbazide (APHA, 2005). To Cr(III) determination, 1,5-diphenylcarbazide method was performed in presence and absence of potassium permanganate ($KMnO_4$) as oxidant (Memon et al., 2006). Cr(III) was estimated as the difference between Cr_{total} [Cr(VI) + Cr(III)] (determined in presence of $KMnO_4$), and Cr(VI) (determined in absence of $KMnO_4$).

In spite of that other Cr species such as Cr(II), Cr(IV) and Cr(V) can be present, they have very short half-life and are unstable at room temperature (Zayed and Terry, 2003). Therefore, in this study was assumed that Cr_{total} corresponds to Cr(VI) + Cr(III) species only. Reliability of colorimetric method was checked by a calibration curve made from $K_2Cr_2O_7$ standard solution in the range of 0.5 mg L^{-1} and 50 mg L^{-1} Cr(VI) concentration in presence and absence of Cr(III). Standard deviation of calibration curve was 0.0044, which indicated a good fit of data and within an error limit <2%. In a previous study carried out in our laboratory, no significant difference was found in Cr(VI) concentration determined either by 1,5-diphenylcarbazide method or atomic absorption spectrometry (AAS) (Prado, 2012). To test whether the McIlvaine buffer interferes with 1,5-diphenylcarbazide assay, a standard curve was made from a $K_2Cr_2O_7$ standard solution prepared in 10 mM McIlvaine buffer. No differences in absorbance values were observed. Further, Cr(VI) was determined in both Cr (VI)-containing tap water and Cr(VI)-containing McIlvaine buffer every day during the experimental period. No change in Cr(VI)

concentration was observed indicating that no spontaneous reduction of Cr(VI) occurred (not shown).

2.3. Biosorption of Cr(VI) on S. minima cell wall

The biosorption process was analyzed using living plants to mimic conditions that occur in natural environments. The progress of Cr(VI) biosorption was calculated as the difference between initial Cr(VI) concentration in treatment solution and Cr_{total} concentration in remaining solution for each sampling time. Biosorption of Cr(VI) was expressed as percentage.

2.4. Effect of pH on Cr(VI) biosorption

To analyze the effect of pH on Cr(VI) biosorption two set of experiments were performed: (i) pH-shift experiment (unbuffered pH), (ii) pH-stat experiment (buffered pH). In pH-shift experiment, Cr(VI) solutions (0, 5 and 20 mg L^{-1}) were initially adjusted to selected pH values (4.0, 6.0 and 7.6) by using 0.5 M HCl or 0.5 M NaOH. Thereafter, pH was not adjusted until the end of the experiment. In pH-stat experiment, Cr(VI) solutions were prepared using a 10 mM McIlvaine buffer. This buffer was chosen to use a unique buffer solution for all selected pH values. Buffer molarity was relatively low to avoid addition of high amount of external ions that may influence Cr(VI) biosorption. Selected pH values were similar to those commonly recorded in wastewater effluents and acid mine drainages of the Tucumán province and other provinces of the northwest region of Argentina (unpublished data). Plants cultivated in tap water and Cr(VI)-containing tap water were used as controls. Every two days, tap water or McIlvaine buffer solution was added to reach initial volume to compensate water loss by evapotranspiration. The pH of treatment solutions was monitored with a digital pH meter (Adwa AD1000, Hungary). Measurements were made at 0 (immediately after to transfer plants to treatment solutions), 3, 5, and 7 days after starting the experiment. After each pH measurement cycle, a 5 mL sample from each treatment solution was collected to determine residual Cr(VI) and Cr(III). Four replications were undertaken for each Cr(VI) concentration and each pH value.

2.5. Recovery of Cr(VI) and Cr(III)

To recovery sorbed Cr, plants were harvested at ending of the experiment, drained on filter paper and transferred to 100 mL deionized water. After incubate at room temperature for 15 min under constant stirring, plants were transferred to 100 mL 0.5 M HCl and incubated again. Finally, plants were transferred to 100 mL 0.5 M NaOH and incubated as described. Washing solutions were used to quantify desorbed Cr(VI) and Cr(III).

2.6. Extrusion of protons

Plants grown in both tap water and Cr(VI)-containing tap water were collected at ending of treatment period, washed thoroughly with deionized water and used to measure proton extrusion (Chen et al., 1990). Briefly, 10 uniform plants with intact leaves were transferred to 50 mL of measuring solution containing 1 mM Tris–HCl buffer (pH 7.0), 0.5 mM calcium chloride ($CaCl_2$) and 50 mM potassium chloride (KCl). This last one was used to get a more significant ΔpH. Measuring solution was constantly stirring using a micro magnetic stir bar. Changes of pH in measuring solution were recorded over 30 min using a digital pH meter (Adwa AD1000, Hungary). Proton extrusion was expressed as percentage, assuming that maximum ΔpH obtained at 0 mg L^{-1} Cr(VI) concentration (Cr-untreated plants) corresponds to 100%. Proton extrusion was also measured after addition sodium vanadate (Na_3VO_4) (15 μM) to measuring solution. Sodium vanadate is a well-known inhibitor of plasma membrane H^+-ATPase activity (Yan et al., 1998).

2.7. Visual symptoms of Cr(VI) effects

At 0, 3 and 7- d cultivation period, plants were carefully observed to establish visual symptoms of metal effects. Analysis of temporal pattern of visual symptoms was performed from a photographic record obtained with a digital camera (Sony DSC-S60, Japan). Photographs of control and Cr(VI)-exposed plants were taken under white light (fluorescent lamps) at the same time. Images were edited to background subtraction and also to correct the contrast and brightness. Image processing was performed using the Corel PHOTO-PAINT X3 software (Corel Inc., USA)

2.8. Statistical analysis

Data are means of three independent replicates. Data were processed by analysis of variance (ANOVA) and means were compared using the Tukey's test at 5% level ($p < 0.05$).

3. Results and discussion

3.1. Biosorption of Cr(VI) on S. minima cell wall at different pH

In a previous work carried out in our Laboratory was demonstrated that *S. minima* cultivated in a Cr(VI)-containing tap water solution was able to accumulate on the surface of lacinias (root-like structures) up to 6 fold more chromium than fronds (Prado, 2012). Agreeing with this result in a previous work Olguín and co-workers demonstrated that the predominant mechanism of Pb(II) removal by living *S. minima* was the surface adsorption (Olguín et al., 2005). The removal of metals from metal-containing solutions by living aquatic macrophytes includes two major processes: (a) a metabolically-independent mechanism that occurs on the plant surface (biosorption), and (b) an energy-dependant process that takes place inside the plant (bioaccumulation) (Chojnacka, 2010). Although adsorption is a binding physicochemical reversible process between dissolved ions from an aqueous solution and binding sites on the adsorbent surface (Nibou et al., 2010), biosorption is a more complex phenomenon that includes adsorption, absorption, surface complexation and ion precipitation onto the cell wall (Fomina and Gadd, 2014). The biosorption of heavy metals by both living and non-living plants closely depends on cell wall composition that varies notably with plant age and among species (Guigues et al., 2014), and physicochemical characteristics of the metal solution (Davis et al., 2003). Proton-metal cation exchange mechanism is the major trait of the metal biosorption process on the cell wall (Meychik and Yermakov, 2001). Depending on the solution pH, binding sites on the cell wall can be pronated or deprotonated giving a positively or negatively charged biosorbent surface (Miretzky and Fernandez Cirelli, 2010). At low pH values the protonation of cell wall binding sites becomes significant, and then an increased availability of anion binding sites occurs. In this condition the biosorption of metal cations such as Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+} and Al^{3+} is often reduced due to competition between dissolved cations and solution protons. Conversely, anionic species like TcO_4^-, $PtCl_4^{3-}$, CrO_4^{2-}, $HCrO_4^-$, $Cr_2O_7^{2-}$and SeO_4^{2-} show an increased biosorption rate (Fomina and Gadd, 2014). When the pH of solution increases, metal binding sites are deprotonated and then the availability of anion binding sites decreases (Elangovan et al., 2008). In strong acidic conditions (pH 2.0–3.6), hydrogen chromate ($HCrO_4^-$) is the prevalent anionic species that subsequently shifts to chromate (CrO_4^{2-}) and ($Cr_2O_7^{2-}$) species under rising pH. At pH higher than 7.0, $Cr_2O_7^{2-}$ is the predominant species

found in aqueous solutions (Kotaś and Stasicka, 2000). Agreeing with these findings our data show that after a 7-d cultivation period under buffered (pH-stat) and unbuffered (pH-shift) conditions, highest and lowest biosorption percentages of Cr(VI) on the cell wall of *S. minima* plants occur at pH 4.0 and 7.6 respectively, in both 5 and 20 mg L^{-1} Cr(VI) (Fig. 1). By comparison with biosorption percentage obtained from a Cr-containing tap water solution (control), biosorption percentage found at pH 4.0 under pH-stat condition was significantly higher than the control whereas under pH-shift was significantly lower. In addition, decreases of biosorption percentages at pH 6.0 and 7.6 were more pronounced under pH-shift condition compared with pH-stat condition (Fig. 1). Time-dependant pattern of Cr(VI) biosorption under pH-stat and pH-shift conditions is shown in Fig. 2. In pH-stat condition the biosorption of metal was faster at pH 4.0 than that at pH 6.0 and 7.6. After a 3-d treatment period, biosorption percentages were 68% and 78% in presence of 5 and 20 mg L^{-1} Cr(VI) concentrations, respectively. In pH-shift condition, Cr(VI) biosorption was faster at pH 6.0 reaching a 43% biosorption after a 3-d treatment period with 5 mg L^{-1} Cr(VI) concentration; while in presence of higher Cr(VI) concentration, it was faster at pH 7.6 (17%). For all cultivation conditions lower biosorption percentages always occurred at higher Cr(VI) concentration. Although this result seems to be unexpected, can be explained by assuming that at low Cr(VI) concentration a relative higher number of binding sites are available on the cell wall. In this condition more Cr ions can bind to adsorption sites giving a higher biosorption percentage. However, it can also be explained by assuming that at high Cr(VI) concentration, Cr ions diffuse to cell wall through an intraparticle diffusion gradient and also greatly through hydrolyzed Cr ions themselves that diffuse slower. On a time-based comparison, at 20 mg L^{-1} Cr(VI) concentration less Cr ions may reach metal binding sites on the cell wall than at 5 mg L^{-1} Cr(VI) concentration, and then *S. minima* becomes more efficient to remove Cr(VI) from dilute solutions.

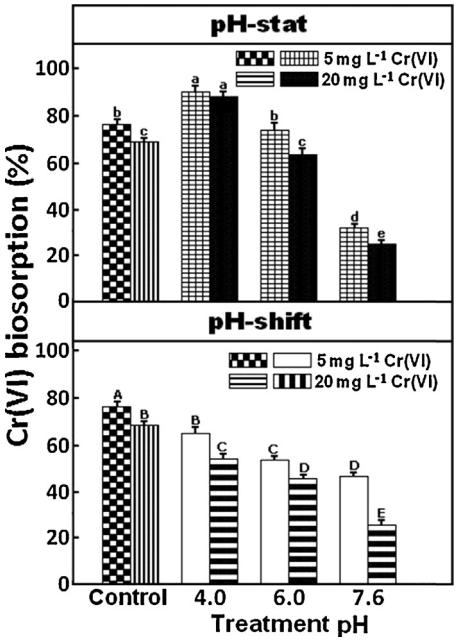

Fig. 1. Biosorption of Cr(VI) on *S. minima* cell wall after a 7-d cultivation period in Cr (VI)-containing tap water (control) and both Cr(VI)-containing buffered and unbuffered solutions. Different letters on bars indicate significant differences at $p < 0.05$ ($n = 6$). Lowercase letters are used to denote significance in buffered solution (pH-stat condition) and uppercase letters to denote significance in unbuffered solution (pH-shift condition).

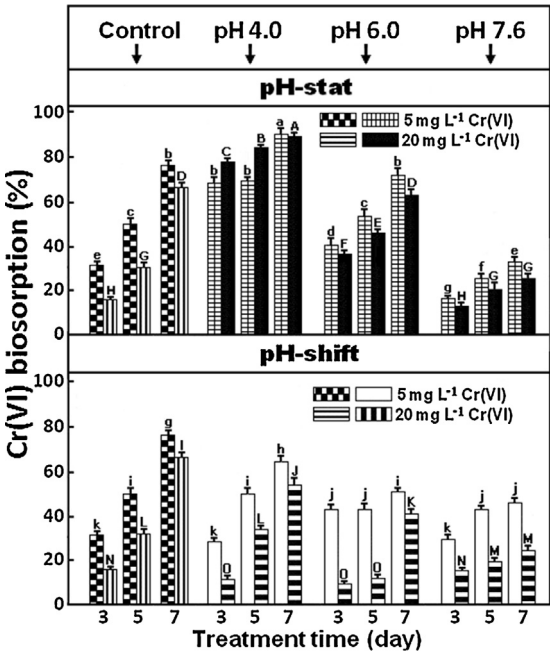

Fig. 2. Time-dependant biosorption of Cr(VI) at different pH in both pH-stat and pH-shift conditions. Cr(VI)-containing tap water was included as control. Bars indicate standard deviation. Different letters on bars indicate significant differences at $p < 0.05$ ($n = 6$). Lowercase letters are used to denote significance at 5 mg L^{-1} Cr (VI) concentration and uppercase letters to denote significance at 20 mg L^{-1} Cr(VI) concentration.

3.2. Changes of solution pH and extrusion of protons triggered by S. minima

Differences observed in biosorption percentages and time-dependant biosorption patterns with all Cr(VI)-containing solutions (Figs. 1 and 2), indicate an indubitable involvement of both buffering capacity of treatment solutions and plant metabolic activity in the biosorption process of Cr(VI) that takes place on the cell wall of living *Salvinia* plants. Under pH-shift condition the buffering capacity of treatment solution does not occur, and then the pH of root surrounding solution (rhizosphere) becomes controlled by metabolic activity. Enhanced secretion of organic anions (e.g., citrate, malate, oxalate) by roots has been recognized as common trait of heavy metal-stressed plants (Zeng et al., 2008; Javed and Greger, 2011). Besides direct complexation with metal

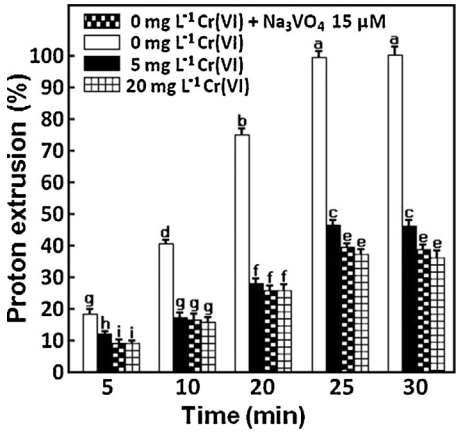

Fig. 3. Temporal evolution of proton extrusion from *S. minima* plants after a 7-d cultivation period in Cr(VI)-containing tap water and tap water without Cr(VI). Different letters on bars indicate significant differences at $p < 0.05$ ($n = 6$).

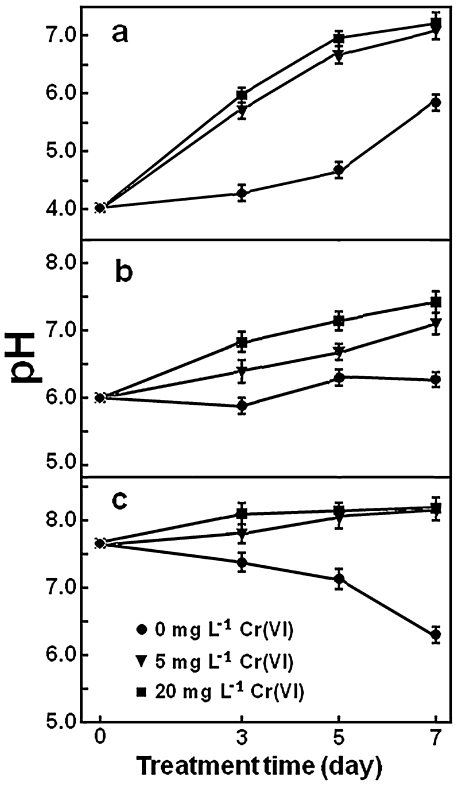

Fig. 4. Time-dependant pH change of treatment solution under pH-shift condition during the cultivation of *S. minima* in presence and absence of Cr(VI). (a) starting pH 4.0; (b) starting pH 6.0; (c) starting pH 7.6. Bars indicate standard deviation at $p < 0.05$ ($n = 6$).

cations, organic anions interact with functional groups (binding sites) of the cell wall affecting their protonation/deprotonation status, and with free protons that modifies the pH of root surrounding solution (rhizosphere) (Hinsinger et al., 2003). Under buffered conditions there are no pH changes and then organic anions only interact with cell wall functional groups giving either protonated or deprotonated binding sites according to the solution pH. In contrary under unbuffered conditions organic anions interact with both cell wall and free protons (Yang et al., 2013). In this context, under unbuffered acidic conditions organic anions bind protons increasing the pH of surrounding solution (rhizosphere alkalization) that affects the protonation/deprotonation status of the cell wall and also the solubility and availability of metal ions. We did not measure the release of organic anions by *S. minima*, and then we cannot confirm their involvement in the regulation of rhizosphere pH.

Beyond the role of organic anions to regulate the rhizosphere pH in heavy metal-exposed plants, the release of protons associated to plasma membrane H^+-ATPase activity also contributes significantly to this regulation (Janicka-Russak et al., 2008). Proton extrusion was significantly inhibited by Cr(VI) in *Salvinia* plants. Maximum inhibitions (−55.4% and −63.9%) for 5 and 20 mg L^{-1} Cr(VI) concentrations were observed 25 min after beginning the recording of ΔpH. From this time on, proton extrusion stayed unchanged (Fig. 3). Addition of Na_3VO_4 also reduced strongly proton extrusion (−62.3%) being similar at the two Cr(VI) concentrations (Fig. 3). This indicate that proton efflux in Cr(VI)-exposed *S. minima* is mainly associated to plasma membrane H^+-ATPase activity. Cr(VI)-induced inhibitions of plasma membrane H^+-ATPase activity and proton extrusion have also been observed in other plant species (Rai et al., 2004; Najafian et al., 2012). By assuming that plasma membrane H^+-ATPase activity contributes to establish the rhizosphere pH in Cr(VI)-exposed plants and depending on initial solution pH, different temporal pH change patterns occurred in treatment solutions under pH-shift condition during the cultivation of *S. minima* (Fig. 4). Starting from pH 4.0, after a 7-d cultivation period, the pH of treatment solution increased by about 3.0 pH units, while in unbuffered solution without Cr(VI) only increased by 1.8 pH units (Fig. 4a). Starting from pH 6.0, the pH of treatment solution increased about 1.5 pH units in the presence of Cr(VI) whereas in absence of metal no significant change was observed (Fig. 4b). Starting from pH 7.6, the value of solution pH only increased slightly (0.2 pH unit) in presence of 20 mg L^{-1} Cr(VI) concentration, while in presence of 5 mg L^{-1} decreased slightly. In absence of Cr (VI) a pronounced decrease of pH value was observed (Fig. 4c). Of interest, in absence of Cr(VI), solution pH tends to reach a similar value independently of starting pH value (Fig. 4a-c). According with Jones (1998) under stress conditions the rhizosphere pH changes strongly whereas under unstressed conditions tends to maintain a constant value. Under pH-stat condition there were no pH changes in presence and absence of Cr(VI) (not shown). Lack of pH changes under pH-stat condition does not necessarily mean that *Salvinia* plants do not release protons, because they may have been consumed in chemical reactions that contribute to buffering capacity of the treatment solution. Further studies are needed to establish contribution of proton extrusion and plasma membrane H^+-ATPase activity to maintain the homeostasis of rhizosphere pH in Cr-exposed aquatic macrophytes.

Table 1
Biosorption of Cr(VI) by *S. minima* plants cultivated in Cr(VI)-containing tap water (control) and both Cr(VI)-containing buffered and unbuffered solutions during 7 days in a controlled environment. Biosorption percentages were calculated based on determinations of Cr(VI) and Cr_{total} [Cr(VI) + Cr(III)] in remaining treatment solutions. Within each row percentage values followed by the same lowercase letter are not significantly different. Within each column values followed by the same uppercase letter are not significantly different, $p < 0.05$ ($n = 6$).

Initial conc. (mg L^{-1})	Biosorption (%)					
	(Based on Cr(VI) determination)			(Based on Cr_{total} determination)		
	Control (tap water)			Control (tap water)		
5	$73.5 \pm 2.9_{aB}$			$73.9 \pm 3.0_{aA}$		
20	$63.7 \pm 2.0_{aC}$			$63.7 \pm 2.5_{aB}$		
	pH-stat (buffered solution)			pH-stat (buffered solution)		
	pH 4.0	pH 6.0	pH 7.6	pH 4.0	pH 6.0	pH 7.6
5	$88.2 \pm 2.4_{aA}$	$71.6 \pm 3.1_{bB}$	$34.1 \pm 1.8_{dF}$	$77.8 \pm 3.1_{bA}$	$69.7 \pm 3.4_{bA}$	$31.6 \pm 2.2_{dE}$
20	$86.7 \pm 2.8_{aA}$	$63.2 \pm 2.1_{cC}$	$23.8 \pm 2.3_{eG}$	$73.6 \pm 2.7_{bA}$	$59.9 \pm 2.2_{cB}$	$24.1 \pm 1.9_{eF}$
	pH-shift (unbuffered solution)			pH-shift (unbuffered solution)		
	pH 4.0	pH 6.0	pH 7.6	pH 4.0	pH 6.0	pH 7.6
5	$63.1 \pm 1.4_{aC}$	$53.9 \pm 1.5_{bD}$	$46.1 \pm 3.7_{cE}$	$63.2 \pm 1.1_{aB}$	$53.9 \pm 1.8_{bC}$	46.4 ± 2.1cD
20	$54.2 \pm 1.2_{aD}$	$46.2 \pm 1.5_{bE}$	$23.5 \pm 2.1_{cF}$	$53.7 \pm 1.5_{aC}$	$42.5 \pm 3.1_{bD}$	$22.1 \pm 2.9_{cE}$

3.3. Reduction of Cr(VI) by S. minima

Metal biosorption percentage obtained on the basis of Cr(VI) concentration remaining in the treatment solution at ending of the experimental period at pH 4.0 and pH.stat condition was significantly higher than that obtained using Cr_{total} [Cr(VI) + Cr (III)] remaining concentration. No significant changes were found at pH 6.0 and 7.6 (Table 1). This lead us to assume that a plant-related reduction of Cr(VI) to Cr(III) must be occurring in *S. minima* grown in a relatively strong acidic solution. Taking account the possibility that the reduction of Cr(VI) can occur spontaneously in Cr(VI)-containing solutions, we determined both Cr(VI) and Cr_{total} in plant-free Cr(VI)-containing solutions maintained under similar growth conditions. No reduction of Cr(VI) was detected at the three pH values (not shown). This confirms that a *Salvinia*-mediated Cr (VI) reduction occurred at the lower pH. Supporting our result, it has been proven that when Cr(VI) comes in contact with biomaterials in strong acidic solutions can be easily reduced to Cr(III) due to its high redox potential (+1.33 V at pH 1.5–3.7). Contrarily in weak acidic solutions (pH 5.5–6.5) there is no reduction of Cr(VI) due to its low redox potential (−0.13 V at pH 6.0) (Park et al., 2006). Agreeing with this finding in the present work Cr(III) was not detected when the pH of remaining Cr(VI)-containing solution was 6.0 (Table 1). Plant-mediated Cr(VI) reductions under strong acidic conditions have also been reported for other plant species (Lytle et al., 1998; Aldrich et al., 2003; Popuri et al., 2007; López-García et al., 2013).

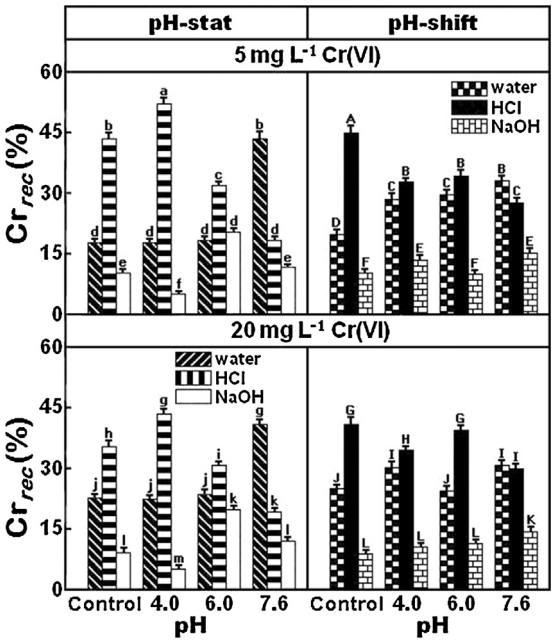

Fig. 5. Total Cr recovered (Cr_{rec}) from *S. minima* plants cultivated under pH-stat and pH-shift conditions after washed with deionized water, HCl and NaOH. Bars indicate standard deviation. For each Cr(VI) concentration and for each pH condition, different letters on bars indicate significant differences at $p < 0.05$ ($n = 6$). Lowercase letters are used to denote significance in pH-stat condition and uppercase letters to denote significance in pH-shift condition.

3.4. Recovery of biosorbed Cr species

Total Cr recovery (Cr_{rec}) from *Salvinia* plants exposed to Cr(VI) during 7 days in buffered and unbuffered Cr(VI)-containing solutions as well as in Cr(VI)-containing tap water, by using deionized water, 0.5 M HCl and 0.5 M NaOH as desorbents is shown in Fig. 5. Higher amounts of biosorbed chromium were released by washing either with deionized water or HCl, whereas the lower amount was released by alkaline washing. Analysis of recovered Cr indicates that both $Cr(III)_{rec}$ and $Cr(VI)_{rec}$ species were present in washing solutions (Fig. 6). In pH-stat condition, desorption of Cr $(VI)_{rec}$ was significantly lower at pH 4.0. A decreased recovery of Cr (VI) surely reflects an increased Cr(VI) reduction. Confirming this assumption, higher amounts of $Cr(III)_{rec}$ were obtained by acid washing from plants grown at pH 4.0 (relatively strong acidic condition) in both 5 and 20 mg L^{-1} Cr(VI) concentrations. By contrast at pH 6.0 (weak acidic condition) the recovery of $Cr(III)_{rec}$ was much lower than that at pH 4.0 (Fig. 6). Although this may indicate that $Cr(III)_{rec}$ desorbed from *Salvinia* plants is mainly produced by a pH-dependant reduction of Cr(VI), the decreased recovery of $Cr(VI)_{rec}$ could also be explained by the statement of Park et al. (2004). According to Park's statement when there is a small number of electron-donor groups on the biomass surface or free protons in the aqueous phase, Cr(VI) can remain bound on biomass surface without reduction, and then a less amount of Cr (VI) is released by wash. Since $Cr(III)_{rec}$ was also recovered from plants grown at pH 7.6 it can assume that a pH-independent

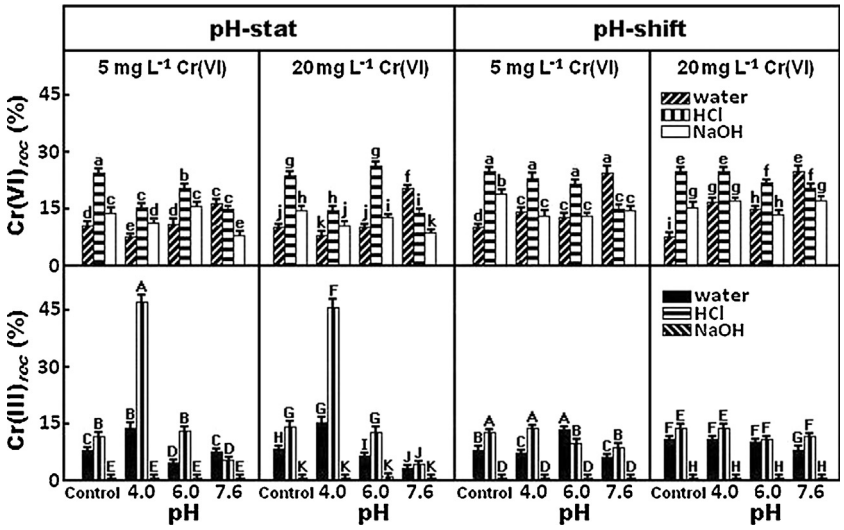

Fig. 6. Recovery of $Cr(VI)_{rec}$ and $Cr(III)_{rec}$ from *S. minima* plants after washed with deionized water, HCl and NaOH in both pH-stat and pH-shift conditions. Bars indicate standard deviation. For each Cr species and for each Cr(VI) concentration in treatment solution, different letters on bars indicate significant differences at $p < 0.05$ ($n = 6$). Lowercase letters are used to denote significance in $Cr(VI)_{rec}$ species and uppercase letters to denote significance in $Cr(III)_{rec}$ species.

reduction of Cr(VI) also occurs on *Salvinia* surface (Fig. 6). pH-dependant and pH-independent reductions of Cr(VI) have been observed in some aquatic macrophytes (Lytle et al., 1998; Zazo et al., 2008; Espinoza-Quiñones et al., 2009). Similarly, the higher release of $Cr(VI)_{rec}$ with deionized water from *Salvinia* plants grown at pH 7.6, could indicate that $Cr_2O_7^{2-}$ ions are maintained on lacinia surface by physical binding (adsorption), by weak binding to cell wall functional groups and/or by slightly complexation with secreted organic acids. In support of the more labile retention of Cr (VI) on *S minima* surface at pH 7.6, recently was communicated that in the hyperaccumulator aquatic macrophyte *Callitriche cophocarpa* growing in presence of Cr(VI), 34% of biosorbed Cr was found in water soluble fraction whereas another 23% occurred as easily mobile fraction (Augustynowicz et al., 2013).

Under pH-shift condition the most remarkable trait was the strong decrease observed in the recovery of $Cr(III)_{rec}$ from plants grown at pH 4.0, when comparing with plants grown at the same pH under pH-stat condition (Fig. 6). Decreased recovery of $Cr(III)_{rec}$ under pH-shift condition could be explained by the Cr-induced rise of solution pH occurring during the plant growth (Fig. 4a). This

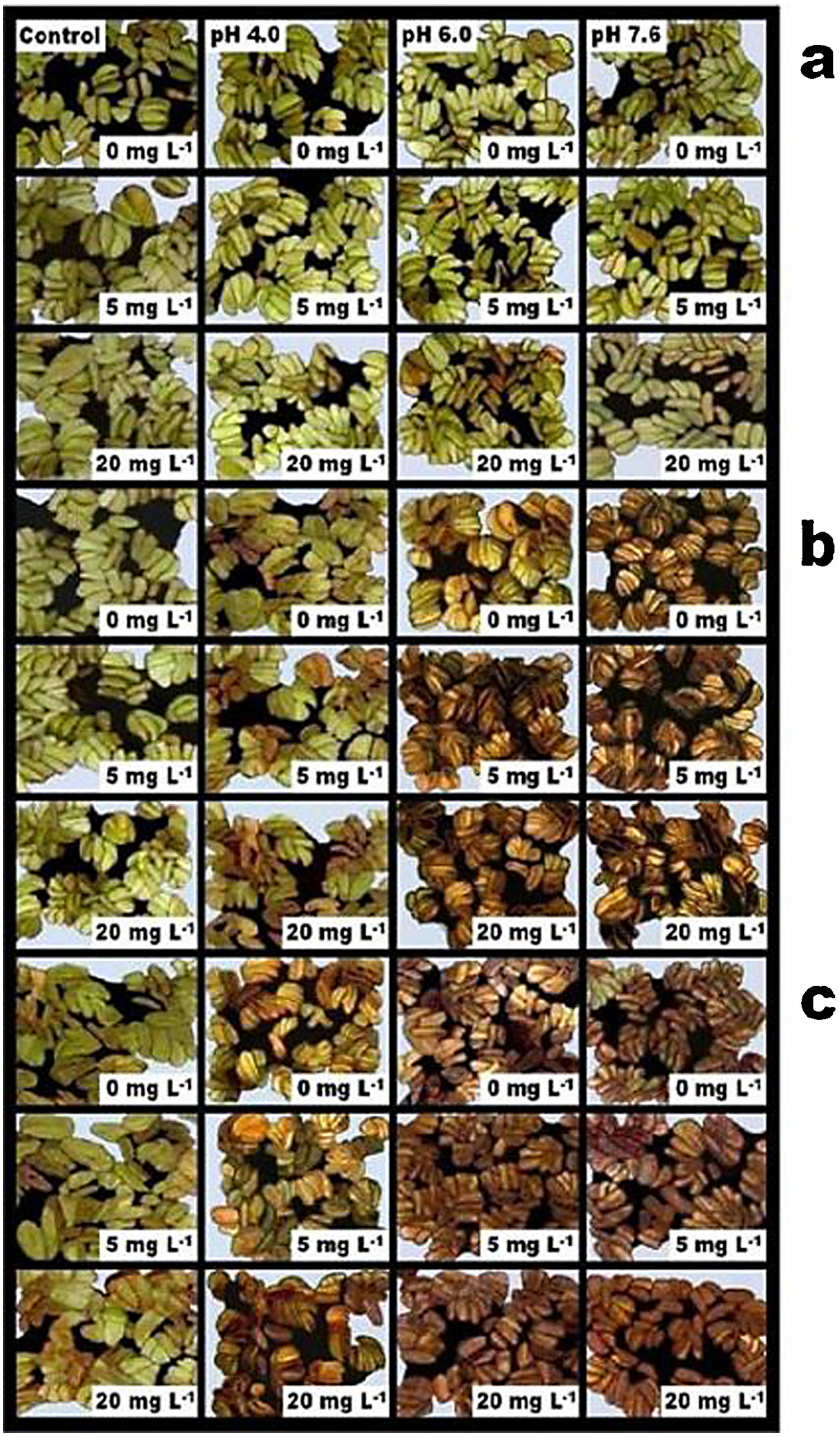

Fig. 7. Temporal pattern of visual symptoms of Cr(VI)-induced damage occurring in *S. minima* plants cultivated in both Cr(VI)-containing buffered solution (pH-stat) and Cr (VI)-containing tap water (control). (a) 0-d treatment time; (b) 3-d treatment time; (c) 7-d treatment time.

would confirm our assumption that in Cr(VI)-exposed *S. minima*, Cr (III) is mainly generated by a pH-dependant reduction of Cr(VI) onto the plant surface. After three desorption washes, total recovered Cr was lower than total biosorbed Cr ($Cr_{total} > Cr_{rec}$) by assuming that Cr_{total} corresponds to 100% of Cr species in both pH-stat and pH-shift conditions (not shown). This indicates that a certain amount of biosorbed Cr was retained either on lacinia surface or accumulated inside the plant. Agreeing with this last, in a previous study we demonstrated a Cr accumulation in fronds of *S. minima* cultivated in Cr(VI)-containing tap water (Prado et al., 2010b).

3.5. Effect of pH on visual symptoms of Cr(VI) damage

Fig. 7 shows visible symptoms of Cr(VI) damage in fronds of *S. minima* cultivated in both Cr(VI)-containing buffered solution (pH-stat condition) and Cr(VI)-containing tap water solution (control). After a 3-d treatment time, symptoms of Cr(VI) damage were clearly visible in plants under pH-stat condition. Plants were less affected at pH 4.0 and 6.0. Cr(VI)-exposed plants at pH 7.6 showed severe chlorosis symptoms that starting from margins were extending toward central portion of lamina. Many fronds show a central brown spot that was extended to whole frond that finally acquired a brownish-red coloration also showing some necrotic spots (Fig. 7b). In control plants visual symptoms of Cr(VI) damage were not observed. After a 7-d treatment period, plants grown at pH 6.0 and 7.6 in Cr(VI)-containing buffered solution acquired a dark brown color and became brittle; while plants grown at pH 4.0 show less damage symptoms. Control plants showed minor damage symptoms (Fig. 7c). This indicates that pH-dependant deleterious effects were stronger than Cr-dependant effects (Fig. 7a-c). Supporting this assumption, plants grown in free-metal buffered solutions showed similar damage symptoms than those grown in Cr(VI)-containing buffered solutions (not shown). Cr-exposed lacinias under pH-stat condition showed a healthier appearance than fronds. Like fronds, symptoms of damage were less severe at pH 4.0 (not shown). This could indicate that lacinias are more tolerant to Cr(VI) than fronds in acidic solutions. Many industrial wastewater effluents and acid mine drainages that contain Cr(VI) are strongly acidic (Barrera et al., 2006), then less occurrence of damage symptoms in lacinias at low pH could signify an acquired trait of plants to enhance Cr(VI) tolerance in polluted aquatic systems. Under pH-shift condition, damage symptoms were very similar to those observed in control plants (not shown). Visual symptoms induced by other heavy metals i.e, Zn, Co, Cd and As have been communicated for *S. auriculata* and *S. cucullata* (Guimarães et al., 2006; Phetsombat et al., 2006; Wolff et al., 2009, 2012). However, most of these studies were made without considering the pH of solution, and then this study can be considered as the first communication on visual symptoms of Cr (VI)-induced damage occurring in a *Salvinia* species grown at different pH values.

4. Conclusions

From the present study *S. minima* appears as suitable species to removal Cr(VI) from acidic aqueous solutions. Results demonstrated a good adaptation of *Salvinia* to tolerate Cr(VI) and also survive at low pH values, frequently occurring in metal polluted wastewater effluents and acid mine drainages. Evaluated parameters represent a relevant approach to enhance the knowledge on plant performance under adverse environmental conditions which can constrain the sustainability of phytoremediation process under a changing pH scenario. Results are sufficiently promising to justify future works to evaluate the performance of *S. minima* to removal Cr(VI) from acidic wastewater at field conditions and even on a pilot scale, with the monitoring of a greater number of parameters than the studied in this work. Results also indicate that selection of suitable aquatic macrophytes for potential application in heavy metal phytoremediation requires an additional focus regarding interactions among metal, solution pH, and plant performance.

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References

Aldrich, M.V., Torresdey, J.L.G., Videa, J.R.P., Parsons, J.G., 2003. Uptake and reduction of Cr(VI) to Cr(III) by mesquite (*Prosopis* spp.): chromate–plant interaction in hydroponics and solid media studied using XAS. Environ. Sci. Technol. 37, 1859–1864.

APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association/American Water Works Association/Water Environment Federation, USA.

Augustynowicz, J., Kyzioł-Komosińska, J., Smoleń, S., Waloszek, A., 2013. Study on chromium-binding capacity of *Callitriche cophocarpa* in an aquatic environment. Arch. Environ. Cont. Tox. 64, 410–418.

Barrera, H., Ureña-Nú, F., ez, Bilyeu, B., Barrera-Díaz, C., 2006. Removal of chromium and toxic ions present in mine drainage by ectodermis of opuntia. J. Hazard. Mater. 136, 846–853.

Barrera-Díaz, C.E., Lugo-Lugo, V., Bilyeu, B., 2012. A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. J. Hazard. Mater. 223–224, 1–12.

Bhatia, P., Ashwath, N., 2005. Effect of medium pH on shoot regeneration from the cotyledonary explants of tomato. Biotechnology 4, 7–10.

Chandra, P., Kulshreshtha, K., 2004. Chromium accumulation and toxicity in aquatic vascular plants. Bot. Rev. 70, 313–327.

Chen, S.L., Chen, C.T., Kao, H., 1990. Acidification of deionized water by roots of intact rice seedlings. Plant Cell Physiol. 31, 569–573.

Chojnacka, K., 2010. Biosorption and bioaccumulation – the prospects for practical applications. Environ. Int. 36, 299–307.

Das, A.P., Mishra, S., 2008. Hexavalent chromium(VI): environment pollutant and health hazard. J. Environ. Res. Develop. 2, 386–392.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37, 4311–4330.

Elangovan, R., Philip, L., Chandraraj, K., 2008. Biosorption of chromium species by aquatic weeds: kinetics and mechanism studies. J. Hazard. Mater. 152, 100–112.

Eleftheriou, E.P., Adamakis, I.D.S., Fatsiou, M., Panteris, E., 2013. Hexavalent chromium disrupts mitosis by stabilizing microtubules in *Lens culinaris* Moench root tip cells. Physiol. Plant. 147, 169–180.

Espinoza-Quiñones, F.R., Martin, N., Stutz, G., Tirao, G., Palácio, S.M., Rizzutto, M.A., Módenes, A.N., Silva Jr., F.G., Szymanski, N., Kroumov, A.D., 2009. Root uptake and reduction of hexavalent chromium by aquatic macrophytes as assessed by high-resolution X-ray emission. Water Res. 43, 4159–4166.

Felle, H.H., 2001. pH: Signal and messenger in plant cells. Plant Biol. 3, 577–591.

Fomina, M., Gadd, G.M., 2014. Biosorption: current perspectives on concept: definition and application. Bioresource Technol. 160, 3–14.

Gopal, R., Sharma, Y.K., 2014. 2014. Effects of chromium (VI) on groundnut (*Arachis hypogeae*) metabolism. J. Plant Physiol. Pathol doi:http://dx.doi.org/10.4172/2329-955X.1000120.

Guigues, Bravin, M.N., Garnier, C., Masion, A., Doelsch, E., 2014. Isolated cell walls exhibit cation binding properties distinct from those of plant roots. Plant Soil 381, 367–379.

Guimarães, F.P., Gomes, C.Q., Magalhães, A.B.S., Freitas, T.V., Oliveira, J.A., Aguiar, R., 2006. Laboratory study of arsenic accumulation and toxicity in *Eichhornia crassipes* and *Salvinia auriculata*. J. Braz. Soc. Ecotoxicol. 1, 109–113 (in Portuguese).

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248, 43–59.

Janicka-Russak, M., Kaba1a, K., Burzyński, M., K1obus, G., 2008. Response of plasma membrane H^+-ATPase to heavy metal stress in *Cucumis sativus* roots. J. Exp. Bot. 59, 3721–3728.

Javed, M.T., Greger, M., 2011. Cadmium triggers *Elodea canadensis* to change the surrounding water pH and thereby Cd uptake. Int. J. Phytoremediation 13, 95–106.

Jones, D.L., 1998. Organic acids in the rhizosphere – a critical review. Plant Soil 205, 25–44.

Kabata-Pendias, A., 2011. Trace Elements in Soil and Plants, 4th ed. CRC Press, Boca Raton, Florida.
Kotaś, J., Stasicka, Z., 2000. Chromium occurrence in the environment and methods of its speciation. Environ. Pollut. 107, 263–283.
Krems, P., Rajfur, M., Wacławek, M., Kłos, A., 2013. The use of water plants in biomonitoring and phytoremediation of water polluted with heavy metals. Ecol. Chem. Eng. 20, 353–370.
Krzesłowska, M., 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol. Plant 33, 35–51.
López-García, M., Lodeiro, P., Herrero, R., Barriada, J.L., Rey-Castro, C., David, C., Sastre de Vicente, M.E., 2013. Experimental evidences for a new model in the description of the adsorption-coupled reduction of Cr(VI) by protonated banana skin. Bioresource Technol. 139, 181–189.
Lytle, C.M., Lytle, F.W., Yang, N., Qian, J.H., Hansen, D., Zayed, A., Terry, N., 1998. Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ. Sci. Technol. 32, 3087–3093.
Madhavi, V., Reddy, A.V.B., Reddy, K.G., Madhavi, G., Prasad, T.N.V.K.V., 2013. An overview on research trends in remediation of chromium. Res. J. Recent Sci. 2, 71–83.
Meychik, N.R., Yermakov, I.P., 2001. Ion exchange properties of plant root cell walls. Plant Soil 234, 181–193.
Memon, S.Q., Bhanger, M.I., Khuhawar, M.Y., 2006. Preconcentration and separation of Cr(III) and Cr(VI) using sawdust as a sorbent. Anal. Bioanal. Chem. 383, 619–624.
Miretzky, P., Fernandez Cirelli, A., 2010. Cr(VI) and Cr(III) removal from aqueous solution by raw and modified lignocellulosic materials: a review. J. Hazard. Mater. 180, 1–19.
Najafian, M., Kafilzadeh, F., Azad, H.N., Tahery, Y., 2012. Toxicity of chromium (Cr^{6+}) on growth: ions and some biochemical parameters of *Brassica napus* L. World Appl. Sci. J. 16, 1104–1109.
Nibou, D., Mekatel, H., Amokrane, S., Barkat, M., Trari, M., 2010. Adsorption of Zn^{2+} ions onto NaA and NaX zeolites: kinetic, equilibrium and thermodynamic studies. J. Hazard. Mater. 173, 637–646.
Olguín, E.J., Sánchez-Galván, G., Pérez-Pérez, T., Pérez-Orozco, A., 2005. Surface adsorption, intracellular accumulation and compartmentalization of Pb(II) in batch-operated lagoons with *Salvinia minima* as affected by environmental conditions, EDTA and nutrients. J. Ind. Microbiol. Biotechnol. 32, 577–586.
Oliveira, H., 2012. Chromium as an environmental pollutant: insights on induced plant toxicity. J. Bot. 8. doi:http://dx.doi.org/10.1155/2012/375843 ID 375843.
Oporto, C., Arce, O., Van den Broeck, E., Van der Bruggen, B., Vandecasteele, C., 2008. Experimental study and modelling of Cr(VI) removal from wastewater using *Lemna minor*. Water Res. 40, 1458–1464.
Paiva, L.B., de Oliveira, J.G., Azevedo, R.A., Ribeiro, D.R., da Silva, M.G., Vitória, A.P., 2009. Ecophysiological responses of water hyacinth exposed to Cr^{3+} and Cr^{6+}. Environ. Exp. Bot 65, 403–409.
Park, D., Yun, Y.S., Park, J.M., 2004. Reduction of hexavalent chromium with the brown seaweed *Ecklonia* biomass. Environ. Sci. Technol. 38, 4860–4864.
Park, D., Yun, Y.S., Park, J.M., 2006. Comment on the removal mechanism of hexavalent chromium by biomaterials or biomaterial-based activated carbons. Ind. Eng. Chem. Res. 45, 2405–2407.
Patnaik, A.R., Achary, V.M.M., Panda, B.B., 2013. Chromium(VI)-induced hormesis and genotoxicity are mediated through oxidative stress in root cells of *Allium cepa* L. Plant Growth Regul. 71, 157–170.
Phetsombat, S., Kruatrachue, M., Pokethitiyook, P., Upatham, S., 2006. Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. J. Environ. Biol. 27, 645–652.
Pittman, J.K., 2012. Multiple transport pathways for mediating intracellular pH homeostasis: the contribution of H^+/ion exchangers. Front. Plant Sci. 3 (11), 8. doi:http://dx.doi.org/10.3389/fpls.2012.00011.
Popuri, S.R., Jammala, A., Reddy, K.V.N.S., Abburi, K., 2007. Biosorption of hexavalent chromium using tamarind (*Tamarindus indica*) fruit shell – a comparative study. Electron. J. Biotechnol. 10, 358–367.
Prado, C., Rosa, M., Pagano, E., Hilal, M., Prado, F.E., 2010a. Seasonal variability of physiological and biochemical aspects of chromium accumulation in outdoor-grown *Salvinia minima*. Chemosphere 81, 584–593.
Prado, C., Rodríguez-Montelongo, L., González, J.A., Pagano, E.A., Hilal, M., Prado, F.E., 2010b. Uptake of chromium by *Salvinia minima*: effect on plant growth, leaf respiration and carbohydrate metabolism. J. Hazard. Mater. 177, 546–553.
Prado, C., 2012. Biochemical and anatomophysiological responses of *Salvinia minima* to chromium. Its potential use as phytoremediator agent of aquatic environments. Ph.D. Thesis. Universidad Nacional de Tucumán, Tucumán (in Spanish).
Rai, V., Poornima, V., Singh, S.N., Shanta, M., 2004. Effect of chromium accumulation on photosynthetic pigments oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. Plant Sci. 167, 1159–1169.
Rodríguez, E., Azevedo, R., Fernández, P., Santos, C., 2011. Cr(VI) induces DNA damage: cell cycle arrest and polyploidization: a flow cytometric and comet assay study in *Pisum sativum*. Chem. Res. Toxicol. 24, 1040–1047.
Saha, B., Orvig, C., 2010. Biosorbents for hexavalent chromium elimination from industrial and municipal effluents. Coordin. Chem. Rev. 254, 2959–2972.
Santos, C., Rodriguez, E., 2012. Review on some emerging endpoints of chromium (VI) and lead phytotoxicity. In: Mworia, J. (Ed.), Botany. InTech, Croatia, pp. 61–82.
Shanker, A.K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S., 2005. Chromium toxicity in plants. Environ. Int. 31, 739–753.
Sharma, D.C., Sharma, C.P., Tripathi, R.D., 2003. Phytotoxic lesions of chromium in maize. Chemosphere 51, 63–68.
Wolff, G., Assis, L.R., Pereira, G.C., Carvalho, J.G., Castro, E.M., 2009. Effects of zinc toxicity on leaves of *Salvinia auriculata* cultivated in nutrient solution. Planta Daninha 27, 133–137 (in Portuguese).
Wolff, G., Pereira, G.C., Castro, E.M., Louzada, J., Coelho, F.F., 2012. The use of *Salvinia auriculata* as a bioindicator in aquatic ecosystems: biomass and structure dependent on the cadmium concentration. Braz. J. Biol. 72, 71–77.
Yan, F., Feurele, R., Schaffer, S., Fortmeier, H., Schubert, S., 1998. Adaptation of active proton pumping and plasmalemma ATPase activity of corn roots to low root medium pH. Plant Physiol. 117, 311–319.
Yang, L.T., Qi, Y.P., Jiang, H.X., Chen, L.S., 2013. Roles of organic acid anion secretion in aluminium tolerance of higher plants. BioMed. Res. Int. 16. doi:http://dx.doi.org/10.1155/2013/173682 ID 173682.
Zayed, A.M., Terry, N., 2003. Chromium in the environment: factors affecting biological remediation. Plant Soil 249, 139–156.
Zazo, J.A., Paull, J.S., Jaffe, P.R., 2008. Influence of plants on the reduction of hexavalent chromium in wetland sediments. Environ. Pollut. 156, 29–35.
Zeng, F., Chen, S., Miao, Y., Wu, F., Zhang, G., 2008. Changes of organic acid exudation and rhizosphere pH in rice plants under chromium stress. Environ. Pollut. 155, 284–289.