Environmental Toxicology and Chemistry—Volume 38, Number 1—pp. 167–176, 2019
Received: 26 June 2018 | Revised: 7 August 2018 | Accepted: 8 October 2018

Environmental Toxicology

Effect of pH on Cr(III) Accumulation, Biomass Production, and Phenolic Profile in 2 *Salvinia* Species

Silvana Chocobar Ponce,[a] Carolina Prado,[a] Eduardo Pagano,[b] Fernando E. Prado,[a] and Mariana Rosa[a,*]

[a]Instituto de Bioprospección y Fisiología Vegetal, CONICET-UNT, Cátedra de Fisiología Vegetal, Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán, Tucumán, Argentina
[b]Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Investigaciones en Biociencias Agrícolas y Ambientales, Facultad de Agronomía, Buenos Aires, Argentina

Abstract: We analyzed the effect of pH on Cr(III) accumulation, biomass production, and phenolic profile of *Salvinia rotundifolia* and *Salvinia minima* plants grown in the presence of increasing concentrations of $CrCl_3$. Biomass accumulation, metal tolerance index, and photosynthetic pigment contents indicate that *Salvinia rotundifolia* seems to be more tolerant of Cr(III) than *S. minima* at different pHs. Increased metal accumulation by *Salvinia* species under increasing pH could be explained by changes of the protonation status of cell wall functional groups because both the highest and the lowest pH values used in the present study were outside of the levels at which Cr(III) species start to precipitate. The metal translocation factor indicates that in buffered conditions *S. rotundifolia* tend to retain more Cr(III) in lacinias than *S. minima*, probably through the involvement of insoluble phenolics. The results of the present study could be useful to the management of solution pH to maximize the removal of Cr(III) by aquatic plants. *Environ Toxicol Chem* 2019;38:167–176. © 2018 SETAC

Keywords: pH; Biomass production; Cr(III) accumulation; *Salvinia* species; Phenolic compounds

INTRODUCTION

Chromium (Cr) is a redox-dynamic transition metal with many industrial uses. It can exist in different oxidation states, the most stable forms being trivalent (Cr[III]) and hexavalent (Cr[VI]), which are commonly found in the environment (Zayed and Terry 2003). However, the hexavalent form can be reduced quickly by soil/water organic matter in the pH range 4.5 to 7.5 (Bartlett and Kimble 1976). In addition, the redox potential of the pair Cr(VI)↔Cr(III) is high, and only a very few oxidants occurring in soils and/or aquatic systems are able to oxidize Cr(III) to Cr(VI) (Kotaś and Stasicka 2000). Therefore, Cr(III) is generally considered to be the stable form in equilibrium with most soil/water systems (Losi et al. 1994). Although Cr(III) appears to be the stable oxidation state in aquatic systems, fluctuations of water physicochemical characteristics such as pH, temperature, dissolved organic matter, and redox potential can affect its speciation and availability (Losi et al. 1994). Among the factors that affect the speciation and availability of Cr(III), pH emerges as the primary control, with cationic species being more soluble under acidic conditions ($pH < 7$) and anionic species more soluble under alkaline conditions ($pH > 7$). Major aqueous species of Cr(III) are Cr^{3+} and $Cr(OH)^{2+}$, which occur at $pH \leq 4.5$; $Cr(OH)_2^{+}$ and $Cr(OH)_{3(aq)}$, which occur at $pH \geq 5.5$; and polyhydroxyl species $Cr(OH)_4^{-}$, $Cr_2(OH)_2^{4+}$, $Cr_3O_4(OH)_4^{3-}$, $Cr_4(OH)_4^{5+}$, and $Cr_2O_2(OH)_4^{2-}$, which occur at high alkaline pHs (Remoundaki et al. 2007).

In rivers, lakes, and lagoons the levels of Cr(III) normally range from >1 to 40 $\mu g\,L^{-1}$, whereas in the oceans is much lower with a mean value of 0.3 $\mu g\,L^{-1}$ (Santonen 2009). However, because of excessive use of Cr(III) compounds in several industrial applications, environmental contamination with this metal has gained substantial attention worldwide. In most polluted waters the concentration of Cr(III) ranges from <1 to 44 $mg\,L^{-1}$, but levels as high as 80 $mg\,L^{-1}$ have also been found in paper mill effluents (Santonen 2009). The trivalent form is regarded by diverse clinical reports as an essential micronutrient for humans and other animals (Levina and Lay 2008; Shadreck and Mugadza 2013), but plant physiologists still debate whether Cr(III) has beneficial or deleterious effects on plants (Prado et al. 2015). In this context, it has been assumed that Cr(III) at low concentrations can be beneficial to plants, although it is highly toxic at high concentrations (Singh et al. 2013). However, increasing evidence indicates that Cr(III) causes more toxic than beneficial effects on both aquatic and terrestrial plants (Karuppanapandian and Kumariah 2008; González et al. 2015; Lukina et al. 2016).

* Address correspondence to rosamd@csnat.unt.edu.ar
Published online 10 October 2018 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4296

Even in some lower and higher plants, such as *Pseudokirchneriella subcapitata*, *Chlorella kessleri*, and *Salsola kali*, Cr(III) seems to be more toxic than Cr(VI) (Gardea-Torresdey et al. 2005; Vignati et al. 2010). Beyond the beneficial or detrimental effects of a determined metal, plants have developed different strategies to avoid the excessive movement of metals in their tissues (Emamverdian et al. 2015). Mechanisms to cope with heavy metals include the binding of metal to cell walls, complexation with secondary metabolites, and vacuolar sequestration, among others (Tong et al. 2004). Soluble phenolics have been recognized as efficient heavy metal complexing molecules (Michalak 2006; Borowska et al. 2018), and there is increasing evidence linking their accumulation with the exposure of plants to heavy metals (Kováčik et al. 2009; Caretto et al. 2015). In addition, different studies have shown that both structural polymerized phenolics (lignin) and cell wall–associated, nonstructural polyphenols (tannins) increase in plants exposed to heavy metals (Pawlak-Sprada et al. 2011).

Fluctuations of pH are considered to be a very important parameter that directly influences plant growth. Changes of pH can alter the availability of essential nutrients and trace metals and even cause direct effects on primary and secondary metabolism at extreme pH values (Lager et al. 2010; Radić et al. 2016). Thus, in heavy metal–polluted waters plant metabolism becomes affected by both heavy metal and pH (Saygideger et al. 2004). A very important question regarding plant–water–metal interaction is how much the solution pH affects metal uptake and plant metabolism. In this way, knowledge of how the solution pH affects plant growth and the amount of Cr removed by the plant becomes of primary importance to maximize the phytoremediation of Cr(III)-polluted aquatic systems.

In living plants, Cr(III) can be retained on the cell wall through cation exchange interactions or sequestered into the vacuoles of epidermal and cortical cells (Mangabeira et al. 2011). Thus, we hypothesized that in *Salvinia* species grown in buffered solutions of Cr(III) significant changes in physiological parameters related to plant growth, metal accumulation, and synthesis of secondary metabolites induced by both heavy metal and solution pH must occur. The aim of the present study was to analyze in plants of *Salvinia rotundifolia* and *Salvinia minima* grown at different pH values 1) Cr(III) accumulation, metal tolerance index, and metal translocation factor; 2) the content of photosynthetic pigments and biomass production; and 3) the accumulation of soluble and insoluble phenolics.

MATERIALS AND METHODS

Plant material and growth conditions

Salvinia rotundifolia and *S. minima* plants were purchased in a local shop of aquarium plants. Because plants grew in the same aquarium media, it is assumed that there are no differences in basal level of mineral nutrients between the 2 species. Plants were thoroughly washed with running tap water to remove plant debris, sediment particles, and/or eventual surface-bound microalgae. Uniform plants with fully expanded floating leaves (fronds) and root-like submerged leaves (lacinias) were selected from both species and transferred to plastic trays containing either unbuffered (tap water) or buffered (McIlvaine buffer) Cr(III) solution. The McIlvaine buffer (citrate-phosphate buffer) was used because of its broad pH range. To avoid the excessive increase of phosphate and citrate anions in treatment solutions, the molarity of buffer was relatively low (10 mM). On the other hand, we did not use zwitterionic N-substituted aminosulfonic acids, usually known as Good's buffers, because these buffers can complex Cr(III) and interact with biological systems (Ferreira et al. 2015). The McIlvaine buffer was prepared using tap water to get an aquatic environment similar to the origin of plants and to avoid posttransplanting stress (Table 1). In addition, plants were not cultivated in Hoagland's solution, to avoid chelation and/or ion competition between Cr(III) species and Hoagland ions for binding sites of the cell wall (Prado et al. 2010). Buffered and unbuffered Cr(III) solutions (5 and 20 mg L^{-1}) were prepared by stepwise dilution of a Cr(III) stock standard solution either with McIlvaine buffer or with tap water. A Cr(III) stock standard solution was prepared at a concentration of 500 mg L^{-1} by dissolving the appropriate amount of pure $CrCl_3 \times 6H_2O$ (analytical grade) in ultrapure water. Selected pHs were 4.0, 6.0, and 7.6 because these are the most frequently found in heavy metal–polluted industrial effluents and acid mine drainages (Shi 2009).

TABLE 1: Physicochemical parameters of the tap water used in the growth of *Salvinia* species[a]

Parameter	
pH	7.0–7.2
Electrical conductivity ($\mu S\,cm^{-1}$)	200
Dissolved oxygen (mg L^{-1})	3
Total dissolved solids (mg L^{-1})	100
Turbidity (nephelometric turbidity units)	<1
As ($\mu g\,L^{-1}$)	0.2
Cu ($\mu g\,L^{-1}$)	2.2
Fe ($\mu g\,L^{-1}$)	12.0
Mn ($\mu g\,L^{-1}$)	0.2
NH_4^+ (mg L^{-1})	<0.02
NO_2 (mg L^{-1})	<0.05
NO_3 (mg L^{-1})	9.0
HPO_4^{2-} (mg L^{-1})	<0.2
HCO_3 (mg L^{-1})	50.0
SO_4^{2-} (mg L^{-1})	11.0
Cl^- (mg L^{-1})	12.0
Ca^{2+} (mg L^{-1})	10.0
Mg^{2+} (mg L^{-1})	7.0
Na^+ (mg L^{-1})	20.0
K^+ (mg L^{-1})	5.0
Cr ($\mu g\,L^{-1}$)	<1.1
Ag^+ ($\mu g\,L^{-1}$)	0.05
Pb ($\mu g\,L^{-1}$)	2.3
Hg ($\mu g\,L^{-1}$)	<0.01
Cd^{2+} ($\mu g\,L^{-1}$)	2.5
CN^- ($\mu g\,L^{-1}$)	<0.001
Zn^{2+} ($\mu g\,L^{-1}$)	3.6
Hardness (mg $CaCO_3\,L^{-1}$)	112.0

[a]Data are the average of the last 10 yr and were provided by the Servicio Provincial de Agua Potable y Saneamiento, Tucumán, Argentina.

For each species and each Cr(III) concentration (buffered and unbuffered), 3 plastic trays (10 × 8 × 4 cm) containing 20 plants (~35 g fresh wt) and 150 mL of treatment solution each were prepared. Trays were transferred to a growth chamber for 7 d under controlled conditions: 200 $\mu mol\ m^{-2}\ s^{-1}$ light intensity, 12:12-h dark: light cycle, 80% relative humidity, and 25/20 ± 1 °C day/night temperature. We chose 7 d as the treatment period because preliminary tests carried out in our laboratory showed that *S. rotundifolia* and *S. minima* plants were able to grow and stay healthy in tap water without nutrient supply for at least 9 d (Prado et al. 2010). Every 2 d, trays were either supplemented with tap water or McIlvaine buffer to compensate for the water loss (evapotranspiration) and maintain the initial volume. The pH of treatment solutions was monitored with a digital pH meter (Adwa AD1000). Measurements of pH were made at 0 d (immediately after transferring plants to treatment solutions) and at 3, 5, and 7 d after starting the experiment. To assess the effect of pH on metal availability, the Cr(III) concentration of each treatment solution was determined colorimetrically at 0, 3, 5, and 7 d.

At the end of the experiment (7 d of Cr[III] treatment) plants were harvested, washed with running tap water to remove the metal attached to plants, rinsed with distilled water, and blotted with paper towels. Ten blotted plants were divided in fronds and lacinias, weighted to obtain the fresh weight, and stored at −20 °C for chemical determinations. Fronds and lacinias from another 10 plants were dried at 80 °C in a hot air oven until constant weight (usually 48 h). After recording the dry weight, fronds and lacinias were ground for Cr(III) determination.

Colorimetric determination of Cr(III)

In buffered and unbuffered solutions Cr(III) was determined colorimetrically following the procedure of Memon et al. (2005) with minor modifications. Briefly, to sample solution (0.1 mL) was added 0.5 mL concentrated H_2SO_4 and 0.25 mL of 20 mM $KMnO_4$. Then it was heated without boiling (~45 °C) for approximately 15 min for complete oxidation of Cr(III) to Cr(VI). The solution was cooled, and 2.5% NaN_3 solution was added dropwise to reduce the excess of $KMnO_4$ (decolorize the pink solution). After that, 0.1 mL of 0.5% 1,5-diphenycarbazide (DPC) reagent (2.5 g DPC dissolved in 50 mL acetone; the solution was prepared fresh daily) was added and made up to 2 mL with distilled water. After standing for 20 min, the absorbance was measured at 540 nm against a reagent blank. A control reaction without $KMnO_4$ was also performed. In the absence of $KMnO_4$ the absorbance value was 0, indicating that no spontaneous oxidation of Cr(III) occurred in buffered and unbuffered solutions. The reliability of the colorimetric method was checked by a calibration curve made from $CrCl_3 \times 6H_2O$ standard solution in the range of 0.2 to 30 $mg\ L^{-1}$ Cr(III) concentration. The standard deviation of the calibration curve was 0.0025, which indicated a good fit of data within an error limit <2%. To test whether the McIlvaine buffer interferes with the DPC assay, a standard curve was also made from a Cr(III) standard solution prepared in 10 mM McIlvaine buffer. No differences in absorbance values were observed between 2 standard solutions.

Biomass production and metal tolerance index

For biomass production another set of experimental trays was made. Immediately after putting the plants in treatment solutions, half of the plants (X_1) were harvested, whereas the remaining plants (X_2) were harvested at the end of the experiment. In both cases, harvested plants were rinsed with distilled water to remove the metal attached to the plants and dried at 80 °C until constant weight. Biomass accumulation corresponds to the difference between X_2(dry wt) and X_1(dry wt) and was expressed as milligrams per plant dry weight. The metal tolerance index was calculated as the ratio between the dry weight of Cr-treated plants and the dry weight of Cr-untreated plants and expressed as a percentage (Reisinger et al. 2008).

Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a* [Chl*a*], Chl*b*, Chl-[*a*+*b*], and carotenoids) were measured at the end of experiment. Briefly, frond samples (0.1 g fresh wt) were added with 2 mL of dimethyl sulfoxide and incubated for 12 h at 45 °C in darkness (Chappelle et al. 1992). Chlorophyll and carotenoid concentrations were calculated from absorbance values at 664, 648, and 472 nm using the Wellburn equations (Wellburn 1994). Concentrations of photosynthetic pigments were expressed as milligrams per gram fresh weight.

Cr accumulation and metal translocation factor

Oven-dried, floating and submerged leaves of Cr-untreated and Cr-treated plants were digested in concentrated HNO_3 at 115 °C for 15 min following the US Environmental Protection Agency (1994) 3051 protocol. Determination of Cr was carried out by atomic absorption spectrophotometry (Perkin-Elmer 373), and Cr content was expressed as micrograms per gram dry weight. A blank of HNO_3 was also measured to ensure the correctness of metal quantification. The accuracy of the sample preparation method was ascertained by adding Cr(III) reference solutions (5 and 20 $mg\ L^{-1}$) to samples. Reference solutions were prepared from a 1000 $mg\ L^{-1}$ Cr(III) stock solution (Certipur®; Merck) by stepwise dilution made with 0.1% v/v HNO_3. The overall recovery associated with the digestion process was found to be in the range 90 to 95%. The error of metal determinations, based on variation in replicate analyses ($n = 2$) on the same sample, was 10% or lower. In Cr-untreated samples, the content of Cr(III) was below the detection limit. The metal translocation factor was calculated as the ratio between the Cr concentration in fronds and the Cr concentration in lacinias (Ton et al. 2015).

Soluble and insoluble phenolics

Soluble phenolics were extracted with ethanol 96% according to Swain and Hillis (1959) with minor modifications.

Briefly, frond and lacinia samples (1.0 g fresh wt) were extracted with 3 mL 96% ethanol, incubated in darkness at room temperature for 48 h, and centrifuged at 3000 *g* for 5 min. Supernatants were recovered and used for soluble phenolic determination. Aliquots of supernatants (0.1 mL) were added with 0.2 mL (1:1, v/v) of diluted Folin-Ciocalteu reagent and 1.8 mL of distilled water. After standing at room temperature for 2 min, 0.8 mL of 7.5% Na_2CO_3 was added and left standing again at room temperature for 5 min. Next, the absorbance was read at 760 nm against a reagent blank using a UV visible spectrophotometer (Hitachi U-2800A). Precipitates from soluble phenolic extraction were washed twice with 2 mL ethanol 96% and centrifugation at 3000 g for 5 min. Washed precipitates were dried a 37 °C for 48 h and used to obtain cell wall-bound insoluble phenolics. Extraction of insoluble phenolic was adapted from Assabgui et al. (1993). Dried samples (0.5 g) were hydrolyzed with 2 mL of 2 N NaOH in a water bath at 60 °C for 60 min. After cooling, solutions were slowly acidified to pH 2 with 5 N HCl and solubilized insoluble phenolics were extracted with ethyl acetate. Following ethyl acetate, fractions were taken near dryness under a stream of N_2 gas and dissolved in 0.5 mL of 96% ethanol. Solubilized insoluble phenolics were determined using the Folin-Ciocalteu reagent, as described. Concentrations of soluble and insoluble phenolics were determined using a standard curve made with pure phenol and expressed as milligrams of phenol equivalent per gram fresh weight.

Statistics

For all determinations at least 3 replicates were done, and 2 independent experiments were performed. Data are presented as the mean of replicates, and bars represent the standard error (SE). Data were subjected to a one-way analysis of variance to confirm the variability of data and the validity of results. The Tukey test was performed to determine significant differences between treatments and between species ($p \leq 0.05$).

RESULTS

Stability of Cr(III) solution and metal availability

No changes in Cr(III) concentrations of both buffered and unbuffered solutions were observed at 0, 3, 5, and 7 d of the experimental period, indicating that no precipitation of Cr(III) species occurred in our experimental condition (data not shown). In a previous study carried out in our laboratory, no significant difference was found in Cr(III) concentration determined either by the DPC method or by atomic absorption spectrophotometry (Prado 2012).

Biomass and metal tolerance index

The biomass of *Salvinia* species was differently affected by Cr(III) and pH (Figure 1). The biomass of *S. rotundifolia* increased with Cr(III) treatment under all assayed pHs, but the higher increment occurred at pH 6.0. Maximum increases were 15.0% (pH 4.0), 38.3% (pH 6.0), and 18.3% (pH 7.6) and occurred at the highest metal concentration. However, in unbuffered plants the biomass accumulation was not affected significantly by the metal treatment. By contrast, in buffered plants of *S. minima* the biomass decreased with Cr(III) treatment. Maximum decreases of 26.9% (pH 4.0), 33.6% (pH 6.0), 35.6% (pH 7.6), and 11.3% (unbuffered plants) were observed in the presence of 20 mg L^{-1} Cr(III). The tolerance index was higher in *S. rotundifolia* than in *S. minima* plants in buffered and unbuffered conditions (Figure 2). The tolerance index values in *S. rotundifolia* ranged between 103.8 and 178.2%, whereas in *S. minima* they ranged between 73.3 and 83.3%. At the end of the experimental period, both species showed healthy-looking plants without visible

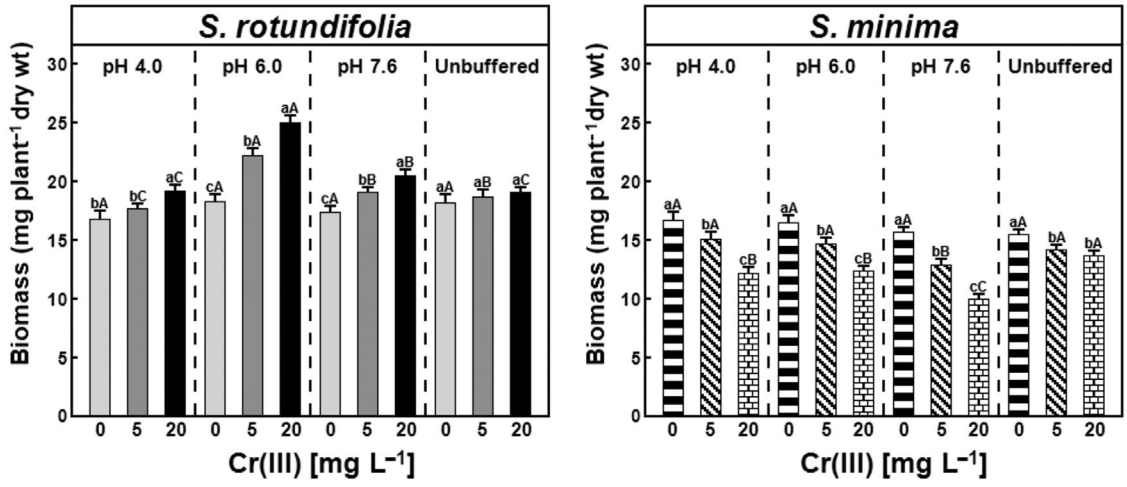

FIGURE 1: Effect of Cr(III) and solution pH on biomass accumulation in buffered and unbuffered *Salvinia rotundifolia* and *Salvinia minima* plants after a 7-d cultivation period. Data are mean ± standard error of 3 replications ($n = 6$). Different lowercase letters on bars indicate significant differences among Cr(III) concentrations for each growth condition and each *Salvinia* species. Different uppercase letters indicate significant differences among growth conditions for each Cr(III) concentration and each *Salvinia* species ($p \leq 0.05$).

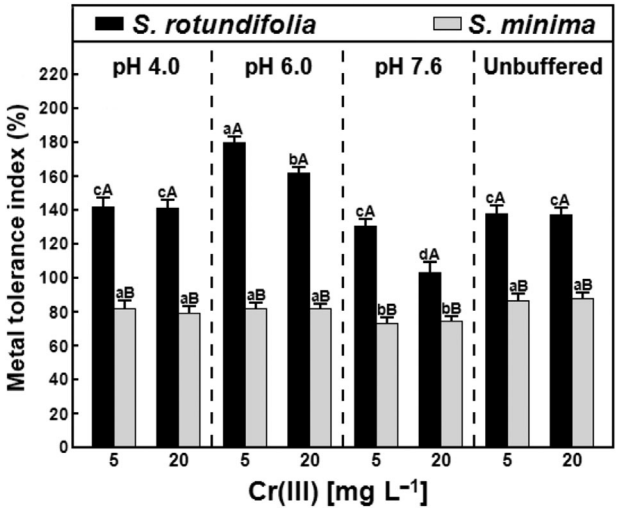

FIGURE 2: Metal tolerance index of buffered and unbuffered *Salvinia rotundifolia* and *Salvinia minima* plants after a 7-d cultivation period. Data are mean ± standard error of 3 replications ($n = 6$). Different lowercase letters on bars indicate significant differences for each *Salvinia* species. Different uppercase letters indicate significant differences between species for each growth condition and each Cr(III) concentration ($p \leq 0.05$).

symptoms of metal toxicity in both buffered and unbuffered conditions (data not shown).

Photosynthetic pigments

Photosynthetic pigments were significantly higher in *S. rotundifolia* fronds than in *S. minima* ones, but the accumulation pattern was differently affected by Cr(III) and solution pH. Under all pH values the presence of Cr(III) increased both chlorophyll and carotenoids in *S. rotundifolia*, whereas in *S. minima* significant decreases occurred (Table 2). Maximum increases of photosynthetic pigments in *S. rotundifolia* were 58.1% (Chl*a*), 36.0% (Chl*b*), 50.1% (Chl[*a*+*b*]), and 43.8% (carotenoids) and were observed at pH 6.0 in the presence of 20 mg L^{-1} Cr(III). Maximum decreases in *S. minima* fronds were 18.9, 27.8, 20.9, and 21.0% and occurred at pH 7.6 under the highest metal concentration. In unbuffered plants of both *Salvinia* species, photosynthetic pigments were not significantly affected by Cr(III) treatment. The Chl(*a*+*b*) to carotenoids ratio of buffered plants ranged between 6.10 and 7.65 in *S. rotundifolia* and between 5.51 and 6.47 in *S. minima*, whereas in unbuffered plants it ranged between 7.02 and 7.08 in the former and between 5.51 and 5.68 in the latter (Table 2).

Cr accumulation and metal translocation factor

The accumulation of Cr in lacinias and fronds of buffered and unbuffered plants of *S. rotundifolia* and *S. minima* increased under increasing Cr(III) concentration, but the amount of accumulated metal was different at all assayed pHs. Independently of the organ and *Salvinia* species, the highest metal accumulation occurred at pH 7.6 (Figure 3A and B). At both the organ and whole-plant levels, the accumulation of Cr(III) was significantly higher in *S. rotundifolia* than in *S. minima*. It is noteworthy that the accumulation of metal in the former was significantly lower in buffered plants, whereas in the latter it was lower in unbuffered plants. In buffered and unbuffered *S. rotundifolia* fronds the amount of accumulated Cr(III) ranged from 0.08 to 1.3 mg g^{-1} dry weight, whereas in *S. minima* fronds it ranged from 0.07 to 0.43 mg g^{-1} dry weight. In lacinias accumulated metal varied between 0.21 and 11.3 mg g^{-1} dry weight in the former and between 0.20 and 1.33 mg g^{-1} dry weight in the latter. Based on the value of pH, the maximum accumulation of Cr in lacinias of plants grown in the presence of 5 and 20 mg L^{-1} Cr(III) showed a different pattern between the 2 *Salvinia* species (Figure 4). In buffered and unbuffered plants the translocation factor was significantly lower in *S. rotundifolia* than in *S. minima*, except at pH 6.0 in the presence of 20 mg L^{-1} Cr(III) concentration. Values of translocation factor ranged between 0.09 and 0.45 in *S. rotundifolia* and from 0.20 to 0.52 in *S. minima*, respectively (Figure 5).

Soluble and insoluble phenolics

Both soluble and insoluble phenolics were significantly higher in *S. rotundifolia* than in *S. minima*, but the accumulation pattern was differently affected by both Cr(III) concentration and solution pH. Comparing with unbuffered plants, soluble and insoluble phenolics in buffered plants of both species showed an increasing accumulation in the presence and absence of Cr(III), but this was more marked for insoluble phenolics (Figure 6A–D). The highest increases of soluble phenolics in buffered plants were noticeable in fronds: 131.6% in the presence of 5 mg L^{-1} Cr(III) and pH 6.0 (*S. rotundifolia*) and 110.5% at pH 7.6 in the absence of metal (*S. minima*). In unbuffered plants maximum increases of soluble phenolics in fronds and lacinias were 37.4 and 51.7% (*S. rotundifolia*) and 24.4 and 133.3% (*S. minima*), respectively (Figure 6A and C). Regarding insoluble phenolics, the most significant increases in both species were observed in lacinias of buffered plants: 2.3-fold (0 mg L^{-1} Cr[III] and pH 7.6) in *S. rotundifolia* and 7.3-fold (20 mg L^{-1} Cr[III] and pH 6.0) in *S. minima*. In unbuffered plants insoluble phenolics increased by 174.4 and 46.3% in fronds and lacinias of *S. rotundifolia* and 9.3 (fronds) and 39.2% (lacinias) in *S. minima* (Figure 6B and D).

DISCUSSION

The trivalent Cr(III) is considered an essential element for animals but has never been recognized as an essential element for plants (Shadreck and Mugadza 2013). However, in a few plants it can stimulate some physiological processes such as growth, photosynthesis, and carbohydrate metabolism (Paiva et al. 2009; Gomes et al. 2017). The increase of biomass is usually used as an indicator of the photosynthetic activity of plants exposed to heavy metals (Azevedo et al. 2005). The present data showed increases of biomass and photosynthetic pigments in plants of *S. rotundifolia* exposed to Cr(III) in buffered solutions, whereas in *S. minima* plants significant decreases were observed (Figure 1 and Table 2). The biomass accumulation is a key indicator of the health of plants in heavy metal–polluted sites

TABLE 2: Effect of Cr(III) on Chla, Chlb, Chl(a+b), and Car contents and the Chl(a+b)/Car ratio in *Salvinia rotundifolia* and *Salvinia minima* fronds after a 7-d cultivation period under buffered and unbuffered conditions[a]

	S. rotundifolia (mg g^{-1} dry wt)					*S. minima* (mg g^{-1} dry wt)				
Cr(III) (mg L^{-1})	Chla	Chlb	Chl(a+b)	Car	Chl(a+b)/Car	Chla	Chlb	Chl(a+b)	Car	Chl(a+b)/Car
Buffered (pH 4.0)										
0	1.114 ± 0.116b,A	0.389 ± 0.031c,A	1.503 ± 0.152c,A	0.213 ± 0.014c,A	7.06 ± 0.63b,A	0.735 ± 0.072a,B	0.269 ± 0.027a,B	1.004 ± 0.097a,B	0.160 ± 0.018a,B	6.27 ± 0.61a,A
5	1.207 ± 0.126b,A	0.461 ± 0.042c,A	1.668 ± 0.153c,A	0.237 ± 0.020c,A	7.04 ± 0.61b,A	0.757 ± 0.061a,B	0.276 ± 0.022a,B	1.033 ± 0.101a,B	0.167 ± 0.012a,B	6.19 ± 0.64a,A
20	1.323 ± 0.138b,A	0.507 ± 0.050c,A	1.830 ± 0.174b,A	0.264 ± 0.021c,A	6.93 ± 0.58b,A	0.624 ± 0.059b,B	0.218 ± 0.017b,B	0.842 ± 0.090b,B	0.140 ± 0.011b,B	6.01 ± 0.59a,A
Buffered (pH 6.0)										
0	1.343 ± 0.141b,A	0.683 ± 0.070b,A	2.026 ± 0.180b,A	0.281 ± 0.026c,A	7.21 ± 0.70a,A	0.678 ± 0.060a,B	0.200 ± 0.018b,B	0.878 ± 0.083b,B	0.140 ± 0.017b,B	6.27 ± 0.60a,A
5	1.863 ± 0.202a,A	0.869 ± 0.081a,A	2.732 ± 0.281a,A	0.357 ± 0.039b,A	7.65 ± 0.77a,A	0.617 ± 0.058b,B	0.182 ± 0.018c,B	0.799 ± 0.070b,B	0.144 ± 0.015b,B	5.55 ± 0.59b,B
20	2.124 ± 0.232a,A	0.929 ± 0.091a,A	3.053 ± 0.311a,A	0.404 ± 0.052a,A	7.56 ± 0.77a,A	0.582 ± 0.050b,B	0.170 ± 0.011c,B	0.752 ± 0.070b,B	0.130 ± 0.015b,B	5.78 ± 0.53b,B
Buffered (pH 7.6)										
0	0.821 ± 0.069c,A	0.339 ± 0.030d,A	1.160 ± 0.104d,A	0.190 ± 0.018d,A	6.10 ± 0.55c,A	0.576 ± 0.054b,B	0.169 ± 0.019c,B	0.745 ± 0.075b,B	0.119 ± 0.009c,B	6.09 ± 0.52b,A
5	1.076 ± 0.098c,A	0.401 ± 0.061c,A	1.477 ± 0.138c,A	0.228 ± 0.029c,A	6.48 ± 0.62c,A	0.524 ± 0.048c,B	0.142 ± 0.011d,B	0.666 ± 0.053c,B	0.103 ± 0.009c,B	6.47 ± 0.59a,A
20	1.147 ± 0.122c,A	0.441 ± 0.063c,A	1.588 ± 0.149c,A	0.257 ± 0.020c,A	6.18 ± 0.57c,A	0.467 ± 0.045c,B	0.122 ± 0.010d,B	0.589 ± 0.060d,B	0.094 ± 0.008c,B	6.27 ± 0.53a,A
Unbuffered										
0	1.324 ± 0.119b,A	0.537 ± 0.058c,A	1.861 ± 0.188b,A	0.263 ± 0.023c,A	7.08 ± 0.65b,A	0.633 ± 0.062b,B	0.215 ± 0.027b,B	0.848 ± 0.071b,B	0.154 ± 0.013a,B	5.51 ± 0.52b,B
5	1.344 ± 0.142b,A	0.548 ± 0.050c,A	1.892 ± 0.165b,A	0.270 ± 0.026c,A	7.01 ± 0.64b,A	0.612 ± 0.060b,B	0.202 ± 0.011b,B	0.814 ± 0.082b,B	0.142 ± 0.014b,B	5.73 ± 0.60b,B
20	1.375 ± 0.136b,A	0.555 ± 0.053c,A	1.930 ± 0.199b,A	0.275 ± 0.026c,A	7.02 ± 0.70b,A	0.598 ± 0.051b,B	0.186 ± 0.011c,B	0.784 ± 0.032b,B	0.138 ± 0.006b,B	5.68 ± 0.54b,B

[a]Data are means of 3 replications ± standard error ($n = 6$). Different lowercase letters in each column for each evaluated parameter and for each species denote significant differences. Different uppercase letters in each row for each evaluated parameter and each Cr(III) concentration denote significant differences between species ($p \leq 0.05$).
Car = carotenoids; Chla = chlorophyll *a*; Chlb = chlorophyll *b*.

(John et al. 2009). Thus, it can be assumed that *S. rotundifolia* is able to grow better than *S. minima* in the presence of Cr(III) at different pHs. Agreeing with this assumption, the tolerance index, an important tool to screen plants based on their tolerance to heavy metals, was significantly higher in *S. rotundifolia* than in *S. minima* (Figure 2). During the exposure to Cr(III) under the unbuffered condition the biomass and photosynthetic pigments in both *Salvinia* species decreased, indicating that the pH becomes a key factor to successfully remove trivalent chromium from polluted waters.

It is well known that plant species suitable for phytoremediation tend to accumulate heavy metals at the root level, avoiding the translocation to aerial parts to prevent and/or avoid their deleterious effects on the photosynthetic machinery (Bonanno et al. 2017). In agreement with this assumption, different studies have demonstrated that the predominant mechanism to remove heavy metals by living species of *Salvinia* is the accumulation of metal ions on root-like submerged leaves (lacinias; Olguín et al. 2005; Chocobar Ponce et al. 2014). Coinciding with this finding, the present data showed that *S. rotundifolia* and *S. minima* plants accumulated much more Cr(III) in lacinias than in fronds (floating leaves) in both unbuffered and buffered conditions (Figure 3A and B). The translocation factor represents the ability of plants to transfer heavy metals from roots to shoots. Values of translocation factor <1 indicate that there is a decreased root-to-shoot translocation (Ebrahimi 2015). In both species, translocation factor values ranged between 0.09 and 0.52, but they were lower in *S. rotundifolia* (Figure 5). This indicates that this species has a higher capacity to retain Cr(III) in lacinias than *S. minima*.

The efficiency of living plants at removing heavy metals mainly depends on plant species, type of metal, and plant metabolism (Tang et al. 2017). Cell wall binding, complexation, and vacuolar sequestration play major roles in heavy metal accumulation and tolerance in plants (Rosa et al. 2014). The capacity of aquatic plants to bind heavy metals mainly depends on cell wall chemical composition, metal solubility and bioavailability, cellular metabolic activity, and presence of ionic species (Yabanli et al. 2014). However, the solubility and bioavailability of metals are greatly influenced by physicochemical characteristics of solution, with pH being one of the most important factors involved in the control of such processes (Weng et al. 2003). Lowering pH increases the concentration of cationic species, whereas increasing pH tends to decrease cationic species and increase anionic species. However, changes of solution pH also affect the protonation status of functional groups on the cell wall (Liu et al. 2017), and then the binding of metals to the cell wall becomes complex and highly dependent on the value of pH.

Different models have been developed to explain the binding of heavy metals to the cell wall, but many of them fail when the study models are living plants. The biotic ligand model (BLM) has been proposed as a tool to predict metal toxicity in aquatic systems by incorporating how water chemistry affects both speciation and the biological availability of the metal (Di Toro et al. 2001). The main assumption of the BLM is that metal toxicity is caused by free metal ions reacting with biological

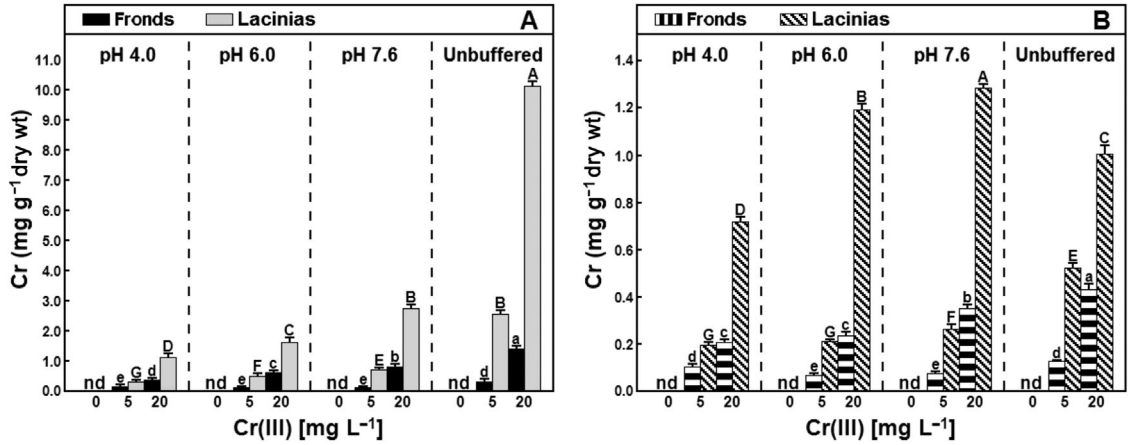

FIGURE 3: Accumulation of Cr(III) in buffered and unbuffered fronds and lacinias of *Salvinia rotundifolia* (A) and *Salvinia minima* (B) plants after a 7-d cultivation period. Data are mean ± standard error of 3 replications (n = 6). For each *Salvinia* species different lowercase and uppercase letters on bars indicate significant differences in metal accumulation by fronds and lacinias, respectively ($p \leq 0.05$). nd = no detected.

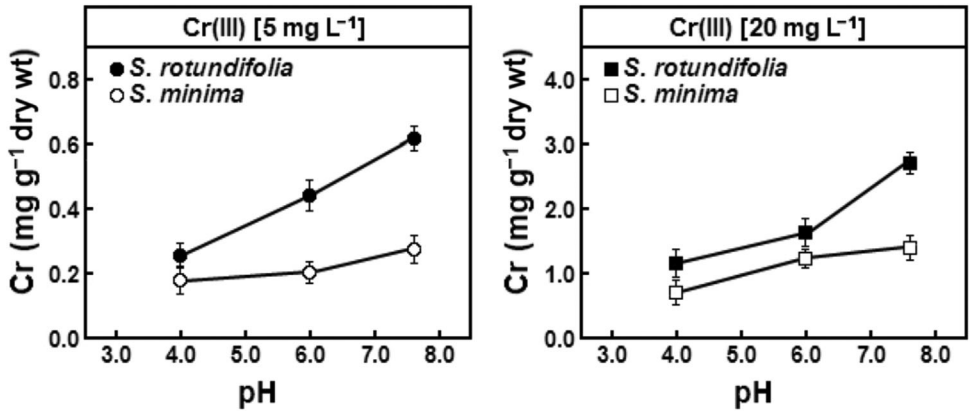

FIGURE 4: Relationship between pH value and accumulated Cr(III) in lacinias of *Salvinia rotundifolia* and *Salvinia minima* plants growing during 7 d at different pHs. Data are mean ± standard error (SE) of 3 replications (*n* = 6). Vertical bars show the SE.

binding sites (Ardestani et al. 2014). According to this model, the binding of Cr(III) to the cell wall is based on metal speciation and competitive binding between Cr(III) species and H^+ of cell wall functional groups, commonly called "biotic ligands." Acid pHs increase the solubility and availability of Cr(III) species (e.g., Cr^{3+} and $CrOH^{2+}$) but also increase the concentration of free H^+ in root surrounding solution, leading to increased competition between Cr(III) species and free H^+ for cell wall binding sites (Song et al. 2014). As the pH is increased, the concentration of free H^+ decreases and the surface charge density of the root cell wall becomes negative (Chathuranga et al. 2013) so that more cationic species (e.g., $Cr[OH]^{2+}$ and $Cr[OH]_2{}^+$) and even polyhydroxyl species (e.g., $Cr_2[OH]_2{}^{4+}$ and $Cr_4[OH]_4{}^{5+}$) can bind to plant roots. In this context an increased accumulation of Cr(III) in *Salvinia* plants is expected to occur under alkaline conditions. Agreeing with this assumption, the present study showed the highest accumulation of Cr(III) in lacinias of both *Salvinia* species at pH 7.6 (Figure 3A and B). However, the trend of Cr accumulation on lacinias as a function of the solution pH was different in both *Salvinia* species (Figure 4). This fact can reflect the presence of different protonizable groups in the cell walls of both *Salvinia* species or that there is a species-specific synthesis and release into the rhizosphere of Cr(III) complexing compounds such as organic acids, amino acids, and phytosiderophores (Hinsinger 2001). In agreement with this assumption, it has been demonstrated that in growing maize plants organic acids contribute to the mobilization of Cr(III) by converting it into a labile, organically bound form, enhancing its availability to the plant (Srivastava et al. 1999). In addition, it has been demonstrated that the presence of heavy metals can trigger aquatic plants to change the pH of root surrounding solution, which may affect both rhizosphere metal bioavailability and plant metal uptake (Javed and Greger 2011). Further in-depth studies are nevertheless required to investigate the binding of Cr(III) species to the cell wall of *Salvinia* species to identify the extent of participation of their respective binding sites.

Secondary metabolites have been recognized as efficient heavy metal–protecting compounds (Skórzyńska-Polit et al. 2004; Michalak 2006). Among secondary metabolites, phenolic compounds can cope with heavy metals either externally by binding the metal to the cell wall or internally by complexing the metal in the cytoplasm and/or by its sequestration inside the

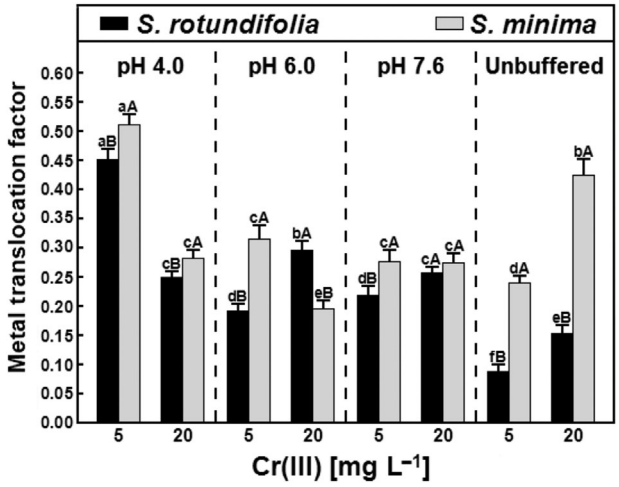

FIGURE 5: Metal translocation factor of buffered and unbuffered *Salvinia rotundifolia* and *Salvinia minima* plants after a 7-d cultivation period. Data are mean ± standard error of 3 replications ($n = 6$). Different lowercase letters on bars indicate significant differences for each *Salvinia* species. Different uppercase letters indicate significant differences between species for each growth condition and each Cr(III) concentration ($p \leq 0.05$).

vacuole (Skórzyńska-Polit et al. 2004). In addition, phenolic compounds can act as antioxidant molecules against reactive oxygen species generated by heavy metal–induced oxidative stress (Michalak 2006). It is known that low and high pHs affect the synthesis and accumulation of secondary metabolites in plants (Schmitzer and Štampar 2010). Increases, decreases, and even no changes of phenolics induced by acid or alkaline pHs have been reported for different plant species (Hawrylak-Nowak 2008; Zhang et al. 2014). However, available data reveal that the effect of pH on the dynamic of phenolic accumulation in heavy metal–exposed plants is not always clear, and contradictory results have been reported (Pal'ove-Balang et al. 2012; Radić et al. 2016). Agreeing with these findings, the present data showed significant differences in the accumulation pattern of soluble and insoluble phenolics in fronds and lacinias of Cr-exposed *S. rotundifolia* and *S. minima* plants growing at different pHs (Figure 6A–D). In both species, insoluble phenolics were significantly higher than soluble phenolics in buffered and unbuffered conditions. The lower content of soluble phenolics observed in lacinias of both species could indicate an increased polymerization of phenolic compounds into the cell wall matrix

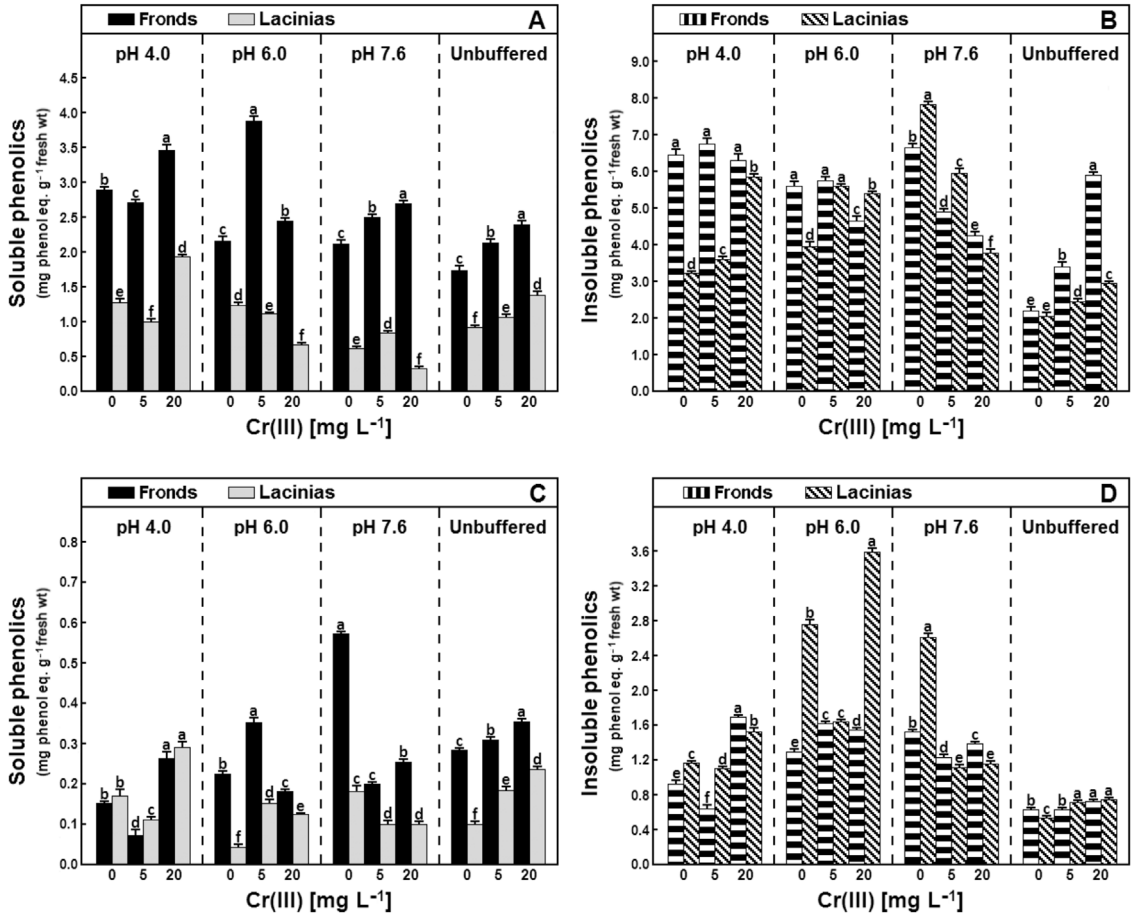

FIGURE 6: Effect of pH and Cr(III) concentration on the content of soluble and insoluble phenolics of buffered and unbuffered *Salvinia rotundifolia* (**A**, **B**) and *Salvinia minima* (**C**, **D**) plants after a 7-d cultivation period. Data are mean ± standard error of 3 replications ($n = 6$). For both soluble and insoluble phenolics, different letters on bars indicate significant differences between floating and submerged leaves for each growth condition ($p \leq 0.05$).

in order to immobilize more Cr(III) at the root level. In this regard, we have demonstrated an increased deposition of insoluble phenolics (i.e., lignin) in the roots of Cd-treated citrus rootstocks (Podazza et al. 2016). Nevertheless, a decreased content of soluble phenolics in lacinias could also indicate either "in situ" reduced synthesis or reduced transport from fronds. By contrast, the highest content of soluble phenolics found in the fronds of *Salvinia* plants could be also related to the cytosolic complexation of Cr(III) species translocated from lacinias. Supporting this assumption, previous reports have shown the complexation of Cr(III) ions with different plant phenolics (Sun et al. 2008; Panhwar and Memon 2014). On the other hand, the high content of insoluble phenolics occurring in fronds of Cr-exposed and Cr-unexposed plants probably can be related to the high content of lignin normally present in the cell wall of floating leaves of aquatic ferns such as *Azolla* and *Salvinia* (Leterme et al. 2009), rather than the binding of Cr(III) ions. Further studies to provide insight into the pH-dependent mechanisms controlling the accumulation of phenolic compounds in Cr-exposed *Salvinia* plants are in progress.

The present results reveal that the solution pH affects the growth and Cr(III) uptake by *Salvinia* species, which indicates that the management of solution pH has important implications for maximizing the removal of heavy metals by aquatic macrophytes and the economic success of the phytoremediation process.

Acknowledgment—The present study was supported by grants from Consejo de Investigaciones de la Universidad Nacional de Tucumán (PIUNT 26G/628) and from Consejo Nacional de Investigaciones Cientficas y Técnicas (CONICET; PIP 14/151). C. Prado, F.E. Prado, and M. Rosa are researchers from CONICET; S.C. Ponce has a fellowship from CONICET. The authors acknowledge the technical assistance of N. Ilina from the Instituto de Investigaciones en Biociencias Agrícolas y Ambientales in the determination of Cr by atomic absorption spectrophotometry.

Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (rosamd@csnat.unt.edu.ar).

REFERENCES

Ardestani MM, van Straalen NM, van Gestel CAM. 2014. The relationship between metal toxicity and biotic ligand binding affinities in aquatic and soil organisms: A review. *Environ Pollut* 195:133–147.

Assabgui RA, Reid LM, Hamilton RI, Arnason JT. 1993. Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. *Phytopathology* 83: 949–953.

Azevedo H, Gomes C, Pinto G, Fernandes J, Loureiro S, Santos C. 2005. Cadmium effects on sunflower growth and photosynthesis. *J Plant Nutr* 28:2211–2220.

Bartlett RJ, Kimble JM. 1976. Behavior of chromium in soils, II. Hexavalent forms. *J Environ Qual* 5:383–386.

Bonanno G, Borg JA, Di Martino V. 2017. Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: A comparative assessment. *Sci Total Environ* 576:796–806.

Borowska S, Brzoska MM, Tomczyk M. 2018. Complexation of bioelements and toxic metals by polyphenolic compounds. Implications for health. *Curr Drug Targets* 19:1612–1638.

Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V. 2015. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. *Int J Mol Sci* 16:26378–26394.

Chappelle EW, Kim MS, McMurtrey JE III. 1992. Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. *Remote Sens Environ* 39:239–247.

Chathuranga PKD, Priyantha N, Iqbal SS, Iqbal MCM. 2013. Biosorption of Cr(III) and Cr(VI) species from aqueous solution by *Cabomba caroliniana*: Kinetic and equilibrium study. *Environ Earth Sci* 70:661–671.

Chocobar Ponce S, Prado C, Pagano E, Prado FE, Rosa M. 2014. Effect of solution pH on the dynamic of biosorption of Cr(VI) by living plants of *Salvinia minima*. *Ecol Eng* 74:33–41.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem* 20:2383–2396.

Ebrahimi M. 2015. Effect of EDTA treatment method on leaching of Pb and Cr by *Phragmites australis* (Cav.) Trin. Ex Steudel (common reed). *Caspian Journal of Environmental Sciences* 13:153–166.

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y. 2015. Heavy metal stress and some mechanisms of plant defense response. *Sci World J* 2015:756120. DOI: 10.1155/2015/756120

Ferreira CMH, Pinto ISS, Soares EV, Soares HMVM. 2015. (Un)suitability of the use of pH buffers in biological, biochemical and environmental studies and their interaction with metal ions—A review. *RSC Adv* 5:30989–31003.

Gardea-Torresdey JL, de la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jimenez G, Cano-Aguilera I. 2005. Differential uptake and transport of trivalent and hexavalent chromium by tumble weed (*Salsola kali*). *Arch Environ Contam Toxicol* 48:225–232.

Gomes MAC, Hauser-Davis RA, Suzuki MS, Vitória AP. 2017. Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations. *Ecotoxicol Environ Saf* 140:55–64.

González CI, Maine MA, Cazenave J, Sanchez GC, Benavides MP. 2015. Physiological and biochemical responses of *Eichhornia crassipes* exposed to Cr (III). *Environ Sci Pollut Res* 22:3739–3747.

Hawrylak-Nowak B. 2008. Changes in anthocyanin content as indicator of maize sensitivity to selenium. *J Plant Nutr* 31:1232–1242.

Hinsinger P. 2001. Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere. In Gobran GR, Wenzel WW, Lombi E, eds, *Trace Elements in the Rhizosphere*. CRC, Boca Raton, FL, USA, pp 25–41.

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

John R, Ahmad P, Gadgil K, Sharma S. 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *Int J Plant Prod* 3:65–76.

Karuppanapandian T, Kumariah K. 2008. Uptake and translocation of tri- and hexavalent chromium and their effects on black gram (*Vigna mungo* L. Hepper cv. Co4) roots. *J Plant Biol* 51:192–201.

Kotaś J, Stasicka Z. 2000. Chromium occurrence in the environment and methods of its speciation. *Environ Pollut* 107:263–283.

Kováčik J, Klejdus B, Kaduková J, Bačkor M. 2009. Physiology of *Matricaria chamomilla* exposed to nickel excess. *Ecotoxicol Environ Saf* 72:603–609.

Lager I, Andréasson O, Dunbar T, Andreasson E, Escobar MA, Rasmusson AG. 2010. Changes in external pH rapidly alter plant gene expression and modulate auxin and elicitor responses. *Plant Cell Environ* 33:1513–1528.

Leterme P, Londoño AM, Muñoz JE, Súarez J, Bedoya CA, Souffrant WB, Buldgen A. 2009. Nutritional value of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell) in pigs. *Anim Feed Sci Technol* 149:135–148.

Levina A, Lay PA. 2008. Chemical properties and toxicity of chromium(III) nutritional supplements. *Chem Res Toxicol* 21:563–571.

Liu ZD, Zhou Q, Hong ZN, Xu RK. 2017. Effects of surface charge and functional groups on the adsorption and binding forms of Cu and Cd on roots of indica and japonica rice cultivars. *Front Plant Sci* 8:1489.

Losi ME, Amrhein C, Frankenberger WT Jr. 1994. Environmental biochemistry of chromium. *Rev Environ Contam Toxicol* 136:91–121.

Lukina AO, Boutin C, Rowland O, Carpenter DJ. 2016. Evaluating trivalent chromium toxicity on wild terrestrial and wetland plants. *Chemosphere* 162:355–364.

Mangabeira PA, Ferreira AS, de Almeida AAF, Fernandes VF, Lucena E, Souza VL, dos Santos AJ, Oliveira AH, Grenier-Loustalot MF, Barbier F, Silva DC. 2011. Compartmentalization and ultrastructural alterations induced by chromium in aquatic macrophytes. *Biometals* 24:1017–1026.

Memon SQ, Bhanger MI, Khuhawar MY. 2005. Preconcentration and separation of Cr(III) and Cr(VI) using sawdust as a sorbent. *Anal Bioanal Chem* 383:619–624.

Michalak A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 15:523–530.

Olguín EJ, Sánchez-Gálvan 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.

Paiva LB, Gonçalves de Oliveira J, Azevedo RA, Rodrigues Ribeiro D, Gomes da Silva M, Vitória AP. 2009. Ecophysiological responses of water hyacinth exposed to Cr^{3+} and Cr^{6+}. *Environ Exp Bot* 65:403–409.

Pal'ove-Balang P, Čiamporová M, Zelinová V, Pavlovkin J, Gurinová E, Mistrk I. 2012. Cellular responses of two Latin-American cultivars of *Lotus corniculatus* to low pH and Al stress. *Cent Eur J Biol* 7:1046–1054.

Panhwar QK, Memon S. 2014. Synthesis of Cr(III)-morin complex: Characterization and antioxidant study. *Sci World J* 2014:845208. DOI: 10.1155/2014/845208

Pawlak-Sprada S, Arasimowicz-Jelonek M, Podgórska M, Deckert J. 2011. Activation of phenylpropanoid pathway in legume plants exposed to heavy metals. Part I. Effects of cadmium and lead on phenylalanine ammonia-lyase gene expression, enzyme activity and lignin content. *Acta Biochim Pol* 58:211–216.

Podazza G, Arias ME, Prado FE. 2016. Early interconnectivity between metabolic and defense events against oxidative stress induced by cadmium in roots of four citrus rootstocks. *Funct Plant Biol* 43:973–985.

Prado C. 2012. Biochemical and anatomophysiological responses of *Salvinia minima* to chromium. Its potential use as phytoremediator agent of aquatic environments. PhD thesis. Universidad Nacional de Tucumán, Tucumán, Argentina (in Spanish).

Prado C, Rosa M, Pagano E, Hilal M, Prado FE. 2010. Seasonal variability of physiological and biochemical aspects of chromium accumulation in outdoor-grown *Salvinia minima*. *Chemosphere* 81:584–593.

Prado FE, Hilal M, Chocobar-Ponce S, Pagano E, Rosa M, Prado C. 2015. Chromium and the plant: A dangerous affair? In Parvaiz A, ed, Plant Metal Interaction: *Emerging Remediation Techniques*. Elsevier, Amsterdam, the Netherlands, pp 149–177.

Radić S, Vujčić V, Glogoški M, Radić-Stojković M. 2016. Influence of pH and plant growth regulators on secondary metabolite production and antioxidant activity of *Stevia rebaudiana* (Bert). *Period Biol* 118:9–19.

Reisinger S, Schiavon M, Terry N, Pilon-Smits EAH. 2008. Heavy metal tolerance and accumulation in Indian mustard (*Brassica juncea* L.) expressing bacterial γ-glutamylcysteine synthetase or glutathione synthetase. *Int J Phytoremediation* 10:440–454.

Remoundaki E, Hatzikioseyian A, Tsezos M. 2007. A systematic study of chromium solubility in the presence of organic matter: Consequences for the treatment of chromium-containing wastewater. *J Chem Technol Biotechnol* 82:802–808.

Rosa M, Prado FE, Hilal M, Pagano E, Prado C. 2014. Phytoremediation: Strategies of Argentinean plants against stress by heavy metals. In Polti M, Alvarez A, eds, *Bioremediation in Latin America: Current Research and Perspectives*. Springer Science+Business Media, New York, NY, USA, pp 123–134.

Santonen T. 2009. Inorganic chromium(III) compounds. Concise International Chemical Assessment Document 76. World Health Organization, Geneva, Switzerland.

Saygideger S, Dogan M, Keser G. 2004. Effect of lead and pH on lead uptake, chlorophyll and nitrogen content of *Typha latifolia* L. and *Ceratophyllum demersum* L. *Int J Agric Biol* 6:168–172.

Schmitzer V, Štampar F. 2010. Substrate pH level effects on anthocyanins and selected phenolics in Rosa × hybrid L. "KORcrisett." *Acta Agric Slov* 95:5–11.

Shadreck M, Mugadza T. 2013. Chromium, an essential nutrient and pollutant: A review. *African Journal of Pure and Applied Chemistry* 7:310–317.

Shi H. 2009. Industrial wastewater—Types, amount and effects. In Yi Q, ed, Point Sources of *Pollution: Local Effects and Control*, Vol 1— Encyclopedia of Life Support Systems. UNESCO, Paris, France, pp 191–203.

Singh HP, Mahajan P, Kaur S, Batish DR, Kohli RK. 2013. Chromium toxicity and tolerance in plants. *Environ Chem Lett* 11:229–254.

Skórzyńska-Polit E, Drążkiewicz M, Wianowska D, Maksymiec W, Dawidowicz AL, Tukiendorf A. 2004. The influence of heavy metal stress on the level of some flavonols in the primary leaves of *Phaseolus coccineus*. *Acta Physiol Plant* 26:247–254.

Song N, Zhong X, Li B, Li J, Wei D, Ma Y. 2014. Development of a multi-species biotic ligand model predicting the toxicity of trivalent chromium to barley root elongation in solution culture. *PLoS One* 9:1–10.

Srivastava S, Prakash S, Srivastava MM. 1999. Studies on mobilization of chromium with reference to its plant availability—Role of organic acids. *Biometals* 12:201–207.

Sun S, Chen W, Cao W, Zhang F, Song J, Tian C. 2008. Research on the chelation between quercetin and Cr(III) ion by density functional theory (DFT) method. *J Mol Struct* 860:40–44.

Swain T, Hillis WE. 1959. The phenols constituents of *Prunus domestica*. I. The quantitative analysis of phenolics constituents. *J Sci Food Agric* 10:63–68.

Tang J, Chen C, Chen L, Daroch M, Cui Y. 2017. Effects of pH, initial Pb^{2+} concentration, and polyculture on lead remediation by three duckweed species. *Environ Sci Pollut Res* 24:23864–23871.

Ton SS, Lee MW, Yang YH, Hoi SK, Cheng WC, Wang KS, Chang HH, Chang SH. 2015. Effects of reductants on phytoextraction of chromium (VI) by *Ipomoea aquatica*. *Int J Phytoremediation* 17:429–436.

Tong YP, Kneer R, Zhu YG. 2004. Vacuolar compartmentalization: A second-generation approach to engineering plants for phytoremediation. *Trends Plant Sci* 9:7–9.

US Environmental Protection Agency. 1994. SW 846 Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils. [cited 2018 March 20]. Available from: http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3051a.pdf

Vignati DAL, Dominik J, Beye ML, Pettine M, Ferrari BJD. 2010. Chromium(VI) is more toxic than chromium(III) to freshwater algae: A paradigm to revise? *Ecotoxicol Environ Saf* 73:743–749.

Wellburn AR. 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 144:307–313.

Weng L, Lexmond TM, Wolthoorn A, Temminghoff EJM, van Riemsdijk WH. 2003. Phytotoxicity and bioavailability of nickel: Chemical speciation and bioaccumulation. *Environ Toxicol Chem* 22:2180–2187.

Yabanli M, Yozukmaz A, Sel F. 2014. Heavy metal accumulation in the leaves, stem and root of the invasive submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae): An example of Kadin Creek (Mugla, Turkey). *Braz Arch Biol Technol* 57:434–440.

Zayed AM, Terry N. 2003. Chromium in the environment: Factors affecting biological remediation. *Plant Soil* 249:139–156.

Zhang Y, Zhang J, Song T, Li J, Tian J, Jin K, Yao Y. 2014. Low medium pH value enhances anthocyanin accumulation in malus crabapple leaves. *PLoS One* 9:1–8.

