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Artículo Original

Bioabsorption and translocation of trivalent chromium in the floating macrophyte *Pistia stratiotes*

Bioabsorción y traslocación de cromo trivalente en la macrófita flotante *Pistia stratiotes*

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Abstract: In this work, the capacity of *P. stratiotes* to absorb, transfer, and remove trivalent chromium in aqueous solution of basic chromium sulfate of different concentrations was evaluated, with a focus on possible applications in phytoremediation of tannery effluents. The accumulation in plant tissues was also evaluated. The concentrations of the solutions used were 2.14, 4.90, 11.98, 25.37 and 43.87 mg.L^{-1} and the exposure period of the macrophyte was 48 hours. At the end of the experiment, the percentages of trivalent chromium removal were 90.6%, 91.4%, 88.6%, 79.9% and 64.6%, respectively. The macrophyte *P. stratiotes* showed high capacity to remove trivalent chromium from acid solutions, and a higher accumulation of the metal was observed in the roots than in the aerial parts; with low translocation from the root to the aerial part: TF= 0.09, 0.06, and 0.05 and bioconcentration factor (BCF) 4416, 1496, and 892 for 2.14 to 25.37, and 43.87 mg.L^{-1} respectively with significant differences between the treatments at 2.14 and 43.87mg.L^{-1}**.** No growth inhibition or senescence was observed during the exposure period, and initial concentrations below 25.37 mg.L^{-1} decreased below 2 mg.L^{-1}. In the calculations of kinetic parameters such as linear correlation coefficient R^2, reaction rate constant K, half-life time $t_{1/2}$, and removal rate V, it was found that most of the concentrations studied fit second-order kinetics best. These data can be used for the design of secondary treatment of industrial wastewater contaminated with trivalent chromium using phytoremediation.

Keywords: *removal kinetics, phytoremediation, metal, bioconcentration factor.*

Resumen: En este trabajo se evaluó la capacidad de P. stratiotes para absorber, transferir y eliminar cromo trivalente en solución acuosa de sulfato básico de cromo en diferentes concentraciones, con un enfoque en posibles aplicaciones en la fitorremediación de efluentes de curtiembres. También se evaluó la acumulación en los tejidos de la planta. Las concentraciones de las soluciones utilizadas fueron 2.14, 4.90, 11.98, 25.37 y 43.87 mg.L^{-1} y el período de exposición del macrófito fue de 48 horas. Al final del experimento, los porcentajes de eliminación de cromo trivalente fueron 90.6%, 91.4%, 88.6%, 79.9% y 64.6%, respectivamente. La macrófita *P. stratiotes* mostró una alta capacidad para eliminar cromo trivalente de soluciones ácidas, y se observó una mayor acumulación del metal en las raíces que en las partes aéreas, con baja translocación desde la raíz hasta la parte aérea: TF= 0.09, 0.06 y 0.05 y factor de bioconcentración (BCF) 4416, 1496 y 892 para 2.14 a 25.37 y 43.87 mg.L^{-1}, respectivamente, con diferencias significativas entre los tratamientos a 2.14 y 43.87 mg.L^{-1}. No se observaron inhibición del crecimiento ni senescencia durante el período de exposición, y las concentraciones iniciales por debajo de 25.37 mg.L^{-1} disminuyeron por debajo de 2 mg.L^{-1}. En los cálculos de parámetros cinéticos como el coeficiente de correlación lineal R^2, la constante de velocidad de reacción K, el tiempo de vida media $t_{1/2}$ y la tasa de eliminación V, se encontró que la mayoría de las concentraciones estudiadas se ajustan mejor a cinéticas de segundo orden. Estos datos pueden ser utilizados para el diseño de un tratamiento secundario de aguas residuales industriales contaminadas con cromo trivalente utilizando fitorremediación.

Palabras clave: *cinética de eliminación, fitorremediación, metal, factor de bioconcentración.*

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Introduction

Water pollution due to human activity has become an ever-increasing global problem that threatens both public health and the integrity of the communities in the receiving environments. Various pollutants can be present in water, and heavy metals represent a group of pollutants difficult to control, since once incorporated into ecosystems, they are difficult to eliminate due to their non-biodegradable nature and capacity for bioaccumulation (Arán *et al.*, 2017). Chromium is considered one of the main inorganic water pollutants and its presence is associated with industries related to leather tanning (Medina *et al.*, 2019; Moretto, 2015and mining (Mohanty *et al.*, 2023). It can be present in two oxidation states: as trivalent Cr(III) and hexavalent Cr(VI). Plants can absorb Cr(III) while maintaining the oxidation state, while Cr(VI) is reduced to the trivalent form when it enters plant cells. Phytoremediation takes advantage of the ability of some plants to remove metals from contaminated environments.

The underlying mechanisms of phytoremediation are based on processes such as phytostabilization, phytodegradation, rhizofiltration, phytoextraction and phytovolatilization (Nedjimi, 2021). Metals can enter through various organs exposed to contaminants, usually through the roots, and from there pass to the rest of the plant; this process that allows the movement of the contaminant in the plant is known as translocation, which occurs from the roots to the aerial part, and is a useful parameter when selecting bioaccumulator plant species (Susarla *et al.*, 2002Cr(III) uptake in plants occurs by simple diffusion at cation exchange sites in the cell wall, whereas Cr(VI) can actively enter through sulfate transporter channels (Gomes *et al.*, 2017; Jovania *et al.*, 2013; Zayed *et al.*, 1998). The process of chromium removal in aqueous solution can be explained by surface adsorption, absorption at the tissue level and other phenomena such as precipitation (Maine *et al.*, 2016). Maine *et al.* (2016) propose that the kinetics of Cr(III) removal involves two processes or components: a fast and a slow one. The fast component occurs during the first hours of contact and is responsible for the major removal of Cr(III) from water, where physical adsorption is the most important removal mechanism; the slow component would be explained by the precipitation and cellular absorption of chromium.

Floating macrophytes comprise a large and varied group of plants, with *Pistia stratiotes* (water lettuce) being one of the most recognized and widely distributed species.The genus Pistia probably originated in the Thetis region (a region that existed prior to the emergence of the Indian Ocean and Mediterranean Sea) (Renner & Zhang, 2004), and currently has a cosmopolitan distribution and is considered invasive in some regions along with *Eichhornia crassipes* (D. Liu *et al.*, 2017). *P. stratiotes* is used in bioremediation of different types of pollutants, through its use in free-flowing or surface wetlands. It can remove toxicity from industrial effluents (Victor *et al.*, 2016) and various metals such as lead (Espinoza-quiñones *et al.*, 2009), chromium (M. A. Maine *et al.*, 2016), and mercury (Molisani et al, 2006). It can also physiological changes induced by iron excess (Coelho *et al.*, 2023) and remove physicochemical parameters indicative of wastewater pollution such as total solids, suspended solids, chemical oxygen demand, biological oxygen demand, total nitrogen, and soluble phosphorus (Abbasi *et al.*, 2022). It has also been used in the treatment of highly toxic biocidal compounds such as chlorpyrifos (Prasertsup & Ariyakanon, 2011) and herbicides at low concentrations (Escoto *et al.*, 2019). Previous chromium removal studies with this macrophyte have focused mainly on Cr(VI) (Maine *et al.*, 2016; Tabinda *et al.*, 2020); while work with Cr(III) has been limited to exposing the plants to low concentrations of the metal, usually less than 5 $mg.L^{-1}$ (Espinoza-Quiñones *et al.*, 2008; Şentürk *et al.*, 2023). Conventional industrial leather tanning processes currently use basic chromium sulfate ($Cr(OH)SO_4$) during basification (Prokein *et al.*, 2020 Cr(III) is still less studied than the Cr(VI) form. The objective of this study was to evaluate the capacity of *P. stratiotes* to absorb, transfer and remove trivalent chromium in aqueous solution of basic chromium sulfate of different concentrations, with prospects for possible applications in phytore-

mediation of tannery effluents.

Materials and methods

Plant material growth and experimental conditions

The plants were collected from Lake Ypacaraí-Paraguay (21J, X:469630.65 m E, Y: 7194572.38 m S). The species was taxonomically identified and its duplicates were deposited in the Herbariums of the Faculty of Exact and Natural Sciences (FACEN) and of the Faculty of Chemical Sciences (FCQ) of the National University of Asunción (UNA), and in the Natural History Museum of the United Kingdom (BM) with the code 1740 (J. De Egea, T. López Arias, 9 mayo 2017). The plants were acclimatized for 60 days in the greenhouse, first with a mixture of tap water and foliar fertilizer (NPK, 20:20:20) and then with the modified APHA medium (Peterson & Moody, 1997). The experiments used seedlings produced and grown in the greenhouse at an average temperature of 25 °C.

Two different experiments were carried out. In the first experiment, the ability of *P. stratiotes* to remove Cr(III) in aqueous solutions of basic chromium sulfate $Cr(OH)SO_4$ at 2.14; 4.90; 11.98; 25.37 and 43.87 $mg.L^{-1}$ was evaluated, with five replicates each. Plants were set to float freely in 0.5 L plastic pots; the pH of the water was maintained at 5.5 to avoid precipitation of Cr(III). They were evaluated for 48 hours, extracting 5 mL aliquots of each solution at 6 times, then the aliquots were acidified to pH=2 with the addition of a drop of 2% nitric acid and stored under refrigerated conditions until further analysis. In the second experiment, the capacity for Cr(III) absorption by the roots and its translocation to the aerial part of the plant were evaluated. For this purpose, experiments were set up under similar conditions to the previous one, with the difference that the plants were kept afloat with a plastic separator, in order to prevent the leaves from coming into contact with the solutions, and thus only the roots had contact with the Cr(III) solution at concentrations of 2.14; 25.37 and 44.87 mg.L-1 The plants were harvested after 48 hours, and the roots were separated from the aerial part for later analysis.

Determination of Cr(III) in aqueous solution and in plant tissues

The determination of Cr(III) in Experiment 1 was carried out directly from the extracted and acidified aliquot. In Experiment 2, the plant samples were washed with an acidified + EDTA solution and tap water, then placed in aluminum trays and dried at room temperature in the laboratory. Then they were dried in an oven at 75±2 ºC for 48 hours. Metal extraction was carried out by microwave-assisted digestion, for which 0.1 to 0.5 grams of the dried plant material samples were weighed and digestion was performed following the methodology indicated by the microwave digestion equipment used (Brand: SINEO, Model: Microwave Reaction system TANK Basic, Manufacturer: Hanon Advanced Technology Group Co., Ltd, City: Jinan, Country: China). Cr (III) quantification for both experiments was done by atomic absorption spectrophotometry readings (Brand: SHIMADZU, Model:AA-7800, Manufacturer: Shimadzu Inc., City: Kyoto, Country: Japan) according to the standardized 3111B direct air-acetylene flame method. (APHA, 2012). Hexavalent chromium was determined at the beginning and end of the experiments to observe whether there was oxidation of trivalent chromium, according to the colorimetric method 3500-Cr D (APHA, 2012).

The bioconcentration factor (BCF) was determined by using the equation (1):

$$BCF = \frac{C\,macrophyte}{Cw\,initial} \qquad (1)$$

where *C macrophyte* is the metal content in the plant organ at the time of harvest ($mg.Kg^{-1}$ dry weight) and *Cw initial* corresponds to the initial metal content in the liquid medium ($mg.L^{-1}$).

The translocation factor (TF) was estimated through the equation (2):

$$TF = \frac{C\,leaf}{C\,root} \qquad (2)$$

where *C leaf* is the chromium content in leaves and

C root is the chromium content in roots.

The removal efficiency (R%) was determined by equation (3):

$$R(\%) = \left[\left(Ci - Cf \right) / Ci \right] \times 100 \qquad (3)$$

where *R* is the decrease of the metal, *Ci* the initial concentration and *Cf* the final concentration.

Determination of kinetic parameters

In our study we observed exponential decay of initial concentrations and evaluated the linearization with zero-order, first-order and second-order chemical reaction kinetic models according to the equations 4, 5 and 6:

$$[A]t = -kt + [A]_0 \qquad (4)$$

$$ln[A]_t = -kt\ ln[A]_0 \qquad (5)$$

$$\frac{1}{[A]_t} = kt + \frac{1}{[A]_0} \qquad (6)$$

where $[A]$ is the Cr(III) concentration, k is the reaction rate constant, and t is the exposure time.

Statistical Analysis of Data

Data analyses were performed using Microsoft Excel and SPSS 21.0 software. One-way analysis of variance ($p < 0.05$) was used to investigate a statistically significant difference in mean pollutant removal efficiencies among CWUs (Clean Water Units). Multiple comparisons were performed using Tukey's HSD tests. Statistical analyses were performed with SPSS Statistics 21.0.

Results and Discussion

Cr(III) removal from aqueous solutions and accumulation in plant tissues of *P.stratiotes*, BCF (bioconcentration factor) and TF (Translocation Factor)

The results of Cr(III) removal capacity in solutions of 2.14, 4.90, 11.98, 25.37, and 43.87 mg.L^{-1} by the macrophyte *P. stratiotes* are presented in Fig. 1A and B. The decrease in the concentration of metal in solution occurs mainly in the first 6 hours, with a slower reduction between 15 and 48 hours. The

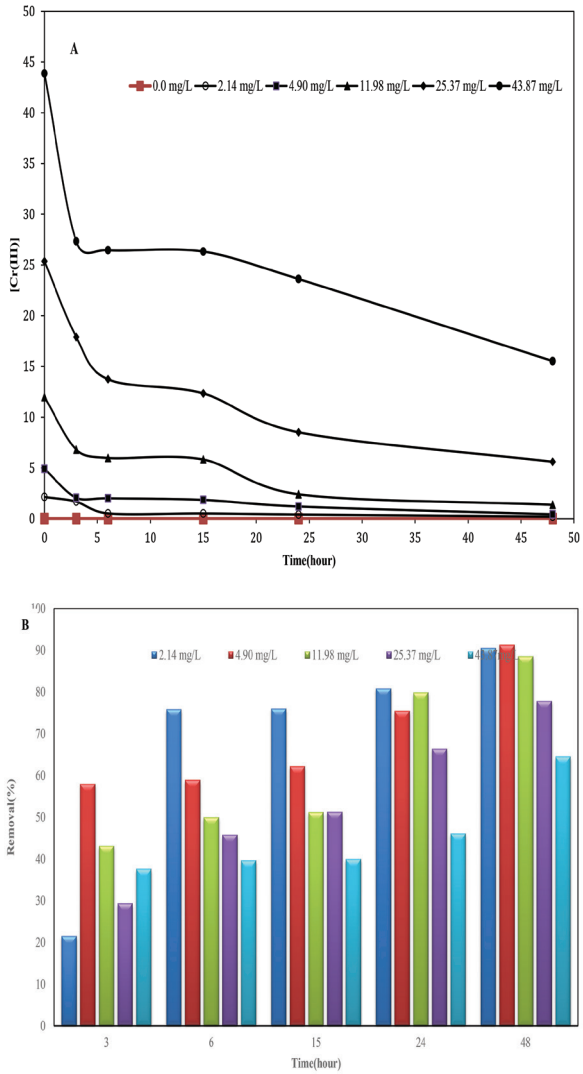

Figure 1. Removal of Cr(III) in aqueous solution by *Pistia stratiotes* **A**. Decrease in Cr(III) concentration over time **B**. Percentage of Cr(III) removal.

observed removal at the first 6 hours was 75.9%, 59%, 50%, 45.8% and 39.7% for the treatments at 2.14; 4.90; 11.98, 25.37 and 43.87 mg.L^{-1} respectively. The final concentrations achieved at 48 hours were 0.20, 0.42, 1.37, 5.61 and 15.52 mg.L^{-1}, corresponding to a removal of 90.6%, 91.4%, 88.6%, 79.9% and 64.6% of the initial concentrations. However, as the concentration of chromium in the treatments increased, a reduction in the removal capacity was observed, mainly in the higher concentrations (25.37 and 43.87 mg.L^{-1}) (Fig. 1B).

Similar removal values of 80% and 75% by exposing *P. stratiotes* to 2 and 6 mg.L^{-1} Cr(III) have been reported in another study (Maine *et al.*, 2016 The results obtained in removal experiment 1 are due to a reduction caused by the direct contact of the whole plant (roots + leaves) with the Cr(III) solution. Maine *et al.* (2004), showed that *P. stratiotes* can adsorb at the leaf level when the leaves come into direct contact with water. These researchers concluded that the adsorption of Cr(III) by direct contact between the leaves and the solution is the main cause of the increase of this metal in the aerial parts, being poorly translocated from the roots to the aerial parts.

The Ministry of Environment and Sustainable Development of Paraguay (MADES) establishes a maximum effluent discharge threshold of 2 mg.L^{-1} for Cr(III) (SEAM, 2002). Our experiments showed that *P. stratiotes* can effectively remove initial concentrations <25.37 mg.L^{-1} Cr(III) of Cr(III) in solution in 48 hours (Fig. 1A) and generate wastewater <2 mg.L^{-1} of the metal.

At the end of the exposure time, 48 hours, the metal concentration values in *P. stratiotes* show that it bioaccumulates mainly in the root. (Table 1) showed that this plant bioaccumulates more in the root (Table 1). An increase in Cr(III) accumulation in roots and aerial parts was observed with increasing Cr(III) concentration. The accumulation values in tissues at low concentrations (2.14 mg.L^{-1}) are higher than those reported by Maine *et al.* (2016), who report values of 0.168 and 1.52 mg.g^{-1} de Cr(III) in leaves and roots of the plant, however the equivalent TF is 0.11, which is higher than the one obtained in this study (TF=0.09, Fig. 2). In a hydroponic system, a high BCF (≥ 1000) for whole plant tissue indicates the phytostabilization potential of plants for heavy metals (Singh & Ma, 2007; Adel *et al.*, 1998). Our experiments showed BCF higher than 1000, so this species can be considered as a good accumulator of Cr(III).

Table 1. Cr(III) concentrations in *Pistia stratiotes* tissues (mg. Kg^{-1} dry weight) obtained in the different treatments. Each value is the mean value of three replicates ± standard deviation.

	2.14 mg.kg^{-1}	25.37 mg.kg^{-1}	43.87 mg.kg^{-1}
Leaf	862±41	2,429±391	2,157±77
Root	9,274±613	35,159±235	39,077±779

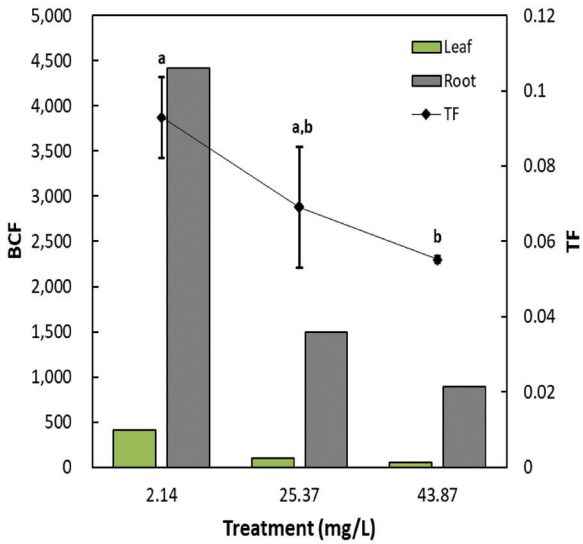

Figure 2. Bioconcentration factor (BCF) and Translocation Factor (TF) Cr(III) in *P. stratiotes*.

The BCF values decreased with increasing concentrations of the metal in aqueous solution. When concentrations increased from 2.14 to 25.37 and 43.87 mg/L, the BCF decreased to 4,416, 1,496, and 892 in roots and to 37.29, 21.19, and 13.54 in leaves (Fig. 2). This is also directly related to a reduction in TF 0.09; 0.06, and 0.05 respectively with significant differences between treatments at 2.14 and 43.87 mg.L^{-1} ($p<0.05$). The bioconcentration factor is an important index to evaluate the potential for phytoremediation of metals (Mendez & Maier, 2008). Similar findings of reduction of BCF and TF of *P. stratiotes* upon increasing Cr(III) concentration from 0.75 to 5 mg.L^{-1} were described by Şentürk *et al.* (2023Our study shows that this behavior is maintained at higher concentrations such as 25.37 and 43.87 mg.L^{-1}. Root-to-shoot translocation of Cr (III) was extremely limited due to the propensity of Cr(III) to bind to cell walls.

Our study confirms the ability of *P. stratiotes.* It is taken up by plants by simple diffusion at cation exchange sites in the cell wall and it accumulates in vacuoles (Gomes *et al.*, 2017) Intake has been

Tabla 2. Kinetic parameters for removal of chromium (III) in aqueous solution.

	Zero order			First order				Second order			
[Cr(III)] ($mg.L^{-1}$)	R^2	K=V ($mg.L^{-1}.h^{-1}$)	$t_{1/2}$ (h)	R^2	K (h^{-1})	$t_{1/2}$ (h)	V ($mg.L^{-1}.h^{-1}$)	R^2	K ($mg^{-1}.L.h^{-1}$)	$t_{1/2}$ (h^{-1})	V ($mg.L^{-1}.h^{-1}$)
2.14	0.529	0.03	33.16	0.871	0.057	12.14	0.12	0.894	0.19	2.51	0.85
4.9	0.581	0.06	38.01	0.897	0.042	16.34	0.21	0.916	0.04	4.89	1.00
11.98	0.705	0.18	34.16	0.909	0.042	16.62	0.50	0.958	0.01	6.23	1.92
25.37	0.732	0.34	37.86	0.904	0.028	24.49	0.72	0.883	0.00	56.32	0.45
43.87	0.635	0.41	53.43	0.787	0.016	42.26	0.72	0.983	0.00	8.14	5.39

shown to occur mainly through Fe(III) channels (J. Liu *et al.*, 2011). Translocation to leaves involves the xylem through the symplastic system, and may subsequently be distributed into the cytoplasm of cortical cells. Previous studies have shown that in plants treated with Cr(III) in aqueous solution, the metal is distributed into the periderm, stem cortex, epidermis and parenchyma of leaf tissues (Mongkhonsin *et al.*, 2011).

Removal kinetics of Cr(III) in aqueous solution with *P. stratiotes*

Among the most widely used models in the literature, three basic kinetic models, zero-order, first-order and second-order, are frequently used to describe the kinetics of environmental systems (Samal & Trivedi, 2020)

In our study we observed exponential decay of the initial concentrations and evaluated the data with linearization of the basic kinetic models, equations (4), (5) and (6) respectively; with values of correlation coefficients, rate constant, half-life and removal rate in Table 2. Figure 3 shows the graphs of the kinetic studies. From the results, it can be concluded that the removal of Cr(III) from aqueous solution with *P. stratiotes* follows first and second order kinetics, although most of the concentrations studied better fit the second order kinetic model, which indicates that the removal rate of chromium (III) in aqueous solution with *P. stratiotes* is directly proportional to the square of its initial concentration. Only the solution of 25.37 mg.L-1 showed a better fit with the first order kinetic model, in this

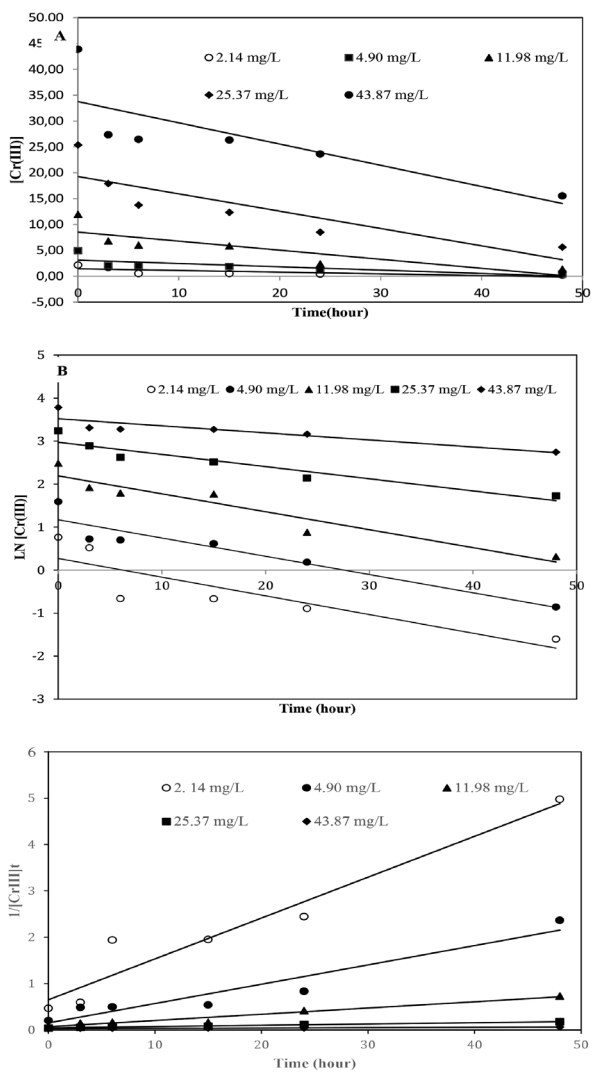

Figure 3. Linearization of kinetic equations. **A)** zero order. **B)** first order. **C)** Second order.

case the removal rate is directly proportional to the initial concentration of Cr(III) in aqueous solution. The rate constant (k) for different Cr(III) removal orders was calculated from the slopes of the line graphs and listed in Table 2.

Conclusions

P. stratiotes favors the rapid removal of Cr(III) in aqueous solution mainly in the first 6 hours of exposure, with final removal values of up to 90.6% at low concentrations. The increase in Cr(III) concentration in plant tissues occurred especially in roots (39.077 $mg.kg^{-1}$ dry weight) and was related to the concentration of the metal in the medium. The translocation factor indicates little migration of Cr(III) from the root to the aerial part, and it decreased with increasing metal concentration in solution. Based on the BCF, *P. stratiotes* can be considered as a hyperaccumulator species and has a high potential as a phytoremediator of trivalent chromium-contaminated effluents. Kinetic parameters such as removal rate and half-life time of the pollutant serve as a basis for future designs of treatment wetlands with *P. stratiotes* or industrial wastewater contaminated with this metal.

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Author contribution

All the authors contributed equally to the elaboration of the paper.

Conflicts of interest

The authors declare having no conflicts of interest.

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