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Original Paper

Cr(VI) absorption in *Salvinia minima* depends of seasonal development and nutrients availability rather than biomass accumulation

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Abstract

We analysed the capacity of *Salvinia minima* plants collected from different seasons to accumulate Cr(VI) in presence or absence of mineral nutrients. Plants were collected in August and November and they were grown in both water and Hoagland solution with and without Cr(VI). August plants showed development of new fronds, a low content of soluble sugars, and an increase in biomass. In November plants, a lower number of new fronds, a higher content of soluble sugars, and a lower increase in biomass were observed. November plants accumulated more Cr than those from August and the growth media showed an increase in DO. These results would indicate that a greater accumulation of biomass (August plants) does not necessarily lead to a greater Cr accumulation. *Salvinia* plants did not show demand for mineral nutrients except for phosphate and magnesium. Changes in the ion composition of growing media during the assay show possible differences in mineral requirement between higher and lower plants. Our results showed that Cr(VI) accumulation in *S. minima* plants depends on the development stage and the mineral nutrients composition of the growth medium.
Key words: Cr removal, developmental stages, lower plants nutrition, phytoremediation, *Salvinia*.

Resumo

Analisamos a capacidade de plantas de *Salvinia minima* coletadas em diferentes estações do ano em acumular Cr(VI) na presença ou ausência de nutrientes minerais. As plantas foram coletadas em agosto e novembro e cultivadas em água e solução de Hoagland com e sem Cr(VI). As plantas de agosto apresentaram desenvolvimento de novas folhagens, baixo teor de açúcares solúveis e aumento de biomassa. Nas plantas de novembro, observou-se menor número de novas folhas, maior teor de açúcares solúveis e menor aumento de biomassa. As plantas de novembro acumularam mais Cr do que as de agosto e o meio de cultivo apresentou aumento de OD. Esses resultados indicam que um maior acúmulo de biomassa (plantas de agosto) não necessariamente leva a um maior acúmulo de Cr. As plantas de Salvinia não apresentaram demanda por nutrientes minerais, exceto fosfato e magnésio. Mudanças na composição de íons do meio de cultivo durante o ensaio mostram possíveis diferenças na exigência de minerais entre plantas superiores e inferiores. Nossos resultados mostraram que o acúmulo de Cr(VI) em plantas de S. minima depende do estádio de desenvolvimento e da composição de nutrientes minerais do meio de cultivo.
Palavras-chave: remoção de Cr, estágios de desenvolvimento, nutrição de plantas inferiores, fitorremediação, Salvinia.

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Introduction

Many heavy metals (*e.g.*, Cr, Pb, As, Cd, Sn) are natural components of the environment as trace metals, but elevated and potentially toxic levels (contamination) sometimes occur. Heavy metal contamination refers to the excessive deposition of metals in the soil and water caused by human activities (Kabata-Pendias 2011). Among heavy metals released to the environment, chromium (Cr) is one of the most toxic occurring in polluted soils and waters. Anthropogenic release of Cr from leather tanning, electroplating, cement plants, stainless steel production, wood preservation, mining, paints and pigments, metal finishing, metal plating, pulp and paper production, and refractory steel industries constitutes the main sources of Cr pollution of both soils and aquatic systems (Milačič & Ščančar 2020). Cr is not destroyed by natural degradation, and it accumulates in the environment for a long time, which leads to an increased contamination and severely increases the threat of this toxic element. Trivalent [Cr(III)] and hexavalent [Cr(VI)] are the most widespread forms of Cr in the environment and have different levels of toxicity. Cr(VI) is more mobile than Cr(III) and has a long persistence time in the environment. Its high oxidizing potential, high solubility, and ease of permeation of biological membranes make it more toxic than the trivalent form. Cr(VI) acts as a strong oxidizing agent on biological systems and affects several metabolic processes through the induction of oxidative stress, that is the main process underlying Cr(VI) toxicity to humans and animals (Gautam *et al.* 2015). Plants are also affected by Cr-induced oxidative stress which impairs their normal development and growth (Wakeel *et al.* 2020).

Water contamination by Cr(VI) is a major problem because many Cr-containing wastes are discharged directly into artificial wastewater reservoirs or natural water bodies without any remediation treatment (Brasili *et al.* 2020). Moreover, this problem becomes more severe in shallow sand and gravel water bodies, because Cr(VI) has low adsorption rate onto soil particles (Linnik & Zubenko 2000). Many aquatic environments face Cr(VI) concentrations that exceed water quality criteria designed to protect the environment, animals and humans. Plant-assisted removal of heavy metals appears as a low-cost effective biotechnology to the treatment of wastewater (Gautam *et al.* 2015; Wani *et al*. 2017). In this sense, submerged, emergent and free-floating aquatic species from the genera *Vallisneria*, *Hydrilla*, *Ceratophyllum*, *Myriophyllum*, *Typha*, *Juncus*, *Scirpus*, *Salvinia*, *Lemna*, *Spirodela*, *Azolla*, *Eichhornia*, *Wolffia* and *Pistia* have the capacity to absorb and accumulate a great number of heavy metals (Olguín *et al*. 2005; Ali *et al*. 2020; Rezania *et al*. 2016) and can well-grow under different stressful conditions including nutrient shortage (Thomaz *et al.* 2009).

The removal of Cr(VI) from polluted waters using free-floating macrophytes has received attention due to its fast growth and easy culture in natural and artificial ponds (Fletcher *et al.* 2020). In many free-floating macrophytes, absorption and accumulation of Cr(VI) takes place in submerged roots (Marbaniang & Chaturvedi 2014), but the translocation of the metal to aerial parts (shoot and leaves) also occurs (Prado *et al.* 2016). Among cosmopolite free-floating macrophytes, *Salvinia* species have the ability to remove high amounts of Cr(VI) from polluted waters (Dhir 2009). Salvinia is fern whith a fast-growing. It produces dark green heartshaped floating leaves called fronds, in pairs and it has no roots. In place of roots present hairy and submerged leaf, called lacinia, which is suspected of functioning like a root by absorbing nutrients and acting as a stabilizer (Gaudet 1973).

In previous studies, Prado *et al*. (2010a, b) showed a higher Cr accumulation in lacinias of *Salvinia minima*, compared with fronds and this accumulation was lower in winter than in summer.

In environments with a marked seasonality, temperature is crucial to induce the stages of development. Phenological studies have been reported only on *S. natans* for the Baltic Sea region (Galka & Szmeja 2013). Other studies have been carried out for the management of *S. molesta* considering development stages, temperature ranges and presence of nutrients (Van Oosterhout 2006). In this sense, for a phytoremediation process, it is important to consider the development stage of the plant, the physiological/metabolic status, and the environmental conditions. Thus, the aim of this study was to analyse the Cr(VI) removal capacity of *S. minima* in different seasonal developmental stages, grown in the presence and absence of mineral nutrients.

Materials and Methods

Plant material and experimental set-up

Healthy *Salvinia minima* Baker plants were collected from a heavy metal non-polluted freshwater pond located at 500 m asl, Tucumán-Argentina (26°50'S, 65°12'W). Collections were performed in August (late winter) and November

(late spring) 2019 (Austral hemisphere) when temperature mean was 14 ± 4 °C (July–August) and 23 ± 4 °C (October–November). Solar radiation mean was 371 ± 113 Wm^{-2} and 559 ± 155 Wm^{-2} in the same period. After collection, plants were thoroughly washed under running tap water to eliminate surface-bound sediments, particles, and microalgae. Next, *Salvinia* plants were transferred to a plastic container filled with tap water during a 24 h period under laboratory conditions (24 °C temperature, 48% relative humidity and 10 h fluorescent lighting provided by fluorescent lamps [~130 µmol m^2 s^{-1}]). After that, plants with uniform weight were selected and transferred to plastic trays (one plant per tray) containing 150 mL of different solution media. Test solutions were as follow: distilled water, distilled water plus 5 mg L^{-1} $K_2Cr_2O_7$, Hoagland mineral nutrient solution (1:4 v/v), Hoagland mineral nutrient solution plus 5 mg L^{-1} $K_2Cr_2O_7$. This concentration value was chosen because previous studies carried out in many development countries reported concentrations of chromium salts (chromate and dichromate) in surface and ground polluted waters from 0 mg L^{-1} to as high as 20 mg L^{-1} (Terry *et al.* 2014). It is worth mentioning that these concentrations are well above the limits established by the WHO (0.05 mg.L) (WHO 2022). Each treatment consisted of eight replicates. Trays were maintained under laboratory conditions for 6 days. We chose this experimental period because preliminary tests showed that *S. minima* plants were able to grow well and stay healthy in distilled water without nutrient supply for at least 9 days. Water loss by evaporation and transpiration was compensated daily by adding distilled water up to the initial volume. Plant samples were collected at 0, 3 and 6 days after Cr(VI) treatment began. Plants were rinsed in distilled water and separated in fronds and lacinias to carry out chemical analyses. To quantify soluble sugars (glucose, fructose, sucrose) plant samples were stored at -20 °C. To minimise any diurnal effect on carbohydrate content, plants were collected at noon. Plant fresh weight (FW) was immediately determined after harvesting, whereas the dry weight (DW) was determined by drying plants at 60 °C in a hot air oven until constant weight.

Accumulation of Cr(VI) in plant tissues

Dried fronds and lacinias were ground in a knife-mill equipped with a 1 mm mesh screen. Powdered samples weighing approximately 0.5 g were ashed in a muffle furnace at 450 ºC for 5 h. Ashed samples were digested in a mixture of HNO_3/$HClO_4$ (3/1, v/v) at 115 ºC for 15 min following the USEPA 3051 protocol (USEPA 1994). Digested samples were analysed for Cr by flame atomic absorption spectrometry (FAAS) using a Perkin-Elmer 373, USA, spectrophotometer. Metal content was expressed as µg g^{-1} DW.

Determination of Cr(VI) in treatment solutions

Cr(VI) was determined colorimetrically by using 1,5-diphenylcarbazide as colour reagent (APHA-AWWA-WEF 2005). To assess there was no reduction of Cr(VI) in treatment solutions, the 1,5-diphenylcarbazide method was performed in presence and absence of $KMnO_4$ to oxidize Cr(III) derived from eventual Cr(VI) reduction (Memon *et al.* 2006). No difference was found between two determinations indicating that no spontaneous reduction of Cr(VI) occurred. Reliability of the 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%.

Bioconcentration factor and translocation factor

The capability of fronds and lacinias of *Salvinia minima* plants to accumulate Cr(VI) from solutions was evaluated using the bioconcentration factor (BCF) and translocation factor (TF) (Yadav *et al*. 2009).

$$BCF = \frac{\text{Metal concentration in plant organ (mg kg-1)}}{\text{Metal concentration in external solution (mg L-1)}}$$

$$TF = \frac{\text{Metal concentration in fronds (mg kg-1)}}{\text{Metal concentration in lacinias (mg kg-1)}}$$

Soluble sugars

Carbohydrates were extracted from 1 g FW of both fronds and lacinias by homogenisation in a mortar and pestle with 2 mL of 80% ethanol (v/v). Resulting homogenate was heated in a water bath at 80 ºC for 10 min, centrifuged at 5000 x g for 10 min, and collected the supernatant. Resulting precipitate was homogenised with 2 mL of 80% ethanol (v/v), heated in a water bath, and centrifuged again. Supernatants were pooled and dried under a stream

of hot air. Dry residue was suspended in 1 mL of distilled water and desalted by filtration through an ion-exchange column (Amberlite MB3, BDH, England). Sucrose was determined according to the procedure of Cardini *et al*. (1955) and fructose by the method of Roe & Papadopoulos (1954). Glucose was determined using a glucose oxidase-peroxidase coupled assay according to Jorgensen & Andersen (1973). Sugar contents were expressed as μmol g^{-1} FW.

Chemical analysis of treatment solutions

Concentrations of Cl^-, SO_4^{2-} and NO_3^- was determined according to 4110B protocol (APHA-AWWA-WEF 2005) by ionic chromatography using an ionic chromatograph (881 Compact IC pro-Anion, Metrohm AG, Switzerland) equipped with a Metrosep A Supp 5 column (150 × 4 mm, 5 μm particle size), 858 Professional Sample Processor, sample filtration system with a 0.2 μm regenerated cellulose membrane, six channel injection valve, low pulsation high-pressure pump, chemical suppression and CO_2 suppression, eluent degasser and conductivity detector. As elution solution a $NaHCO_3$ 1 mM and Na_2CO_3 3.2 mM (CertiPUR®, Merck, Darmstadt, Germany) mixture was used. Multi ion standard solutions (Cl^-, SO_4^{2-} and NO_3^-) were prepared from anion standard stock solutions (1,000 mg L^{-1}). All solutions were prepared using Milli-Q water, (Mill-Q Direct 8, Merck Millipore; resistivity > 18.2 MΩcm, equipped with a Millipack 0.22 μm filter). Before measurements samples (5 ml) were filtered by MF-Millipore™ membrane filter with pore size of 0.45 μm (Merck KGaA, Darmstadt, Germany). Chromatographic analysis was performed using 2.5 ml of each eluent sample. Phosphate-phosphorus pool (PO_4–P) or soluble reactive phosphorus (SRP) was determined by the colorimetric molybdenum blue method (Murphy & Riley 1962).

Cation ions (K^+, Ca^{2+}, and Mg^{2+}) were quantified by ionic chromatography without chemical suppression using a Metrosep C 4 column (150 × 4 mm, 5 μm particle size) and dipicolinic acid (2 mM) and HNO_3 (2 mM) mixture as eluent.

Physicochemical parameters of treatment solutions

Measurements were performed at 0, 3 and 6 days after beginning of Cr(VI) treatment. Temperature and electrical conductivity (EC) were measured using a portable conductivity meter (Hach Sension 156, USA). pH was measured using a portable pHmeter (Metrohm 826 pH mobile, Switzerland). Dissolved oxygen (DO) was measured with a portable DOmeter (Horiba OM-14, Japan).

Statistical analysis

For all determinations at least three replicates were performed, and two independent experiments were carried out. Data are presented as the mean of all replicates. The effect of mineral nutrients on the removal and partitioning of Cr(VI) between fronds and lacinias of *S. minima* plants, was analysed using the ANOVA test. The analysis was performed using the SIGMA STAT Program (version 3.0, 2003) at $p < 0.05$.

Results

Plant growth

Under all treatments, *Salvinia minima* plants showed a similar appearance (Fig. 1, water plus Chromium treatment is shown). A high percentage of young fronds in August plants was observed (Fig. 2a, maximum value 77%), meanwhile in November plants, the highest value was 16% (Fig. 2b). Damaged fronds were not observed in any treatment.

Changes of biomass (expressed as % DW respect to 0-d) of *S. minima* plants are shown in Figure 3. August and November plants grown in Hoagland solution showed significant increases of DW at 6-d. No significant differences were observed in presence or absence of Cr(VI). In water-grown plants a less increase of biomass occurred in both months. The dry weight to fresh weight ratio (DW/FW) did not show significant seasonal variations neither in water nor in Hoagland-grown plants (data not shown).

Cr(VI) in plant organs and growth solutions

Cr(VI) accumulation in fronds and lacinias of August and November plants is shown in Figure 4. The highest Cr(VI) contents were observed at 6-d treatment in November plants grown in Hoagland solution. In August plants these values were: 942 ± 92 μg g^{-1} DW (lacinias) and 230 ± 18 μg g^{-1} DW (fronds). Maximum contents of metal in lacinias and fronds of water-grown plants were: 1,018 ± 99 and 250 ± 22 μg g^{-1} DW (November), and 955 ± 90 and 258 ± 18 μg g^{-1} DW (August).

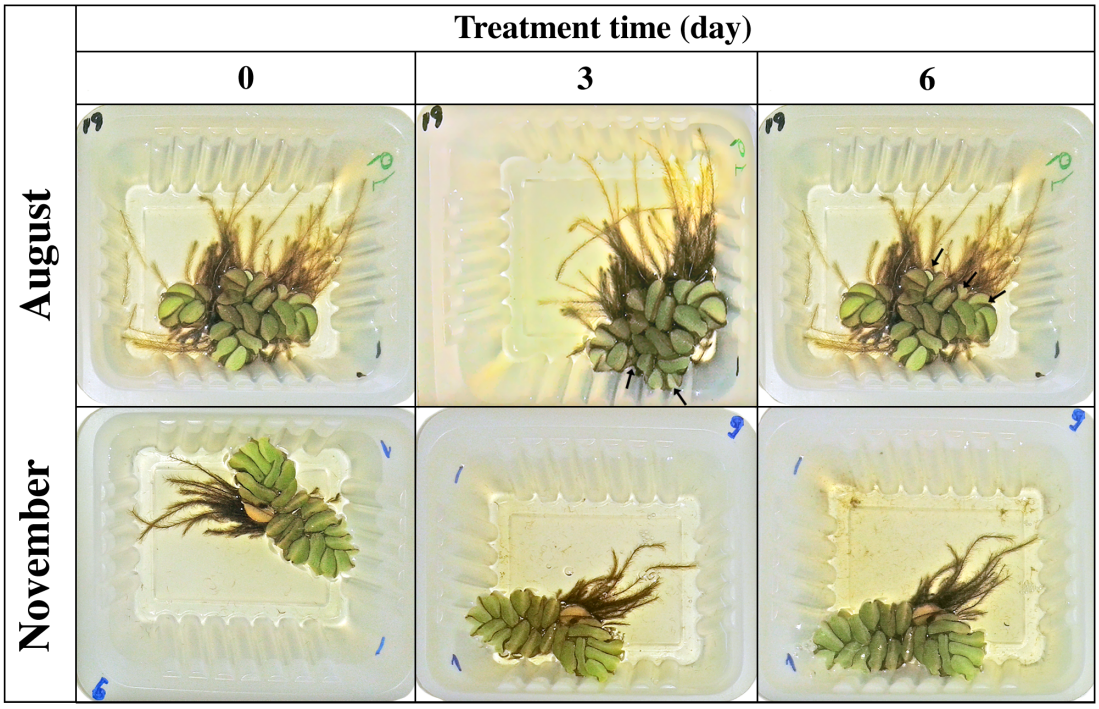

Figure 1 – Appearance of August and November plants. *Salvinia minima* plants grown in water plus Cr are representatively shown. Arrows indicate new fronds developed during the assay.

Cr(VI) remainder in treatment solutions is shown in Figure 5. At the ending of the experimental period, residual Cr(VI) concentrations in water and Hoagland solutions were: 4.24 and 3.94 mg L^{-1} (August), and 4.43 and 4.23 mg L^{-1} (November), respectively. Regarding chromium speciation, no statistical differences ($p < 0.05$) were observed in Cr(VI) concentrations determined in presence and absence of $KMnO_4$ along the experimental period in both treatment solutions (data not shown).

Bioconcentration factor (BCF) and translocation factor (FT)

BCF values in August and November plants increased with exposure time but were higher in the latter. Furthermore, the increase in BCF

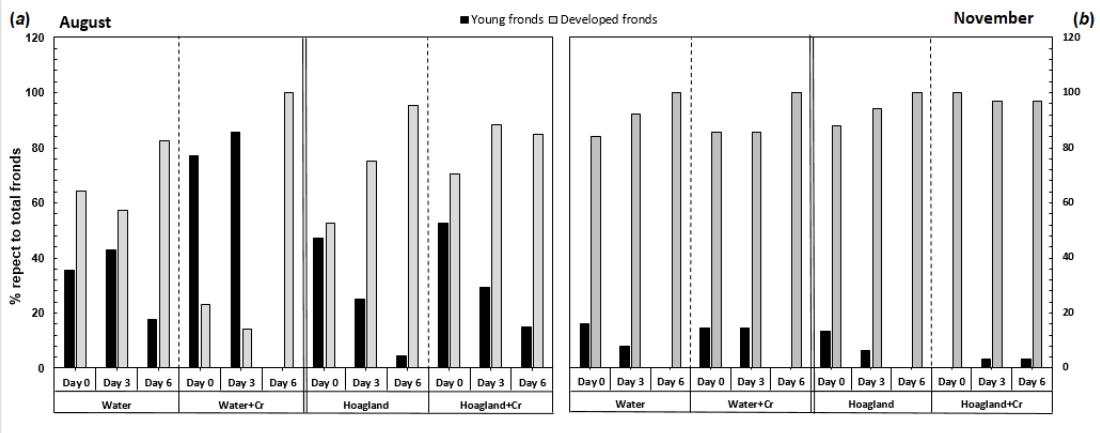

Figure 2 – a-b. Percentage of developing and developed fronds, at 0, 3, and 6 days of the assay – a. August plants; b. November plants.

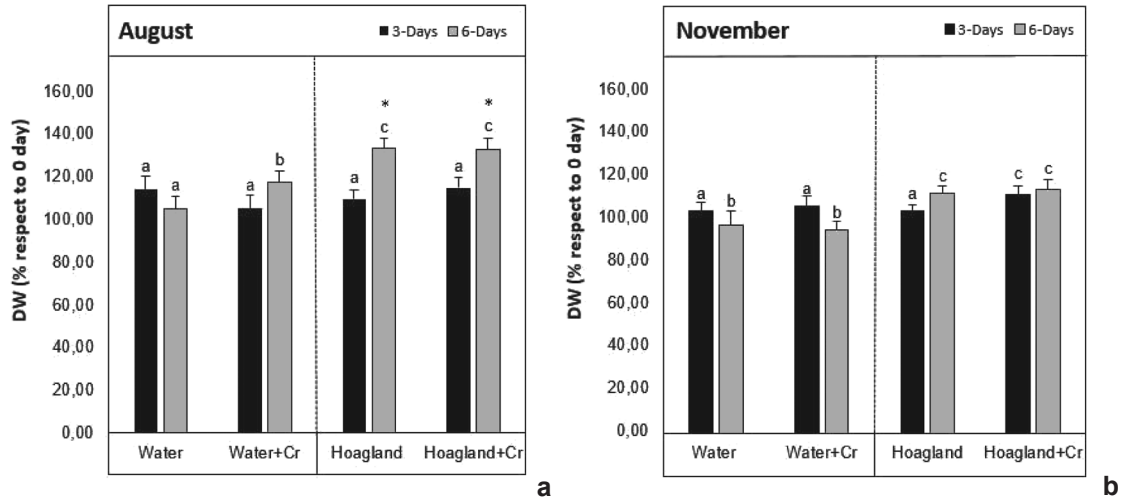

Figure 3 – Biomass variation with respect to 0 day in (a) August and (b) November plants. Different letters indicate significant differences ($p < 0.05$).

values was greater in the fronds and laciniae of Hoagland-grown plants than in water-grown plants in both months. Regarding Cr(VI) distribution in plant organs, the highest BCF values were found in lacinias. Maximum BCF value was 286 ± 25 and was observed on day 6 in lacinias of November plants grown in Hoagland solution, while the minimum value (35 ± 3) was found on day 3 in fronds of Hoagland-grown plants during August. In both growth media, BCF values for August and November lacinias were 3 to 4 times higher than values for fronds. TF values did not show significant seasonal differences in both water- and Hoagland-grown plants (Tab. 1).

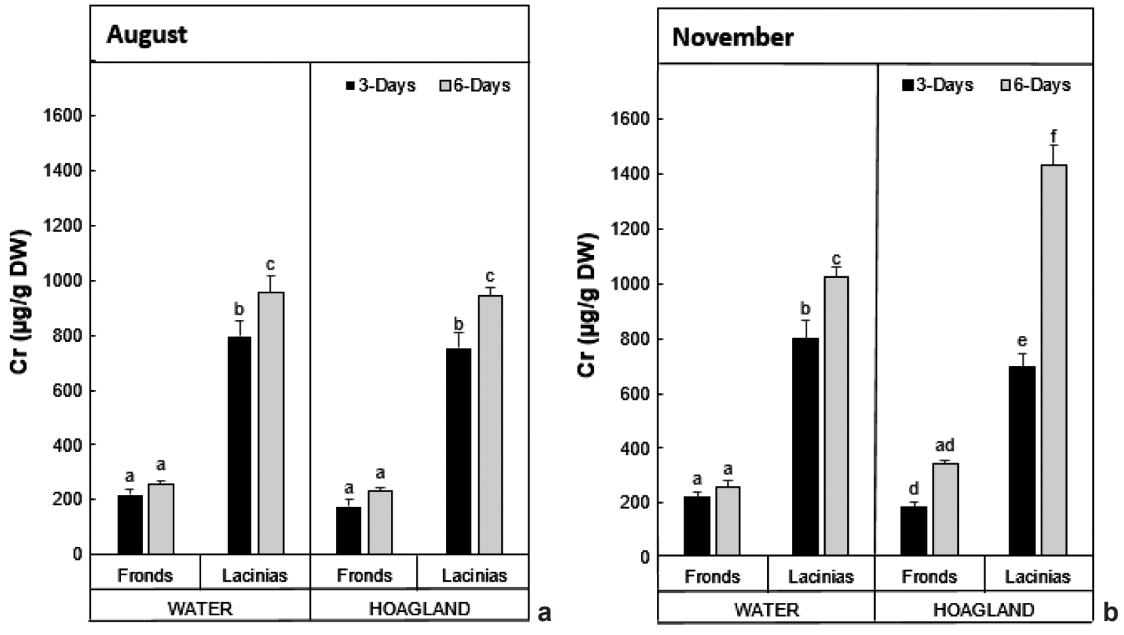

Figure 4 – Cr(VI) accumulation in lacinias and fronds in (a) August and (b) November plants. Different letters indicate significant differences between organs and treatments ($p < 0.05$).

Soluble sugars

Table 2 shows soluble sugars content at 6 d of treatment in all media tested. In general, higher sugar contents were found in November than in August. In fronds and lacinias of August plants were found to have the lowest values of glucose and sucrose.

In lacinias of November plants grown in Hoagland plus Cr were found to have lower soluble sugar content compared to plants grown in Hoagland solution. In both, fronds and lacinia of plants grown in water plus Cr fructose content increase 5.4 times.

Physicochemical parameters of treatment solutions

Physicochemical parameters of treatment solutions are shown in Figure 6. Dissolved Oxygen (DO) in each medium test is shown in Figure 6a-b. Under all treatments, the temporal profile of DO was similar, differing between months only. In August, DO values remained without significant changes around 7.3 mg O_2 L^{-1}; while in November, DO values increased between 33 and 50% reaching between 8.0 and 9.8 mg O_2 L^{-1} at 6-d in the different media.

Salvinia minima significantly increased the EC values (Fig. 6c-d) in Cr(VI)-containing and Cr(VI)-uncontaining treatment solutions, but increases were more pronounced for the former. Maximum values in water plus Cr at 6-d were 32 and 40 µS cm^{-1} in August and November, respectively. In Hoagland plus Cr, EC increased around 17% in both months.

Throughout the treatment period, in both months, the pH levels tend to reach values close to neutrality. Maximum pH values observed at 6 d ranged between 6.2 and 6.8 (August) and between 6.4 and 6.8 (November) (Fig. 6e-f). The pH value of the pond where the plants were collected is maintained around 6.00 +/- 0.3 throughout the year (Prado 2012).

Chemical analysis

In both seasons, ion concentrations in treatment solutions with and without Cr(VI) were affected by *S. minima* plants. All of analysed ions, with the exception of Mg^{2+} and SRP, underwent significant increase in distilled water solution with and without Cr(VI) at 6 d (Tab. 3).

In the Hoagland solution with and without Cr(VI), significant increases occurred in Cl^- concentrations, while SRP showed significant decreases in both months. NO_3^- concentration increased in the Hoagland solution with and without Cr(VI) in August, while in November did not show changes.

Overall, ion concentrations were less affected by *S. minima plants* in Hoagland solution than in distilled water.

Discussion

Data from this study gave interesting results about the effects of development stage and nutrients availability on the biomass production and Cr(VI) removal in *S. minima*.

At the latitude at which the study was carried out, during August begins the vegetative growth period (appearance of new fronds) due to the average maxima temperatures are around 27 ± 3 °C, while in November reach to 37 ± 2 °C and the fronds are already fully expanded and functional.

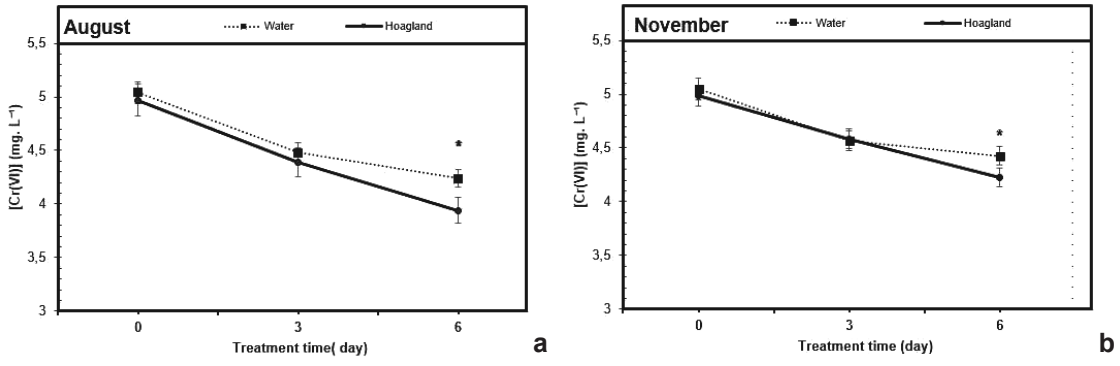

Figure 5 – Cr(VI) remained in treatment solutions (water and Hoagland solution) during the experimental period. (a) August; (b) November. Asterisks indicate significant differences regarding 0 day of experimental period ($p < 0.05$).

Table 1 – Bioconcentration factor (BCF) and translocation factor (TF) of Cr(VI) in *Salvinia minima* plants grown in water and Hoagland solution. Data are mean of three replicates of two independent experiments (n = 6).

BCF	**August**		**November**	
	3 days	**6 days**	**3 days**	**6 days**
Fronds				
Distilled water	44 ± 3^{b}	52 ± 5^{b}	43 ± 3b^{c}	49 ± 5^{b}
Hoagland nutrient solution	35 ± 3^{c}	46 ± 4^{b}	36 ± 3^{c}	67 ± 8^{a}
Lacinias				
Distilled water	159 ± 17^{bc}	191 ± 21^{b}	159 ± 19^{bc}	204 ± 18^{b}
Hoagland nutrient solution	150 ± 12^{c}	188 ± 17^{b}	138 ± 15^{c}	286 ± 25^{a}
TF	**August**		**November**	
	3 days	**6 days**	**3 days**	**6 days**
Distilled water	0.26 ± 0.02^{a}	0.27 ± 0.03^{a}	0.27 ± 0.03^{a}	0.25 ± 0.02^{a}
Hoagland nutrient solution	0.23 ± 0.02^{a}	0.24 ± 0.03^{a}	0.26 ± 0.03^{a}	0.23 ± 0.02^{a}

Different letters denote significant differences in all treatment and month (TF) and between each organ (BCF).

Table 2 – Soluble sugars content (glucose, fructose and sucrose) in August and November plants grown in water and Hoagland solution, in presence or absence of Cr(VI). Data are mean of three replicates of two independent experiments (n = 6).

Solube sugars	**August**		**November**	
	Fronds	**Lacinias**	**Fronds**	**Lacinias**
Glucose (µmoles g^{-1} FW)				
Water	0.09 ± 0.01	0.03 ± 0.00	0.14 ± 0.03	0.30 ± 0.02
Water + Cr(VI) 5 mg/L	0.03 ± 0.00*	0.04 ± 0.00	0.30 ± 0.05*	0.39 ± 0.08
Hoagland	0.04 ± 0.01	0.07 ± 0.02	0.33 ± 0.05	0.66 ± 0.09
Hoagland + Cr(VI) 5 mg/L	0.07 ± 0.00*	0.04 ± 0.01	0.40 ± 0.04	0.23 ± 0.02*
Fructose (µmoles g^{-1} FW)				
Water	0.94 ± 0.10	0.45 ± 0.03	0.75 ± 0.09	1.20 ± 0.15
Water + Cr(VI) 5 mg/L	0.74 ± 0.05*	0.52 ± 0.04	0.69 ± 0.05	6.55 ± 0.83*
Hoagland	0.39 ± 0.06	0.91 ± 0.12	0.66 ± 0.08	4.09 ± 0.53
Hoagland + Cr(VI) 5 mg/L	0.75 ± 0.11*	0.46 ± 0.08*	0.51 ± 0.06	1.46 ± 0.22*
Sucrose (µmoles g^{-1} FW)				
Water	0.10 ± 0.02	0.16 ± 0.02	0.20 ± 0.03	0.46 ± 0.05
Water + Cr(VI) 5 mg/L	0.08 ± 0.01	0.06 ± 0.01*	0.46 ± 0.06*	0.39 ± 0.04
Hoagland	0.06 ± 0.00	0.31 ± 0.06	0.30 ± 0.04	0.64 ± 0.05
Hoagland + Cr(VI) 5 mg/L	0.11 ± 0.01*	0.08 ± 0.01*	0.44 ± 0.04*	0.22 ± 0.02*

* = denote significant difference between presence or absence of chromium ($p < 0.05$).

Temperature and mineral nutrients are key chemical factors that affect the plant growth and development (Zhang *et al.* 2019) but many floating aquatic macrophytes such as *Salvinia* species exhibit extremely plastic adaptations to well-grown in different water solutions and fluctuating temperature regimes (Henry-Silva *et al*. 2008). In agreement, our data show that, regardless of the media tested, new fronds appeared in August plants (Fig. 2a) and DO of growing media did not show changes throughout the treatment, possibly due to a balance between the high respiratory activity of the developing fronds and the photosynthetic O_2 production (Fig. 6a; Tab. 2). On the contrary, in November, an increase in the DO of growing media was observed, probably due to a high photosynthetic activity and a low respiratory activity of fully developed fronds, which was in coincidence with a high soluble sugars content observed under all treatments and organs in this month (Fig. 6b; Tab. 2).

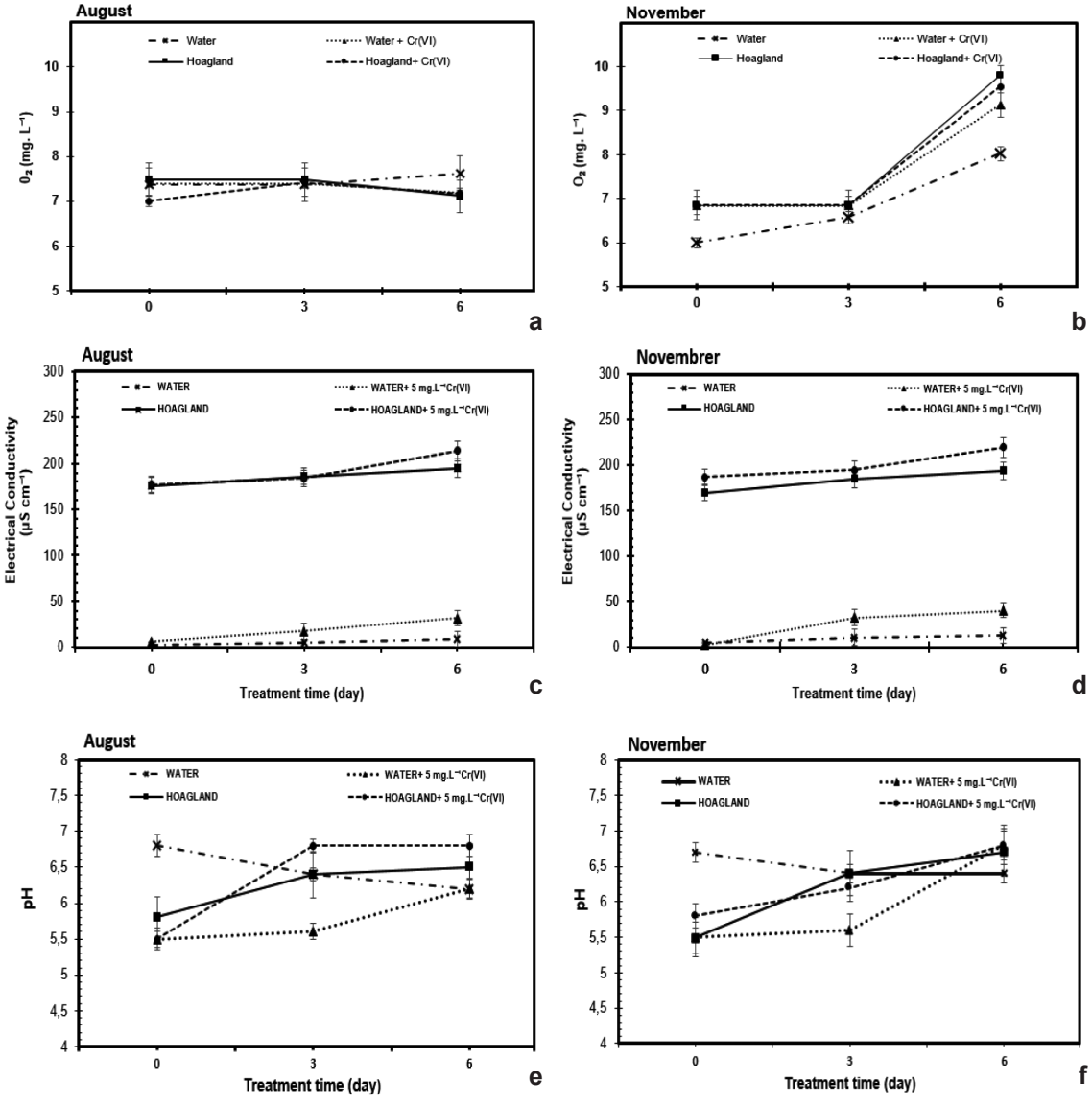

Figure 6 – a-f. Physicochemical parameters in treatment solutions during the experimental period – a-b. dissolved Oxygen (OD); c-d. electrical conductivity (EC); e-f. pH values. Asterisks indicate significant differences regarding 3-d ($p < 0.05$).

Table 3 – Variations of chemical composition of treatment solutions during the experimental period. Data are mean of three replicates of two independent experiments (n = 6).

Treatment solution	August			November		
	Treatment time (day)			Treatment time (day)		
Water + 0 mg L^{-1} Cr(VI)	0	3	6	0	3	6
Cl^- (mg L^{-1})	ND	1.05 ± 0.11*	1.04 ± 0.22*	ND	2.36 ± 031*	2.90 ± 0.22*
SO_4^{2-} (mg L^{-1})	ND	2.11 ± 0.41*	1.56 ± 0.31*	ND	1.01 ± 0.11*	1.41 ± 0.13*
NO_3^- (mg L^{-1})	ND	0.2 ± 0.22*	0.29 ± 0.23*	ND	0.05 ± 0.01	0.2 ± 0.01***
SRP (mg L^{-1})	ND	ND	ND	ND	0.14 ± 0.01*	0.07 ± 0.01**
K^+ (mg L^{-1})	ND	2.59 ± 0.21*	2.50 ± 0.20*	ND	2.59 ± 0.14*	2.50 ± 0.12*
Ca^{2+} (mg L^{-1})	ND	0.27 ± 0.13*	0.29 ± 0.08*	ND	0.92 ± 0.06*	0.57 ± 0.04***
Mg^{2+} (mg L^{-1})	ND	ND	ND	ND	0.04 ± 0.02	0.13 ± 0.07
Water + 5 mg L^{-1} Cr(VI)						
Cl^- (mg L^{-1})	ND	0.88 ± 0.06*	1.03 ± 0.08*	ND	3.4 ± 0.12*	3.75 ± 0.23*
SO_4^{2-} (mg L^{-1})	ND	1.74 ± 0.09*	1.94 ± 0.21*	ND	1.17 ± 0.11*	1.14 ± 0.08*
NO_3^- (mg L^{-1})	ND	0.20 ± 0.02*	0.56 ± 0.04***	ND	0.20 ± 0.02*	0.20 ± 0.02*
SRP (mg L^{-1})	ND	0.22 ± 0.03*	0.12 ± 0.02***	ND	0.33 ± 0.03*	0.07 ± 0.03 **
K^+ (mg L^{-1})	6.26 ± 0.38	3.81 ± 0.12*	8.54 ± 0.44 **	6.07 ± 0.52	5.92 ± 0.43	3.48 ± 0.47*
Ca^{2+} (mg L^{-1})	ND	0.28 ± 0.01*	0.30 ± 0.02*	ND	0.9 ± 0.07*	0.2 ± 0.01***
Mg^{2+} (mg L^{-1})	ND	ND	ND	ND	0.51 ± 0.07*	0.3 ± 0.06 **
Hoagland + 0 mg L^{-1} Cr(VI)						
Cl^- (mg L^{-1})	0.60 ± 0.02	0.70 ± 0.05	1.16 ± 0.10***	0.64 ± 0.02	1.50 ± 0.12*	1.72 ± 0.14*
SO_4^{2-} (mg L^{-1})	26.31 ± 2.30	26.92 ± 4.22	25.66 ± 3.38	26.35 ± 2.25	25.48 ± 1.34	24.89 ± 2.30
NO_3^- (mg L^{-1})	16.00 ± 1.00	30.03 ± 2.05*	20.14 ± 3.28**	16.14 ± 1.75	17.50 ± 2.34	16.84 ± 1.95
SRP (mg L^{-1})	13.20 ± 1.54	12.35 ± 1.11	10.05 ± 1.15*	13.19 ± 1.23	10.75 ± 1.88	7.75 ± 0.80*
K^+ (mg L^{-1})	3.42 ± 0.30	3.56 ± 0.28	4.20 ± 0.39	3.35 ± 0.31	3.40 ± 0.20	3.50 ± 0.28
Ca^{2+} (mg L^{-1})	0.07 ± 0.02	0.28 ± 0.02*	0.31 ± 0.03*	0.06 ± 0.01	0.17 ± 0.02*	0.19 ± 0.03*
Mg^{2+} (mg L^{-1})	0.65 ± 0.07	0.50 ± 0.04	0.49 ± 0.06*	0.65 ± 0.03	0.52 ± 0.03	0.52 ± 0.05*
Hoagland + 5 mg L^{-1} Cr(VI)						
Cl^- (mg L^{-1})	0.50 ± 0.02	0.74 ± 0.08*	0.94 ± 0.13*	0.52 ± 0.04	1.21 ± 0.13*	1.50 ± 0.24*
SO_4^{2-} (mg L^{-1})	26.10 ± 0.32	26.37 ± 0.36	29.65 ± 0.28	26.00 ± 0.25	24.47 ± 0.63	26.73 ± 0.29
NO_3^- (mg L^{-1})	16.06 ± 1.37	29.50 ± 2.08*	26.24 ± 2.86*	16.15 ± 1.10	17.50 ± 1.54	18.23 ± 1.84
SRP (mg L^{-1})	15.72 ± 1.02	11.30 ± 1.65*	11.00 ± 1.08*	13.00 ± 1.52	12.00 ± 1.28	11.50 ± 1.90
K^+ (mg L^{-1})	3.42 ± 0.30	3.50 ± 0.32	4.50 ± 0.41***	3.34 ± 0.25	3.40 ± 0.33	4.02 ± 0.45
Ca^{2+} (mg L^{-1})	0.07 ± 0.02	0.27 ± 0.03*	0.30 ± 0.31*	0.06 ± 0.30	0.31 ± 0.04*	0.38 ± 0.05*
Mg^{2+} (mg L^{-1})	0.62 ± 0.03	0.50 ± 0.03	0.49 ± 0.03*	0.64 ± 0.03	0.50 ± 0.03	0.54 ± 0.03*

SRP = soluble reactive phosphorus

* = denote significant difference respect to 0 day

** = denote significant difference between 3 and 6 days

Since metals are taken up by roots in ionic form, their uptake by plants can be affected by the presence of other ions (Greger 2004; Ephraim *et al*. 2018). In this context, is expected the occurrence of differences in Cr(VI) accumulation patterns between water and Hoagland grown plants. Greger (2004) claimed that the uptake of heavy metals decreases with decreasing metal/mineral nutrient ratio and Yadav *et al*. (2016) assume that mineral nutrients influence the plant growth which, in turn, also affects both metal uptake and metal accumulation. Our results showed that in November, plants grown in Hoagland at 6d accumulated 35–40% more Cr than water-grown plants (Fig. 4). In August this effect was not observed, probably because in this period the metabolism of the plant is focused on the development of new tissues (new leaves). Metal accumulation in aquatic plants also depends on the growing season, but available data are controversial (Polechońska *et al*. 2017). Some studies indicate higher metal accumulation in autumn than in spring, while others indicate high content during spring or summer and low accumulation in winter (Duman *et al*. 2006). Metal accumulation and BCF in November plants grown in Hoagland presence was significantly higher than biomass increase, indicating that plant development stage is a determining factor for its accumulation capacity. The opposite occurs when the plants are in the sprouting stage (August) possibly because the new tissues, regardless of nutrients presence, do not have a high accumulation capacity. Tissues under active growth would be a stronger C sink than other processes such as ion accumulation and protecting compounds. This shows that a higher biomass accumulation would not necessarily imply a higher Cr accumulation.

According to their ability to translocate absorbed metals, plants can be considered accumulator species as they actively take up metal through their roots and translocate it to aerial parts; or tolerant species by restricting metal transfer from root to stem (Yoon *et al*. 2006). The efficiency of plants to translocate heavy metals from the root to the shoot is evaluated by the Translocation factor (TF). When TF is higher than one indicates an effective metal transfer to aerial parts whereas TF is less than one indicates ineffective metal transfer, suggesting that these plant types accumulate metals in the roots more than in shoots or leaves (Usman *et al*. 2019). Data of TF values suggest that *S. minima* did not transfer the metal to the fronds, that is, it accumulated it preferentially in lacinias. However, in this type of plant, which has the capacity to absorb by both structures (fronds and lacinia), this parameter is not conclusive, but based on the Cr content observed in lacinia, we can conclude that it is there where it preferentially accumulates.

In both seasons, the EC of treatment solutions did not show different patterns along the experimental period. When comparing EC values between Cr-containing and Cr-uncontaining treatment solutions, significant increases were found in Cr-containing water medium indicating a possible oxidative damage of plasma membrane induced by the metal. Contrarily, there were no differences in EC values between Cr-containing and Cr-uncontaining Hoagland solution. This fact probably reflects a better functionality of the antioxidant mechanism due to the nutrient supplementation of Cr-containing solution as has been reported for other species subjected to heavy metals stress (Cheng *et al.* 2012; Yadav *et al*. 2016).

Salvinia minima tends to stabilize the pH at values close to neutrality under all treatments, demonstrating that at low concentrations of Cr this species has mechanisms to increase or decrease the pH of the medium, adjusting it to a narrow range (6.2–6.8) for best performance (Gaudet 1973; Chocobar-Ponce *et al*. 2014). One of these mechanisms could involve an increased excretion of organic anions (*e.g.*, citrate, malate, oxalate) as has been reported for other aquatic plants (Javed & Greger 2011; Yang *et al*. 2013). In this context, it is expected that released acid anions interact with both, protonated binding sites (functional groups) of the cell wall and free protons of treatment solution, which lead to rising of the pH value of root-surrounding solution (Chocobar-Ponce *et al*. 2014).

In water growth media under all conditions, an increase in the ion contents was observed, except K^+ in the media Cr-containing in November. This fact could be related to the maintenance of an osmotic balance between plant and the growth media.

In Hoagland presence, significant changes in SRP, Cl^-, Ca^{2+} and Mg^{2+} contents were observed. SRP and Mg^{2+} contents decreased in both seasons between 20–40%, which could indicate these are limiting ions to the *Salvinia* metabolism. Ca^{2+} content increased in both months under all conditions. This fact would indicate that *Salvinia* would not be using it, possibly because this ion could be replaced in its functionality by other divalent cations such as Mg^{2+}, which decreased in the growth media (Tab. 3). These results could

suggest a difference in essentiality criteria between higher and lower plants, although more specific assays are necessary to confirm this hypothesis.

NO_3^- content increased in all growth media, except in Hoagland of November. In this sense, passive or active efflux (*e.g.*, transporter NXT1) have been described in higher plants (Segonzac *et al*. 2007); however, the physiological significance of nitrate extrusion remains to be elucidated.

According to our results, the Cr(VI) accumulation in *Salvinia minima* plants depends on the development stage and the presence of mineral nutrients. It is important to consider the phenological stage of plants used in phytoremediation. A higher biomass accumulation would not necessarily involve a higher Cr accumulation, because in the sprouting stage (August) the metabolism is more addressed to growth and not towards the uptake and protection mechanisms against heavy metals. Changes in the ion composition of growing media show possible differences in essentiality criteria between higher and lower plants, for which further studies are important.

Finally, considering the use of the species in phytoremediation processes, we suggest: the use of fully developed plants and a plant-contaminated environment contact time of at least 6 days to ensure that the greatest possible metal removal is achieved.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data supporting the findings of this study are available within the paper and from the corresponding author, Mariana Rosa, upon request.

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