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Biomass of the macrophyte remedies and detoxifies Cd(II) and Pb(II) in aqueous solution

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Abstract Aquatic plants are considered to be important remedial agents in aquatic environments contaminated by metals. The *Salvinia biloba* macrophyte was evaluated in relation to its removal kinetics, adsorption capacity, and toxicology, aiming at its application in the removal of Cd^{+2} and Pb^{+2} ions from aqueous solutions. A batch-type system was used, in which the plants were cultivated in microcosms containing nutritive solution and metallic ions, stored in a controlled environment (pH, temperature, and luminosity). The removal kinetics consisted in the analysis of efficiency, varying the concentrations of the metals, and time of cultivation of plants in solution. To describe the process, adsorption isotherms were constructed with the equilibrium data, which were later adjusted to Langmuir and Freundlich models. The toxicological trial was performed by sub-acute exposure test of *Caenorhabditis elegans* nematode to phytoremediated solutions. The results highlight the remedial effect of the plant in solutions contaminated with both metals. The kinetic study demonstrated that the plant responds differently to metals, and physical-chemical and biological processes can be attributed to the removal of metals from the solution by the plant. The equilibrium time obtained was 48 h for both metals, and the adsorption capacity was higher for Cd^{2+}. The toxicological evaluation indicates that there was a reduction in toxicity after the remediation of the solutions by *S. biloba*, for all times and concentrations evaluated. *Salvinia biloba* was efficient for the removal of Cd^{2+} and Pb^{2+} metals from aqueous solution. The plant is a low-cost metal biosorbent and can be considered promising for phytoremediation strategies in liquid effluents and water bodies.

Keywords Plants · Remediation · Removal kinetics · Toxicity · Trace metals · Water

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Introduction

Trace metals are recognized for their toxicity to different ecosystems, including human health, negatively impacting animal, plant, and microbial diversity (Pietro-Souza et al., 2017; Mata et al., 2019; Mello et al., 2019; Mariano et al., 2020; Martyniuk et al., 2020; Yang et al., 2020). Some metals (e.g., Cu, Zn, Mn & Cd) are used in cellular metabolism at low concentrations, and exposure at high concentrations results in several harmful effects at the cellular, individual, population, and even community levels (Järup, 2003). They are detected in natural environments and frequently in concentrations below the limit capable of offering risk to the environment. Unfortunately, these metals have accumulated in environments over the years in areas under anthropic influence (Ciszewski & Grygar, 2016; Hanfi et al., 2019; Zhu et al., 2019).

Among the metals of environmental interest, cadmium (Cd) and lead (Pb) are known for their polluting potential and adverse effects on environmental and human health (Sljivic Husejnovic et al., 2018; Silva et al., 2018; Nong et al., 2020). The main dissemination routes of Cd and Pb to the aquatic environment are by way of mining effluents from mine drainage, agricultural effluents from the use of fertilizers and pesticides, and effluents from the paint and lubricant industry (Campaner & Luiz-Silva, 2009; Wolff et al., 2012; Taylor et al., 2014; An et al., 2017). Specifically regarding Pb, other sources of contamination for the aquatic environment include automobile batteries (Freitas et al., 2009) and electronic equipment (Damasceno et al., 2016) due to improper disposal in the environment.

In the search for alternatives aiming to reduce or eliminate contamination and the impacts generated by metals, there has been a growing interest in the use of technologies that are capable of removing or mitigating these substances to levels considered tolerable, such as phytoremediation (Nair & Kani, 2016; Casagrande et al., 2018; Freitas et al., 2019, Yang et al. 2019). Phytoremediation is based on detoxification processes through the incorporation of contaminants and subsequent metabolization, or immobilization within the plant, or through the promotion or support of rhizospheric microorganisms (Alkimin et al., 2020). Understood as a "green" technology, phytoremediation has been considered a technology capable of overcoming, in terms of economic and environmental cost-benefit, the traditional physical-chemical systems of water treatment (Costa et al., 2018; Anand et al., 2019; Paruch et al., 2019).

The phytoremediation systems used for aquatic environments are generally presented in configurations of wetlands, be they natural or man-made (*Wetlands*), or lagoon systems, in which the remedial agents are plants typical of wetlands, such as aquatic macrophytes (Fia et al., 2017; Prabakaran et al., 2019; Yang et al., 2020; Zhao et al., 2020). Macrophytes are considered promising for water treatment, mainly due to their rapid growth, large biomass production, and direct contact with the pollutant in the environment (Sood et al., 2011; Akhtar et al., 2017; Al-Homaidan et al., 2020; Eid et al., 2020). To deal with the metals, macrophytes present varied biological mechanisms, of which are highlighted the processes of adsorption, absorption, and translocation (Tran & Van, 2016; Rodrigues et al., 2016; Freitas et al., 2018a; Jiang et al., 2018). Due to the biological processes facing the toxic effects of metals on plants, the efficiency of a given species for remediation purposes depends on its biological characteristics, as well as technical aspects adopted in the phytoremediation project.

The *Salvinia* Ség. (Salviniaceae) are macrophytes which thrive in diverse environments such as wetlands, reservoirs, lagoons, and rivers, especially in tropical regions where they find the ideal climatic conditions for their development (Esteves, 1998; Pitelli et al., 2014; Gomes et al., 2017), and studies have evidenced their use in remediation processes for metal-contaminated environments (Guimarães et al., 2006; Wolff et al., 2012; Casagrande et al., 2018; Freitas et al., 2018a, b; Loría et al., 2019). And thus, this study evaluated the application of *Salvinia biloba* Raddi (Salviniaceae) in the removal of Cd^{+2} and Pb^{+2} in aqueous solutions, contributing to a reduction of environmental toxicity. *Salvinia biloba* was submitted to kinetics and adsorption capacity tests by way of experimental and theoretical models, and the remediation effect of the plant on metal solutions was evaluated by means of the toxicological test using the nematode *Caenorhabditis elegans* Maupas, 1900 (Secernentea, Nematoda) as a model.

Material and methods

Sampling and preparation of plants

The plants were collected in a lagoon in the municipality of Alta Floresta, north of Mato Grosso, Brazil (9° 51′ 11.81″ S and 56° 4′ 50.61″W), in February 2017 and sent to the laboratory. In the laboratory, the plants were put through a screening process, which consisted of washing them in natural running water and then selecting those with similar morphological conditions (size, mass, and vitality). Plants that presented characteristics expected for a healthy condition, such as the green pigmentation characteristic of the species and absence of damage to their structures, were selected. For subsequent steps, the plants were weighed in an analytical balance, considering a fresh biomass of approximately 2.0 grams (for each sample unit) for each experiment. The size was standardized during weighing, prioritizing leaf conformation, and root size for a better homogenization of the samples.

Characterization of functional groups by way of IR

After sorting, the plants were dried in an air circulation drying oven at 60 ºC until constant weight. The material was crushed, sieved, and separated, and particles of average size of 0.267 nm were obtained (Freitas et al., 2018b). The absorption spectrophotometry in the infrared region with Fourier transform (FTIR) (Shimadzu Iraffinity-1 (Shimadzu)) was carried out as an auxiliary technique in the characterization of *S. biloba* samples. The spectra were obtained using potassium bromide pellets containing about 1–2 % of the dry plant, which were recorded at room temperature in the range of 3.900–500 cm^{-1}. For each sample, 20 scans were recorded with a resolution of 4 cm^{-1}.

Kinetic study

The kinetic study was carried out adopting a batch-type system in a controlled environment. Each *S. biloba* plant, previously weighed, was inserted in 1 L microcosm with nutritive solution containing $(NH_4)_2CO_3$ (ammonium carbonate) and KNO_3 (potassium nitrate). The pH of the solutions was defined at 6.5, considering the pH of the water from the plant's place of origin and was adjusted using a buffer formed by mixing K_2HPO_4 (monobasic potassium phosphate) and Na_2HPO_4 (bibasic sodium phosphate) (Freitas et al., 2018a). The microcosms were kept in a BOD incubator (ELETROLAB, 122FC) with environment adjusted for a 12-h photoperiod and temperature at 25 ± 2 °C.

The treatments contained the metals Cd^{2+} ($Cd(NO_3)_2{\cdot}4H_2O$) and Pb^{2+} ($Pb\ (NO_3)_2$) in concentrations of 1.0 mg L^{-1} and 7.0 mg L^{-1}, respectively. Control flasks were kept free from metal addition. These concentrations were stipulated in order to guarantee that the aqueous medium was considered contaminated according to the levels considered by Brazilian legislation, specifically, by Resolution N° 430 of the National Environmental Council which stipulates on conditions, parameters, and standards for management of effluent discharge into receiving water bodies (CONAMA, 2011).

The remediation capacity of the metals was determined in microcosm samples collected at intervals of 1, 2, 3, 6, 12, 24, 48, 72, and 168 h. The concentration of metals in the samples was evaluated by atomic absorption spectroscopy with flame atomization (Varian AA140). The calculation of the removal efficiency was performed according to Freitas et al. (2019).

Adsorption isotherms

The adsorption isotherm experiment was performed in microcosms under the same conditions described for the kinetic assay, except for initial metal concentrations and time. For characterization of adsorption curves, five points were used referring to initial concentrations of 0.5, 1.0, 3.0, 5.0, and 7.0 mg L^{-1} of each metal. Time was stipulated based on the kinetic test, being 72 h for both metals. The adsorption capacity (qe) and construction of the Langmuir and Freundlich isothermal models were carried out according to Nascimento et al. (2014). In both experiments, the analyses were performed in triplicate for each metal concentration and equilibrium time. The calculations and construction of models and figures were performed in Microsoft Excel software.

Toxicological evaluation of solutions after assisted bioremediation

The toxicity of the solutions collected from the microcosm was determined using *C. elegans* line N2. *Caenorhabditis elegans* is a free-living nematode that colonizes water sheets from soil particles by nourishing itself mainly with edaphic bacteria (Chen et al., 2006). It is an important model of environmental toxicity for aquatic environments and soil (Leung et al., 2008).

The assay was carried out on 96-well plates with synchronized worm population at stage L4 maintained in M9 medium (Porta-de-la-Riva et al., 2012). Each assay was conducted with approximately 20 L4 individuals and performed in triplicate. The plates were sealed and incubated for 72 h at 20 ºC. The survival percentages of the worms were submitted to homoscedasticity tests by Kolmogorov-Smirnov. The averages between treatments were compared by the unpaired *T* test ($p<0.05$). The analyses were performed in BioEstat 5.3 software (Ayres et al., 2007).

Table 1 Functional groups identified in *Salvinia biloba* biomass and respective absorption bands

Absorption bands (cm^{-1})	Functional groups
540	–NH (oscilação)
920	–CH
1030	–C–O
1375	$-CH_3$
1427	–CH
1630/1635	–OH
1726	–C=O
2850	–CH
2920	–CH
3420	Overlay –OH, –NH, $-NH_2$

Results

The characterization of *S. biloba* biomass by infrared spectroscopy indicates the presence of important functional groups for metal adsorption (Fig. 1; Table 1).

With regard to the kinetic test, it can be observed that in the first hour of the experiment, the removal efficiency of Cd^{2+} ions was 20%, with 50% of removal

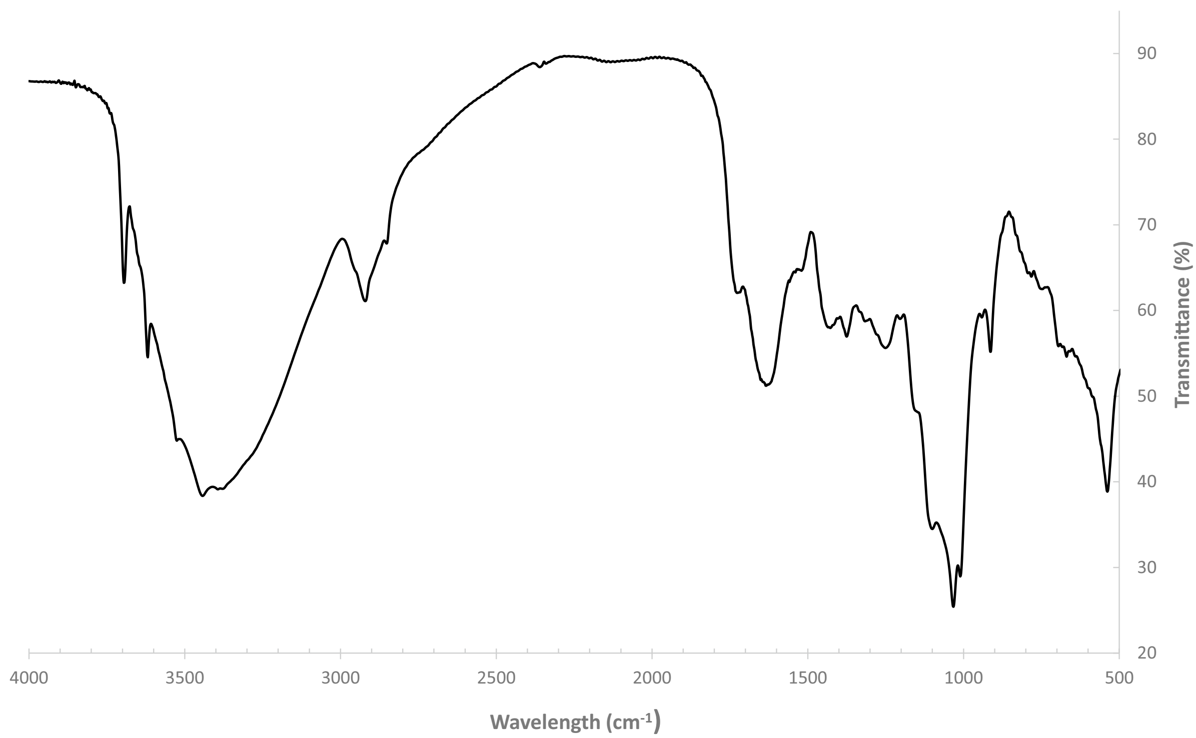

Fig. 1 Infrared spectra by Fourier transform of *Salvinia biloba* biomass

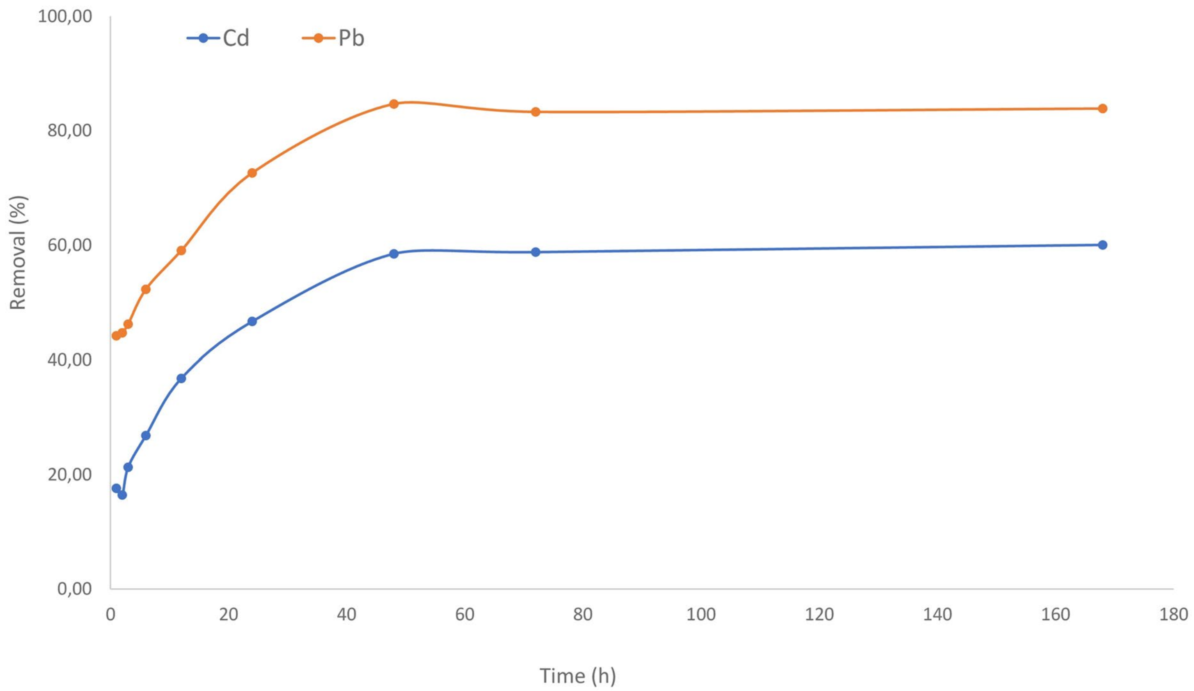

Fig. 2 Removal efficiency of *S. biloba* plants exposed to a culture solution at initial concentrations of 1 mg L^{-1} of Cd^{2+} and 7 mg L^{-1} of Pb^{2+}, at time intervals over 168 h (7 days)

achieved in 24 h, along with maximum efficiency of 60% obtained in 48 h (Fig. 2). For Pb^{2+} ions, a 45% removal efficiency was obtained in the first hour of the trial. As from 2 h onward, there was a reduction in the speed of the process, reaching 50% efficiency in up to 6 h, with maximum removal efficiency of 85%, obtained in 48 h. The equilibrium time was reached in 48 h for both metals (Fig. 2).

The experimental isotherms obtained with equilibrium data assumed a form characterized as unfavorable for the adsorption process (Fig. 3). These results demonstrated a better performance of the plant, under equilibrium conditions, in the removal of Cd^{2+} if compared to Pb^{2+}. This is evidenced by the higher amount of Cd^{2+} accumulated by biomass, for the same initial concentrations of metals. The isotherms adjusted to the Langmuir and Freundlich models are shown in Figs. 4 and 5, respectively, for Cd^{2+} and Pb^{2+}. As can be observed, the theoretical models evaluated were not satisfactory for the adjustment of the experimental isotherms.

The effect of plant exposure to metals was visually identified. The plants of the control environment (no metal presence) did not present morphological changes throughout the experiment (Fig. 6a, d). In plants submitted to Cd^{2+}, loss of pigmentation and foliar lesions was mainly observed, for both concentrations (Fig. 6b, c). Foliar necrosis was registered in plants submitted to 7 mg L^{-1} (Fig. 6c). In plants submitted to Pb^{2+}, no significant changes were observed for concentration of 1 mg L^{-1} (Fig. 6e). However, plants submitted to concentration of 7 mg L^{-1} showed loss of pigmentation in leaf edges (Fig. 6f).

Data indicate that *C. elegans* is more sensitive to the increase of Cd^{2+} concentration than to Pb^{2+} in evaluated concentrations, and there were no significant differences in percentage of mortality with time, as observed in Fig. 7. There was an increase in mortality rate when concentration of Cd^{2+} went from 1 to 7 mg L^{-1} (*T* test $p<0.05$). For Pb^{2+}, the mortality rate did not vary significantly from initial metal concentration or from phytoremediation time variable (Fig. 7).

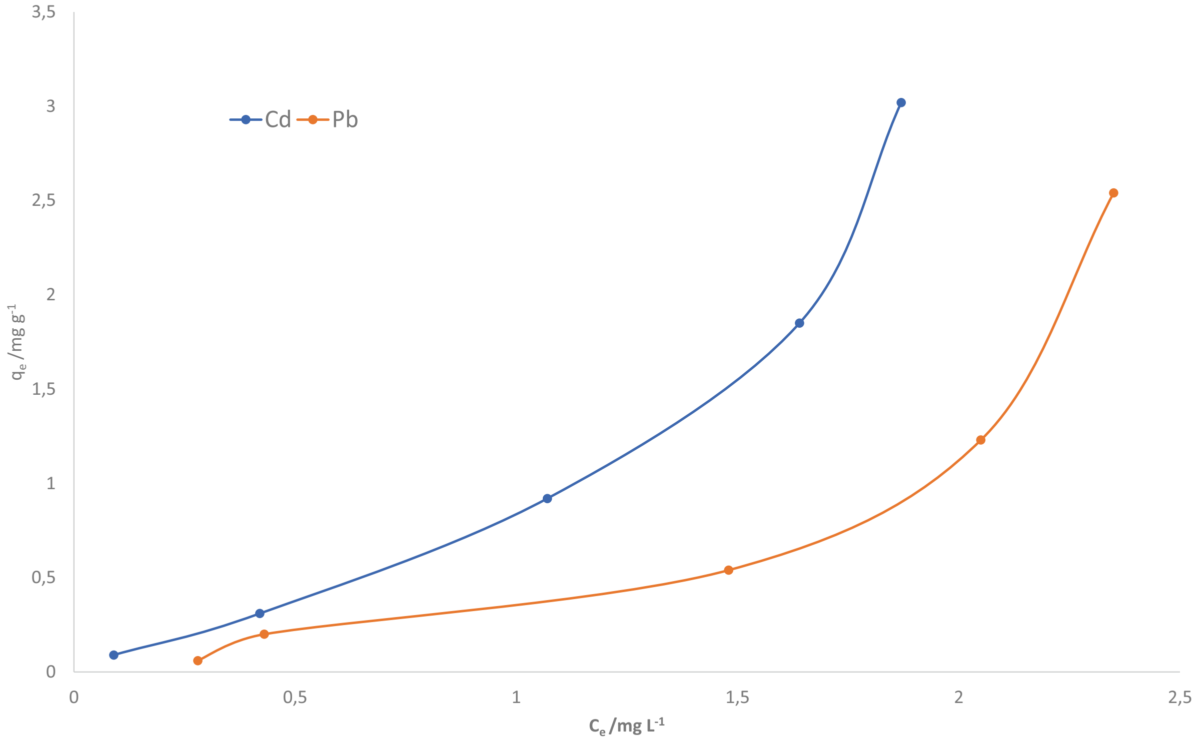

Fig. 3 Experimental isotherms of *S. biloba* in the adsorption of metals Cd^{2+} and Pb^{2+} at different concentrations and at an equilibrium time of 72 h (q_e adsorption capacity; C_e equilibrium concentration)

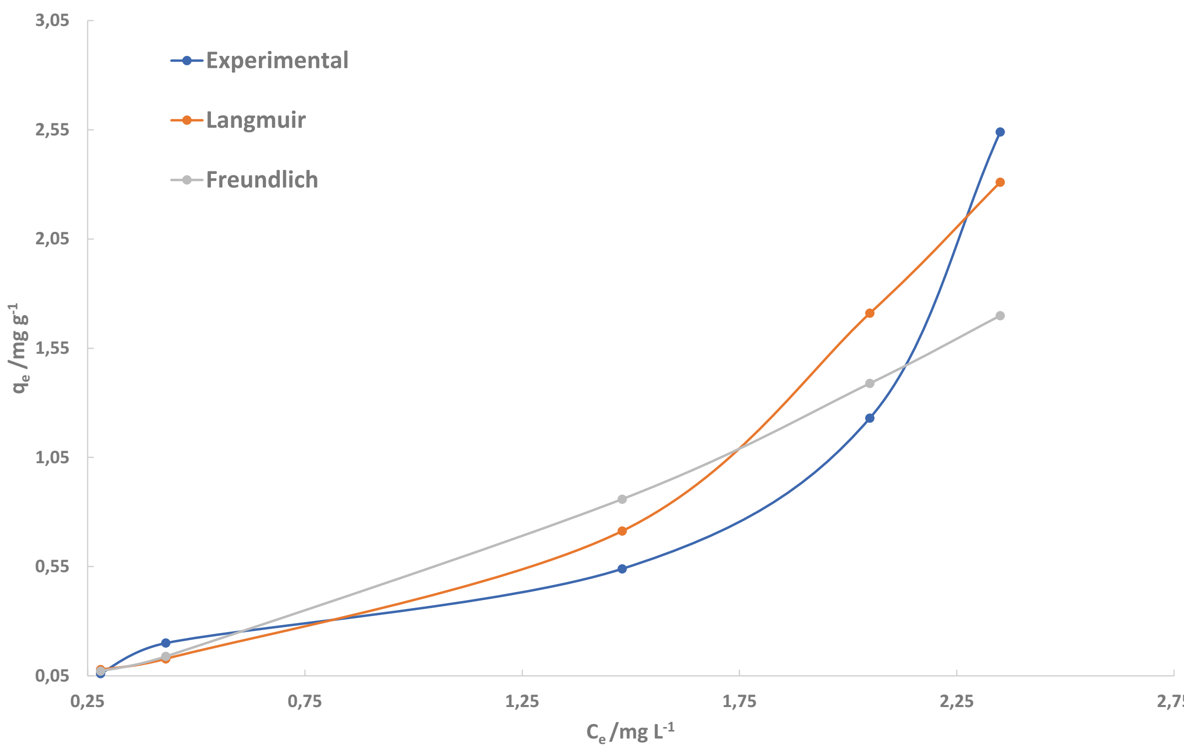

Fig. 4 Experimental isotherms of Cd^{2+} adsorption by *S. biloba*, compared with those adjusted to Freundlich and Langmuir models

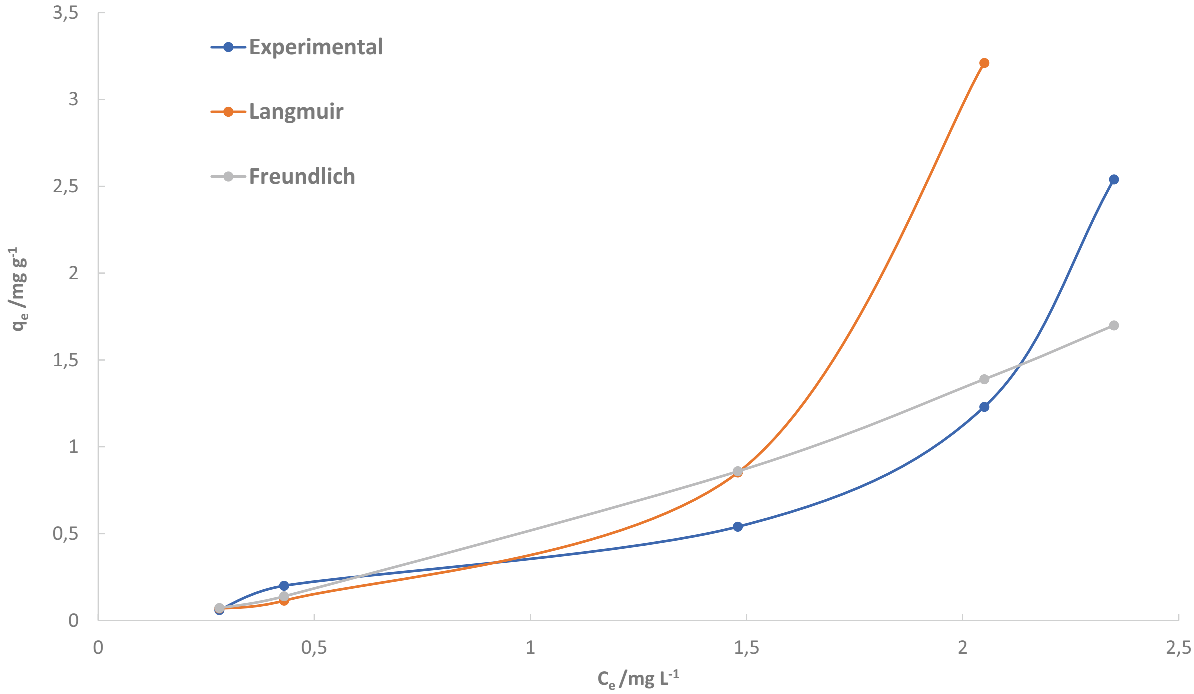

Fig. 5 Experimental isotherm of Pb^{2+} adsorption by *S. biloba*, compared to Freundlich and Langmuir models

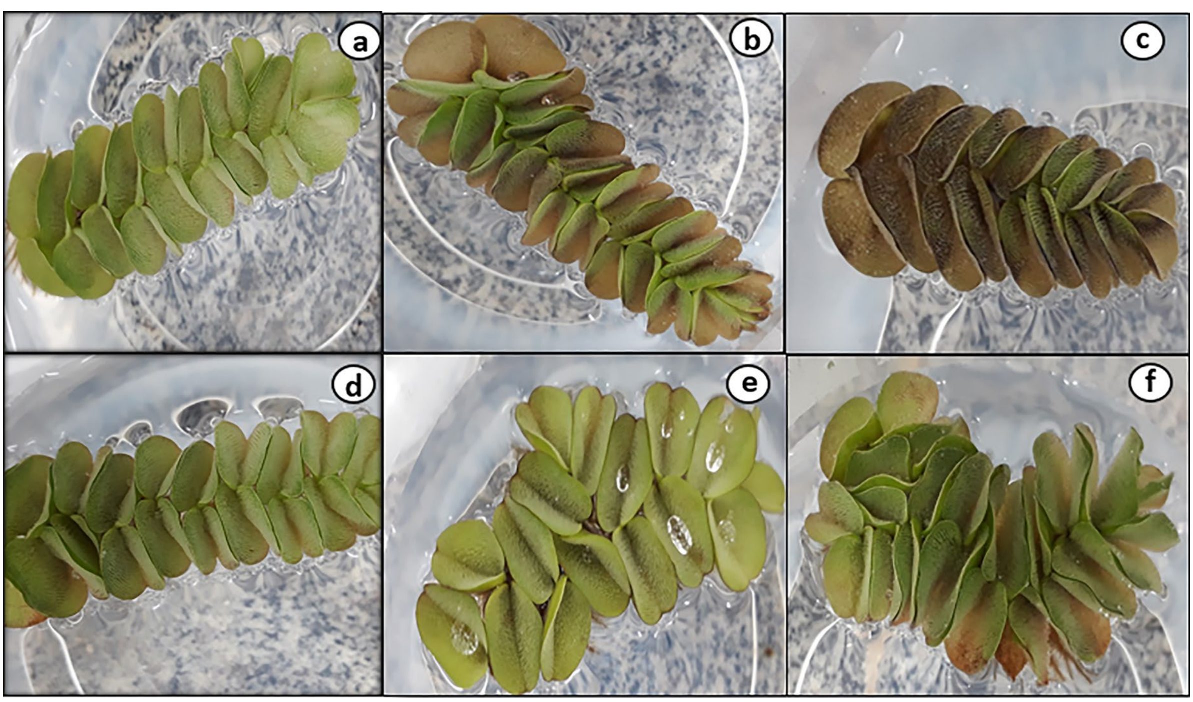

Fig. 6 Effect of *S. biloba* plant exposure, in microcosms simulating a metal-free aqueous environment (**a**, **d**) and in solution of Cd^{2+} 1 mg L^{-1} (**b**) and 7 mg L^{-1} (**c**) and of Pb^{2+} 1 mg L^{-1} (**e**), and 7 mg L^{-1} (**f**), in 168 h (7 days)

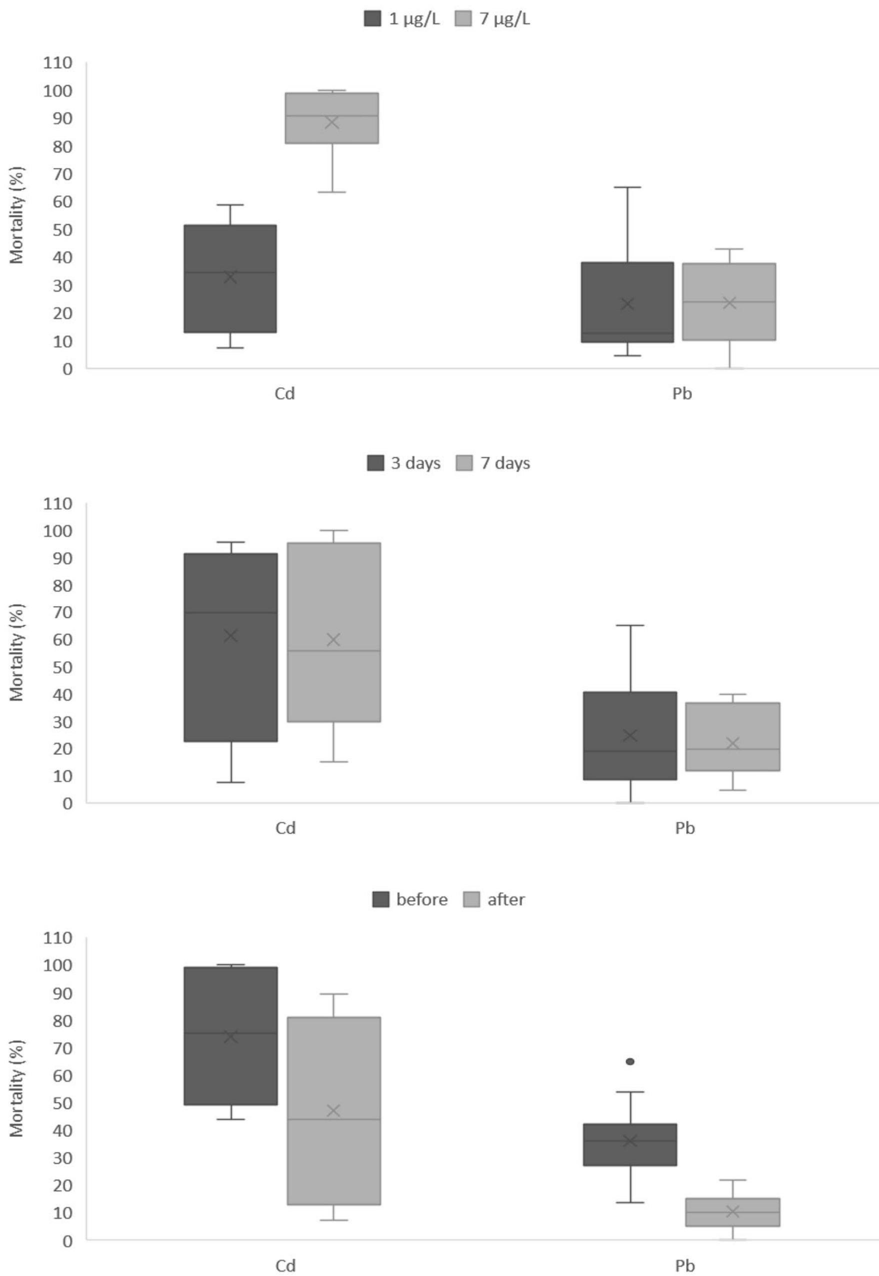

Fig. 7 Toxicological analysis of *S. biloba* to remedy Cd^{2+} and Pb^{2+} solutions at different concentrations (1 and 7 mg L^{-1}) and exposure days (3 and 7 days) evaluated without (before) and with (after) the presence of the plant

Discussion

The use of macrophytes in remediation processes is favored by biology and physiology, associated with a high productive potential of these plants (Rodrigues et al., 2016; Fia et al., 2017; Prabakaran et al., 2019). These characteristics are evident in species of the Salviniaceae family (Casagrande et al., 2018; Freitas et al., 2018a, b, 2019; Loría et al., 2019). Our data indicate that *S. biloba* is an aquatic plant which is capable of remediating contamination of Cd^{2+} and Pb^{2+}, with a consequent reduction in environmental toxicity caused by metals in *C. elegans*.

The characterization of *S. biloba* biomass revealed the presence of important functional groups for adsorption, as well as proving effective in reducing the toxicology of the solutions evaluated. The FTIR spectrum of *S. biloba* biomass presented a broad band in the region of 3.400 cm^{-1} indicating an overlap of such groups as hydroxyl (OH), primary amines ($R-NH_2$), and secondary amines (R_1R_2NH), characteristic of this region (Silverstein & Bassler, 1962). The presence of the carbonyl group (C=O) was also observed, with characteristic absorption around 1.800 cm^{-1} (Rodrigues et al., 2006). The absorption in the regions of 1.375 cm^{-1} and 1.427 cm^{-1} is typical of carboxyl groups (C–H and CH_3) as well as those observed in 2.850 and 2.920 cm^{-1} (Barros et al., 2006). Stretch-compatible vibrations (–C–O) with simple connections are assigned to the 1.030 cm^{-1} band (Zhao et al., 2019).

The absorption observed in 540 cm^{-1} was attributed to the –NH grouping (Silverstein & Bassler, 1962), while those observed below the 540 cm^{-1} region and above 3.600 cm^{-1} were considered as noises, not being possible to identify them according to the technique used. The groupings identified in the FTIR analysis, highlighting carboxylic, carbonyl, and hydroxyl, are included among the essential components for the interaction of the adsorbate with the biological material in the adsorption process (Honorato et al., 2015). Such functional groups can be deprotonated depending on the pH of the solution, thereby acquiring negative charge, becoming an active site for the adsorption of positively charged substances. Thus, the presence of these groups in *S. biloba* is an important indicator of its effectiveness for application in the adsorption of metals in the solution.

The results of the kinetic study evidenced a rapid removal of both metals in the first hours of the experiment, followed by a decrease in removal over time until the adsorption equilibrium was reached. The behavior described could be expected, and it is related to surface adsorption provided by physical-chemical forces between the adsorbate and the adsorbent material (Nascimento et al., 2014; Freitas et al., 2019). The removal efficiencies for Cd^{2+} and Pb^{2+} ions were not compared in this step, because the initial concentrations of the metals used were different, being 7.0 mg L^{-1} for Pb^{2+} and 1.0 mg L^{-1} for Cd^{2+}. In general, the relation of higher plant accumulation is recurrent with the higher concentration of the metal in the solution (Casagrande et al., 2018; Freitas et al., 2018a).

The kinetic equilibrium by *S. biloba* occurred in 48 h. After the plant reached this condition, the level of adsorption was reached, and an increase was no longer expected. The equilibrium time is an important parameter to be estimated, considering that the incorporation of the metal by the plant does not guarantee its definitive elimination, since the decomposition of biomass can promote its feedback to the environment (Barros & Henares, 2015; Jesus et al., 2015; Freitas et al., 2018a). Thus, according to the kinetic criterion, the useful contact time of the plants with the solution to be treated was determined, indicating that the use of *S. biloba* for remediation purposes requires a biomass harvesting system, considering that from the time of equilibrium, the plant is no longer efficient for the removal and, even more, will be under toxic effects of the metal which may accelerate its decomposition.

The isotherms evidenced the highest adsorption capacity (experimental isotherm) for Cd^{2+}, which may be related to the highest ionic radius of Pb^{2+} (Lide, 2005). The larger the ionic radius, the more difficult it is for an ion to access the pores of an adsorber, even if the ion in question has a high affinity with the surface, since it is a physical impediment (Liu et al., 2009). The study by Rodrigues et al. (2006) also discussed the possibility of loading a certain metal in the adsorbent as being better, the smaller the ionic radius was, which according to the authors would justify the better intra-particle absorption. However, the consideration of the effect of the ionic radius of the metal crystal on adsorption should be made with caution, considering that other factors may interfere with this evaluation (Aguiar et al., 2002).

Experimental isotherms have shown an increase in adsorption capacity with an increase in equilibrium concentration. The occurrence of this pattern in the relationship between time and concentration is commonly attributed to species with bioaccumulative potential (Giri & Patel, 2011; Oliveira et al., 2001; Guimaraes et al., 2012; Casagrande et al., 2018). As regards adsorption description, equilibrium data were not well represented by both theoretical models. Considering that Langmuir and Freundlich are characteristic models of surface adsorption (Nascimento et al., 2014), it can be assumed that they were not adequate for the equilibrium data due to the occurrence of bioaccumulation phenomenon, in other words, the participation of the plant metabolism stage for the removal of Pb^{2+} and Cd^{2+} metals from the solution. Bioaccumulation involves cellular energy expenditure due to internal transport of metal in membranes (Vullo, 2003) and differs from adsorption at interfaces that occur predominantly at surface sites of biosorbent material (Chojnacka, 2010; Sood et al., 2011).

Cadmium and lead are elements known to cause physiological stress in plants. Cadmium is considered an extremely toxic element for plants even in concentrations considered to be low (Oliveira et al., 2001). Its toxicity is associated with several effects in plants, such as chlorosis, alteration in pigmentation, deformities, senescence, and leaf necrosis (Souza et al., 2009; Wolff et al., 2012). Other effects include changes in reproductive structures, epidermis and endodermis, root system, and aerenchyma (Silva et al., 2013). Some of the symptoms mentioned were visible in *S. biloba* plants exposed to Cd, mainly for the concentration of 7 mg L^{-1}.

For Pb, the morphological symptoms were not expressive in *S. biloba*, even for the highest concentration evaluated. Lead is commonly reported as a low-toxicity metal, depending on its concentration (Alves et al., 2008; Ribeiro et al., 2015). In some cases, the absence of effects or low toxicity of Pb can be attributed to certain mechanisms of tolerance that some plant species demonstrate in the presence of this metal (Hu et al., 2010; Souza et al., 2011; Chen et al., 2015; Loría et al., 2019). Therefore, the results obtained may indicate a greater tolerance of the plant in relation to this metal, when compared to Cd.

The morphological effects of higher toxicity of Cd^{2+} ions, visually identified in plants of *S. biloba*, were also observed in toxicological analysis evaluating the mortality rate of nematodes *C. elegans*_N2 submitted to solutions contaminated with the metals, before and after the application of *S. biloba* in the phytoremediation process. For Cd^{2+}, the significant increase of mortality of animals with increase of metal concentration from 33 to 88% was evidenced. This result implies that, despite the toxicity and consequent effects of Cd on plants, *S. biloba* was efficient for the remediation of the aqueous environment, which was corroborated by the lower mortality rate of nematodes submitted to phytoremediation solutions by macrophytes.

For Pb, both the morphological effects observed in plants and mortality rates of nematodes were low for the concentrations studied, with no variation in mortality rate of animals. This result may be associated with a good capacity of *S. biloba* to support Pb, possibly associated with mechanisms of tolerance of the plant to this metal (e.g., Loría et al., 2019). Another possibility is that this result is due to the low concentrations evaluated, i.e., it is possible that the effects of Pb, both for the plant and nematodes, can be better observed for higher concentrations than those used in this study.

Caenorhabditis elegans is sensitive to metals Cd and Pb resulting from alterations in the differential expression of genes as well as in mortality of nematodes (Cui et al., 2007; Wang & Yang, 2007; Sudama et al., 2012; Jiang et al., 2016). The phytoremediation of Cd by *S. biloba* reduced the mortality of *C. elegans*, indicating a reduction in solution toxicity, as seen in mercury phytoremediation assisted by endophytic bacteria (Mello et al., 2019).

For both metals, the phytoremediation time variable did not significantly affect the mortality rate of nematodes, corroborating with the equilibrium time obtained in the kinetic trial. In other words, after the equilibrium time of 2 days, there is no more variation in the rate of metal removal by the plant and, consequently, no differences in mortality rates of the nematode are observed. In this sense, the toxicological evaluation reinforces the relevance of harvesting the *S. biloba* biomass after reaching the equilibrium condition, considering that, after this time, the removal of the metal ceases to take place and, from then on, the possibility of the solution being fed back by the metal may occur, due to plant decomposition, especially for Cd, whose harmful effects were more expressive.

The results obtained show that *S. biloba* are promising for the remediation of Cd^{2+} and Pb^{2+} aqueous solutions and can contribute to the reduction of the toxicity of these aquatic environment metals. The analysis of the plant biomass, as well as the metal removal behavior, expressed in the kinetics and adsorption capacity, demonstrated important macrophyte characteristics for phytoremediation including different processes of plant interaction with the aqueous environment. The remedial effect of the plant was confirmed by the decrease in mortality rate of nematodes submitted to phytoremediation solutions by *S. biloba*, showing the most critical toxicity of the Cd according to the increase in initial metal concentration. From an application perspective, *S. biloba* can be indicated as a biosorbent of Cd^{2+} and Pb^{2+} ions, with the advantages of low cost of application, rusticity in its management, wide geographical occurrence, and high productivity of the species and its biological properties pertinent to the removal of metal ions from the aqueous environment. Its use is recommended in controlled remediation systems, where it is possible to properly manage the plant.

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Data availability All data generated or analyzed during this study are included in this published article. Moreover, the raw datasets generated during and/or analyzed during the current study are available from the authors on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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