Evaluation of the autochthonous free-floating macrophyte *Salvinia biloba* Raddi for use in the phytoremediation of water contaminated with lead

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ABSTRACT

Plant-based systems for the treatment of contaminated environments (phytoremediation) have been proved to be highly efficient in removing pollutants, especially heavy metals. However, in strictly aquatic and high-flow treatment systems, the use of free floating plants could be more adequate. For a treatment system based on phytoremediation strategies to be feasible and sustainable, it is essential that the plants used are not only efficient in pollutants removal, but also abundant in the region, easily accessible, and do not require special culture conditions. In this work, we evaluate the capacity of four different autochthonous macrophytes obtained from the Paraná river (Argentina) to adapt and reproduce without any additional nutrient supply or temperature special conditions (laboratory «indoor» environment). Only those specimens that doubled their biomass in a two-week period without any visual signs of deterioration (loss of turgor, chlorosis and/or necrosis of leaves) were considered for further analysis. From different pre-selected species, only *Salvinia biloba* Raddi showed a wide capacity to adapt and reproduce under these conditions. Moreover, the ability to remove lead (Pb^{2+}) by *S. biloba* was evaluated in water samples contaminated with three metal concentrations (4.8 ± 0.3, 9.1 ± 0.4 and 19.6 ± 0.5 mg/L) at different exposure times (0–24 h), showing a high efficiency in the pollutant elimination. A compartmentalization analysis indicates that surface adsorption was the predominant mechanism for Pb^{2+} removal at the first 24 h. Finally, the bioconcentration factor (BCF) was calculated at the end of the exposure time, reflecting both hyperaccumulation capacity and high metal tolerance by this plant. Our results suggest that incorporation of *S. biloba* in wastewater treatment systems could be a successful strategy to efficiently remove heavy metals by bioremediation processes.

Keywords: Heavy metals; Industrial wastewater; Lead; Phytoremediation; *Salvinia biloba* Raddi

1. Introduction

In the last decades, contamination of natural waters by the release of large volumes of aqueous effluents containing heavy metals has been increased related to anthropogenic activity and industrialization. Heavy metals are a serious threat to the environment and to human health since these ions are inert to chemical or biological degradation, highly toxic at very low concentrations, and can be transferred

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through the trophic chain posing a serious risk to living beings [1].

Lead (Pb) is omnipresent and one of the earliest metals discovered by the human race. Its unique properties (high malleability, low melting point, resistance to corrosion, among others) have led to its widespread use for different industrial applications, triggering the unfortunate consequence of free lead occurrence in the environment [2]. Lead is present in the wastewaters of petrochemical, painting and coating, founding, metal electroplating, mining, plumbing and battery industries. In most cases, lead is discharged into the environment without any adequate treatment [3]. Lead poisoning has been recognized as a major public health risk, particularly in developing countries. Exposure to lead produces several deleterious effects on the hematopoietic, renal, reproductive, and central nervous system, inducing neurotoxic effects at blood concentrations as low as 20 µg/dL [4]. Although lead toxicity is a highly-explored topic, complete control and prevention over lead exposure is still far from being achieved. While various occupational and public health measures have been undertaken in order to control lead exposure, cases of lead poisoning are still reported [2].

Conventional technologies for the treatment of industrial residual waters containing heavy metals are usually expensive and inefficient because current legislation demands very low metal concentrations as maximum permissible levels [5]. The use of aquatic phytoremediation for the removal of pollutants from wastewater and industrial effluents is a sustainable and promising alternative rather than classical physicochemical procedures [6]. This eco-friendly technology is based on the plant ability to adsorb, accumulate, and tolerate high concentrations of pollutants, including heavy metals [5,7]. Phytoremediation-based technologies have been shown to be competitive compared to more conventional methods, especially for the removal of low metal concentrations wherein toxic effects to plants are minimal or absent. One of its major advantages is that plants can be used as low-cost extract pumps to purify contaminated water, thus being an appropriate solution to remediate large areas or to complete decontamination in longer periods of time [8]. Its success depends on the physicochemical properties of the surface of the plants utilized, and some other biological features such as productivity, geographical distribution and tolerance to toxic compounds [9].

Aquatic phytoremediation has been extensively implemented using helophytes (*i.e.*, emergent plants) in constructed wetlands [10]. However, a second approach may utilize aquatic free-floating plants in lagoons. In such sense, several macrophytes have been used for heavy metal remediation from water samples with the additional advantage that plant biomass can be easily harvested and treated for metal recovery before its final disposal [11]. Vascular floating plants are widely distributed in tropical and subtropical regions, thus, the implementation and management of lagoons for treating metal-containing effluent at these regions appears to be a viable solution since environmental conditions favor high plant productivity [12].

In Argentina, the ecosystem surrounding the Paraná river is characterized by an extensive wetland area with abundant and diverse aquatic vegetation. These regional macrophyte species, which have high efficiency in heavy metal uptake, could be used for the removal of ions in artificial systems or as bioindicators. Therefore, the aim of the present work was to assess the potential of several autochthonous free-floating macrophytes obtained from the Paraná river to be used in the phytoremediation of industrial wastewater contaminated with lead.

2. Materials and methods

2.1. Collection and characterization of macrophytes

Specimens of *Hydrocotyle ranunculoides*, *Eichhornia crassipes*, *Lemma minor*, and *Salvinia* sp. were collected from a shallow lake at the middle Paraná river (32°52'37" S – 60°40'36" O), with no known pollution load. The collected plants were transported to the laboratory at ambient temperature in plastic recipients containing river water. Only mature plants with uniform size were selected for experimental purposes. Taxonomic classification was carried out by the *Instituto de Botánica del Nordeste* (IBONE-CONICET, Universidad Nacional del Nordeste, Corrientes, Argentina) based on morphological characteristics. *Salvinia* sp. specimens were classified as *Salvinia biloba* Raddi (synonym name *Salvinia herzogii* de la Sota) according to the criteria established by [13].

2.2. Plant material cultivation and selection

All plants collected were cultivated and maintained in greenhouse conditions under natural light to allow acclimation. Temperatures ranged from 24 to 28°C. Glass aquaria of 10 L capacity were used. Fresh plants and water from the sampled natural environment were placed in every aquarium. No further nutrients or special requirements were added to the medium. Only those specimens that doubled their biomass in a two-week period without any visual signs of deterioration (loss of turgor, chlorosis and/or necrosis of leaves) were considered for further analysis.

2.3. Stock solution of Pb(II)

The stock solution of Pb^{2+}(1000 mg/L) was prepared by dissolving analytical grade $Pb(NO_3)_2$ (Cicarelli, Santa Fe, Argentina) in deionized water and stored at 4°C. Standard solutions of Pb^{2+} for calibration purposes (1.0, 5.0, 10, 15, 20, 25, 30 mg/L) were prepared as recommended for Varian AA240FS operational manual, by diluting the stock solution with the necessary volume of deionized water in glass calibrated containers. All calibration procedures showed reproducible linear relationships ($R^2 = 0.995$).

2.4. Batch kinetic studies

The plants selected as the best adapted to growth in un-supplemented conditions were rinsed gently with deionized water and placed in rectangular glass containers (20 cm × 35 cm) with different initial concentrations of Pb^{2+} (4.8 ± 0.3, 9.1 ± 0.4 and 19.6 ± 0.5 mg/L) in 1.5 L final volume. The metal concentrations selected were

in accordance with previously reported data [3,14,20]. The experiments were accomplished at an average room temperature of 23 ± 2°C in the presence of fluorescent tubes (OSRAM DULUX L HE, Germany) light intensity (100 l m/W) for a photo period of 12 h. The batch kinetic study was conducted with 20.0 g of fresh plant in each recipient. The fresh weight of plant biomass was determined after removing the excess of liquid over a filter paper. Eight experimental units were used as replicates for each exposure time (0.5, 2, 4, 6, 12 and 24 h) and the data were reported as mean value ± standard deviation (S.D.). Additionally, two controls were carried out. One, containing Pb^{2+} at the same initial concentration of the treatment without biomass, was used to determine the possible adsorption of the metal onto the surface of the containers. The content of lead in the water column did not change along the experimental time, indicating that no lead adsorption occurred in the system. Another control, containing biomass in water without Pb^{2+}, was used as blank of the experiment in which no lead was quantified either in plant or medium.

In all cases, pH was adjusted and maintained at 6.0 ± 0.5 with 1 N HNO$_3$ (Cicarelli, Santa Fe, Argentina) in order to avoid Pb(OH)$_2$ precipitation. Water samples were withdrawn after the consecutive hours of contact period and residual concentration of Pb^{2+} was measured. Total biomass was removed and treated for compartmentalization analysis after each exposure time.

2.5. Lead adsorption kinetics and lead adsorption equilibrium

First and second order models were applied to evaluate the kinetics of Pb^{2+} elimination from water samples. The linear form of the first (1) and second (2) order rate equations are expressed as follows:

$$\ln C_t = \ln C_0 - K_1 C_t \tag{1}$$

$$\frac{1}{C_t} - \frac{1}{C_0} = k_2 t \tag{2}$$

where C_t = residual concentration of Pb^{2+} (mg/L) at time t, C_0 = initial Pb^{2+} concentration (mg/L), K_1 = first order reaction rate constant, K_2 = second order reaction rate constant.

Pseudo-first order or Lagergren model [15] and pseudo-second order [16] rate equations were used for evaluating the kinetics of Pb^{2+} adsorption to *S. biloba* biomass. The linearized form of the pseudo-first order kinetic model proposed by Lagergren [15] is:

$$\ln (q_e - q_t) = \ln q_e - k_1 t \tag{3}$$

where q_e = amount of solute adsorbed per unit weight of adsorbent at equilibrium, q_t = amount of solute adsorbed per unit weight of adsorbent at time t, k_1 = pseudo-first order reaction rate constant.

The linear form of the pseudo-second order equation proposed by Ho and Mckay [16] is:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{4}$$

The above equation is the integrated rate law for a pseudo-second order reaction, which after rearrangement is expressed in the following form:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \tag{5}$$

where $h = k_2 q_e^2$ (k_2 = pseudo-second order reaction rate constant), q_e = amount of solute adsorbed per unit weight of adsorbent at equilibrium, and q_t = amount of solute adsorbed per unit weight of adsorbent at time t.

The Langmuir and the Freundlich isotherm models were chosen to describe the adsorption equilibrium of Pb^{2+} [3]. The Langmuir adsorption model can be derived from Eq. (6):

$$q = \frac{q_{max} b C_e}{1 + b C_e} \tag{6}$$

where q is the mass of sorbate adsorbed per unit mass of sorbent (mg/g), b is the Langmuir constant, C_e is the equilibrium concentration (mg/L), and q_{max} is the adsorption capacity (mg/g).

The Freundlich adsorption isotherm is mathematically expressed as Eq. (7):

$$q = K_F C_e^{1/n} \tag{7}$$

Once again, q is the adsorption capacity (mg/g), C_e is the equilibrium concentration (mg/L), $1/n$ is the sorption intensity, and K_F is the measure of sorption capacity.

2.6. FTIR-ATR analysis

The spectra of dry *S. biloba* biomass were obtained using a Fourier Transform Infrared (FTIR) spectrophotometer Shimadzu IR Prestige-21 (Tokyo, Japan) with Attenuated Total Reflectance (ATR) attachment. Measurements were recorded in the scanning range of 500–4000 cm^{-1}. A total of 20 scans were performed at 2 cm^{-1} resolution.

2.7. Compartmentalization analysis

To assess the distribution of Pb^{2+} among compartments (remainder in the water column, adsorbed on the plant surface and accumulated in the plant tissue), an analysis of compartmentalization was carried out as described by [14]. Samples from the water column were collected at the end of each exposure time in the batch kinetic experiments and filtered using Whatman paper (pore size = 11 μm). Biomass was collected and washed with deionized water to remove residues from the medium. After removing the excess of liquid over a filter paper, plant biomass was washed with 400 mL of 1.7 mM EDTA solution (concentration equivalent to a molar ratio EDTA/Pb^{2+} ≥ 15) for 60 min in an orbital shaker at 180 rpm. Samples from this washing were taken to quantify Pb^{2+} adsorbed on the plant surface. The EDTA-washed biomass was then rinsed with deionized water and dried at 90°C to constant weight. Samples of 0.05 g of this dried-biomass were treated overnight with 10 mL 65% HNO$_3$ analytical grade (Cicarelli, Santa Fe, Argentina). Subsequently, samples were heated at 120°C in a digestion system for 2 h.

Aliquots of those samples were diluted with deionized water to quantify Pb^{2+} accumulated into the plant tissue. All the samples were analyzed in an atomic absorption spectrophotometer Varian AA240FS (Varian Inc., Palo Alto, CA, EEUU) at 207 nm using an air-acetylene flame [17].

2.8. Calculation of the bioconcentration factor

The bioconcentration factor (BCF) was calculated as an indicator of plant lead removal efficiency according to [18] as follows:

$$BCF = \frac{Pb^{2+} \text{ in total biomass } \left(mg/Kg \text{ dry biomass}\right)}{Pb^{2+} \text{ initial concentration in solution } \left(mg/L\right)}$$

2.9. Statistical analysis

Linear regression, non-linear regression and variance analyses (ANOVA) were carried out using the SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA) at $p = 0.05$ level.

3. Results and discussion

3.1. Plants selection for metal remediation studies

In order for an aquatic system based on phytoremediation strategies to be feasible for contaminants elimination, it is essential that the macrophytes used in the system design do not require special culture conditions. Properties such as high biomass productivity and high sorption capacity are essential features when applying these technologies. In this study, from the different autochthonous aquatic macrophytes collected from Paraná river that were analyzed, only *S. biloba* showed a remarkable capacity to grow and reproduce under greenhouse conditions. Among various species, *Salvinia* holds a distinctive position due to several advantages including high yield and tolerance to a wide range of temperatures. It is widely spread in tropical zones but it is also found in subtropical and temperate zones. In Argentina, it grows abundantly in wetlands. Specimens from this genus can be easily and inexpensively handled. The capability of *Salvinia* for mass-scale use in phytoremediation must be highlighted since different species, including *S. minima*, *S. auriculata*, *S. natans*, and *S. rotundifolia*, have demonstrated high potential to remove heavy metals from wastewaters [19]. Based on this background and our observations, the free-floating aquatic plant *S. biloba* was selected for further experiences.

3.2. Batch sorption kinetics

In order to establish the capacity of *S. biloba* to remove Pb^{2+}, a series of batch experiments were carried out using deionized water as the experimental medium and three different metal concentrations (4.8 ± 0.3, 9.1 ± 0.4 and 19.6 ± 0.5 mg/L). In all cases, Pb^{2+} in water decreased over time and most of this reduction took place during the first 24 h (Fig. 1). As can be seen in Table 1, metal elimination by *S. biloba* reached more than 95% removal at 24 h for 4.8 ± 0.3
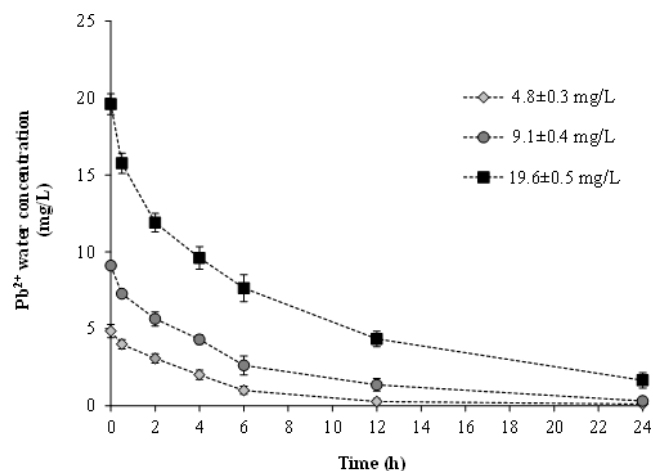

Fig. 1. Lead (Pb^{2+}) removal kinetics from water column by *S. biloba*.

Table 1
Percentage of removed Pb^{2+} at different exposure times for various metal initial concentrations

Time (h)	Pb^{2+} removal (%)		
	4.8 ± 0.3 mg/L	9.1 ± 0.4 mg/L	19.6 ± 0.5 mg/L
0	0	0	0
0.5	17.7 ± 0.3[a]	20.1 ± 1.4[a]	19.7 ± 4.0[a]
2	36.8 ± 1.4[b]	38.3 ± 6.1[b]	39.4 ± 4.0[b]
4	58.8 ± 3.7[c]	52.7 ± 0.3[b]	51.1 ± 5.5[bc]
6	80.0 ± 10.5[d]	71.7 ± 10.7[c]	61.1 ± 7.0[cd]
12	94.5 ± 3.3[de]	84.9 ± 4.6[cd]	77.9 ± 3.4[d]
24	97.7 ± 0.1[e]	96.6 ± 0.4[d]	91.6 ± 2.1[e]

Results are presented as mean ± S.D. Different letters in the same column represent significant statistical difference ($p < 0.05$); *e.g.*, "a" and "b" are statistically different from each other but not from "ab".

and 9.1 ± 0.4 mg/L of initial Pb^{2+} concentration, but this percentage slightly declined at 19.6 ± 0.5 mg/L. After a contact period of 7 days with the highest initial Pb^{2+} concentration, approximately 98.7% removal was observed [20]. It is interesting to note that, in all cases, remnant Pb^{2+} in water samples were below the limit of admissible lead discharge in industrial waste liquids (<0.50 ppm), established by resolution No. 1089/82 (Santa Fe, Argentina). Throughout all the bioassays, plants general appearance was good and no growth differences or evidence of necrosis was observed.

The elimination of Pb^{2+} from the water column was around 50% after 4 h for each initial metal concentration assayed (Table 1). During the following 8 h, at the two lowest Pb^{2+} concentrations, the metal was almost fully eliminated from the water column (~85–95% of the metal), whereas at the highest Pb^{2+} concentration, the removal continued until 24 h, when about 90% metal elimination was reached. In addition, Pb^{2+} elimination from the water column fits a first order reaction model, indicating that the rate of this process depends of the initial concentration of the metal. That is,

Table 2
Rate constants of first (K_1) and second (K_2) order kinetic models obtained for Pb^{2+} elimination from the water column by *S. biloba* biomass at different metal concentrations

Pb^{2+} initial concentration (mg/L)	First order kinetics		Second order kinetics	
	K_1 (1/h)	R^2	K_2 (L/mg·h)	R^2
4.8 ± 0.3	0.163	0.9930	0.379	0.8900
9.1 ± 0.4	0.137	0.9873	0.126	0.9077
19.6 ± 0.5	0.098	0.9751	0.022	0.9638

Fig. 2. Lead (Pb^{2+}) adsorption kinetics of *S. biloba* at different initial concentrations.

as Pb^{2+} concentration increases, its removal rate (K value) decreases (Table 2).

The data depicted in Fig. 2 reveal a biphasic nature of Pb^{2+} uptake with a first rapid sorption process up to a period of 6 h, reaching almost equilibrium after 12 h. These observations suggest that the rate of the sorption process is diffusion controlled by metal concentration differences between the bulk solution and the roots microcosm.

The rapid rate of removal at the earlier period may be explained due to the high availability of active sites at the plant root membranes but, as time increases, removal rate decreases due to the saturation of these sites. Similar patterns have been described by other authors for the removal of numerous metal ions using a wide variety of aquatic macrophytes, including *Salvinia* species [3,14,19,21–23]. Plant cells have an abundance of negatively charged sites on their walls, and hence several interactions between metals and carboxyl, sulphate, amino and other groups are likely to occur [24]. Surface sorption is a combination of physical and chemical processes such as chelation, ion exchange and chemical precipitation of metal ions [25]. Biological processes (intracellular uptake and traslocation to the aerial parts) and root-mediated precipitation of metal (particularly pronounced at high metal concentrations) are probably responsible for the slower components of *S. biloba* lead removal mechanism [21].

Our results demonstrate that metal concentration and exposure time directly affect pollutant removal efficiency. Removal of Pb^{2+} using *S. biloba* proved to be fast enough to reach a state of equilibrium between the first 12–24 h of exposure at all metal concentrations evaluated. When the aim is to control the operation cycles of an effluent treatment system that utilizes aquatic plants as part of the bioremediation mechanism, it is important to know the time required to reach certain degree of contaminant removal. In this way, it is possible to adapt the periodicity of the discharges that impact the system, establishing a minimum of resting time that allows the system to recover part of its phytoremediation capacity.

The batch kinetic results were also tested in two models in order to evaluate the best fit sorption reaction mechanism: pseudo-first order equation on the basis of adsorbent uptake capacity [15] and pseudo-second order reaction model based on the solid-phase sorption [16]. Kinetic parameters obtained from both proposed models at different metal initial concentrations are presented in Table 3. Metal adsorption by *S. biloba* biomass was satisfactorily adjus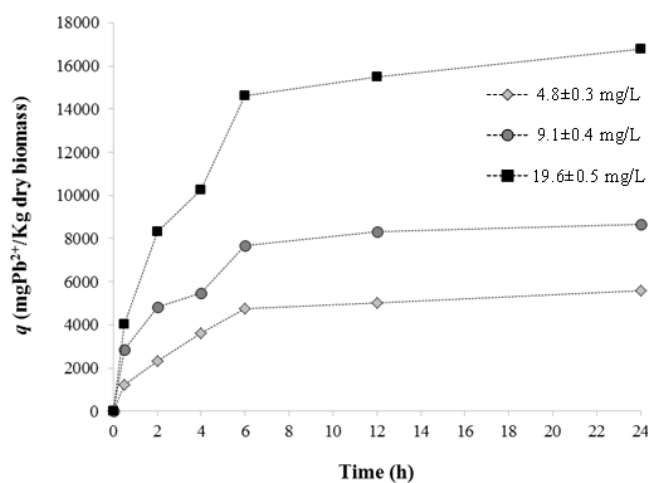ted to a pseudo-second order kinetic model for all Pb^{2+} concentrations tested, with high coefficients of determination (Table 3). The adsorption capacity at equilibrium (q_e) and the initial adsorption rate (h) significantly increased as the metal content in the solution increased. These results indicate that increasing metal concentrations in water samples cause more interactions between the metal and the biomass to occur. In contrast, the pseudo-second order rate constant (k_2) decreased as Pb^{2+} concentration increased. In this case, when the same amount of bio-adsorbent is used (20.0 g at this work), active adsorption sites may be more rapidly saturated with increasing pollutant concentration, reducing the speed of the adsorption process. Similar values to those described here for q_e, h and k_2 have been previously described by [3] for an analogous batch system operating with *S. minima*.

Pseudo-second order kinetics model has been extensively used to describe the sorption of divalent metal ions onto highly heterogeneous surfaces [26]. Most adsorption systems follow this model in which chemisorption took place during the adsorption process [27]. Therefore, it is possible to assume that the mechanism of adsorption of Pb^{2+} by *S. biloba* involves the formation of strong chemical bonds, mainly by ionic exchange. In fact, several authors have reported that other ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+} and Mn^{2+} are liberated into the solution during the adsorption of Pb^{2+}, Cd^{2+}, Zn^{2+}, or Ni^{2+} by different aquatic macrophytes [28–30].

3.3. Adsorption isotherms

There are several isotherm equations available for analyzing the behavior of adsorbent materials during the removal of contaminants in wastewater, the Langmuir and Freundlich models being the most common. The Langmuir isotherm model assumes that a finite number of active sites are uniformly distributed over the adsorbent surface. These active sites have the same affinity for adsorption of a mono molecular layer and there is no interaction between adsorbed molecules. In contrast, the Freundlich isotherm model applies to adsorption on heterogeneous surfaces

Table 3
Parameters of pseudo-first order and pseudo-second order kinetic models obtained for Pb^{2+} adsorption by *S. biloba* biomass at different metal concentrations

Pb^{2+} initial concentration (mg/L)	Pseudo-first order kinetics			Pseudo-second order kinetics			
	q_e (mg/g)	k_1 (1/h)	R^2	q_e (mg/g)	k_2 (g/mg·h)	h (mg/g·h)	R^2
4.8±0.3	4.162	0.187	0.8968	6.169	0.064	2.418	0.9961
9.1±0.4	6.538	0.252	0.9559	9.328	0.062	5.435	0.9953
19.6±0.5	12.473	0.205	0.9071	18.349	0.025	8.264	0.9959

with interaction between the adsorbed molecules, and is not restricted to the formation of a monolayer. This model assumes that as the adsorbate concentration increases, the concentration of adsorbate on the adsorbent surface also increases [31]. The parameters and correlation coefficients obtained for the Langmuir and Freundlich isotherms were calculated for Pb^{2+} adsorption by *S. biloba* and are presented in Table 4. Our results show that lead adsorption fits well with the Freundlich model, suggesting a heterogeneous distribution of active sites on the plant surface. These results are compatible with the presence of different functional groups responsible for metal adsorption in *Salvinia* [3,14,19, 28].

The Freundlich (K_F) and heterogeneity (n) constants are two parameters associated with the adsorption capacity and the intensity, respectively. If n value is less than one, the adsorption process is unfavourable, and if it is above unity, adsorption is favourable [32,33]. In the present study, the value of n at equilibrium was 2.256 suggesting favourable adsorption (Table 4). This value also suggests the establishment of strong interactions between the metal and the biomass. Using similar experimental set-ups, [3] reported lower K_F (8.31) and n (1.57) values for Pb^{2+} adsorption by *S. minima* from water samples than those obtained in the present work. Hence, our results suggest that *S. biloba* may be a better choice than *S. minima* for the phytoremediation of waters contaminated with Pb^{2+}.

A successful metal phytoremediation partially depends on the surface characteristics of the plant. *Salvinia* biomass has a great specific surface area rich in carbohydrates and carboxyl groups, which may explain its high metal removal capacity [3,14,19,28]. The content of macromolecules (carbohydrates, protein, lipids), acidic groups (carboxylic groups) and surface area are key factors which determine the binding mechanism between metal and biomass. Recently, [34] obtained the FTIR-ATR spectrum for *S. natans* biomass before and after the biosorption process of Pb^{2+} and Cr^{3+} ions. These authors reported the presence of different functional groups at *Salvinia* biomass such as carboxyl, phosphate, amide, hydroxyl and others suggesting the involvement of these functional groups (particularly phosphonates groups) in metals adsorption. In this study, the FTIR-ATR spectrum obtained for *S. biloba* biomass (Fig. 3) is closely related to that reported by [34]. The bands at 3400–3250 cm^{-1} are assigned to the OH stretch of polymeric compounds, the band at 2940–2830 cm^{-1} refer to the asymmetric and symmetric vibration of methylene (CH$_2$), respectively. The peak at 1630 cm^{-1} corresponds to C=O of amide. The peak at 1130–1000 cm^{-1} is the vibration of C-O-C and O-H of polysaccharides. The strong peaks at 1034–1036 cm^{-1} cor-

Table 4
Equilibrium parameters for Pb^{2+} adsorption by *S. biloba* according to Freundlich and Langmuir isotherms

Langmuir isotherm			Freundlich isotherm		
q_{max} (mg/g)	b (L/mg)	R^2	K_F (mg/g)(L/mg)$^{1/n}$	n	R^2
21.786	2.623	0.9685	15.020	2.256	0.9921
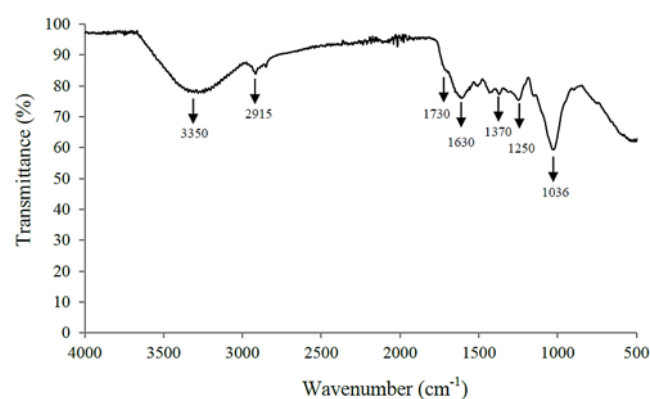
Fig. 3. FTIR-ATR spectrum of *S. biloba* biomass.

respond to alcohol groups. The 1250 cm^{-1} peak is the C-O stretch of carboxylic acids. The bands <800 cm^{-1} are the fingerprint zone which include phosphate and sulphur functional groups [35]. So, it is expected that all macrophytes of *Salvinia* family share similar biosorption mechanisms.

3.4. Analysis of compartmentalization

To understand the fate of lead within the biomass microcosm, a compartmentalization analysis was carried out according to the methodology proposed by [14]. The greatest amount of Pb^{2+} removed from the water column was adsorbed onto plant roots, regardless of the initial metal concentration and exposure time (Fig. 4), indicating that adsorption on the surface of the plant was the main mechanism observed under the experimental conditions tested. The second most important mechanism for Pb^{2+} removal was accumulation within the cells. However, metal accumulation was found in a lower proportion. After 24 h, Pb^{2+} accumulated within plant cells represented near 25% of the total initial concentration. Moreover, within the range of concentration tested, the Pb^{2+} intracellular accumulation
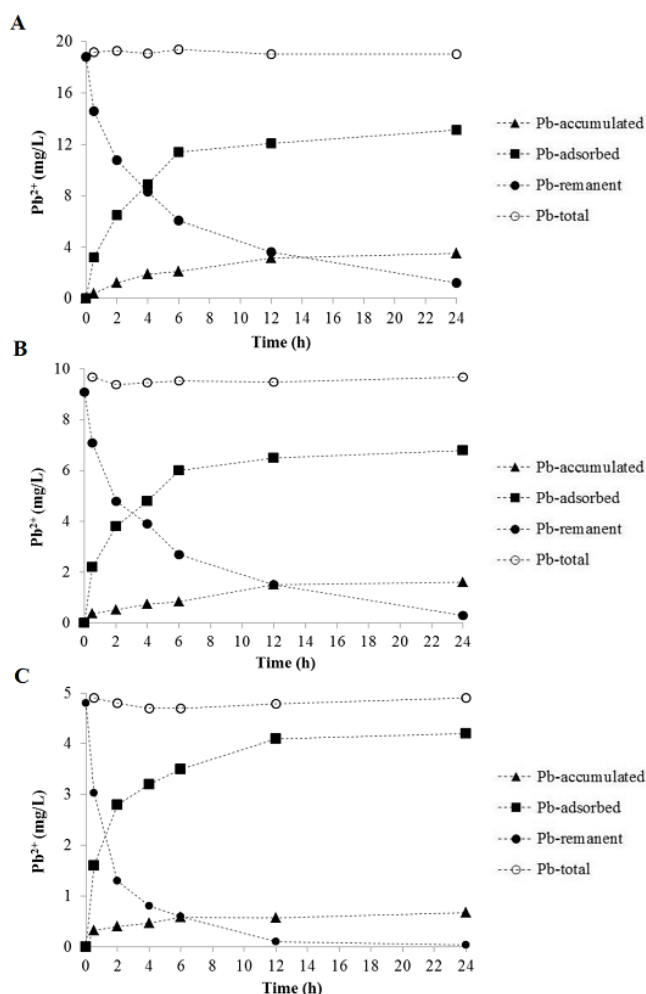
Fig. 4. Compartmentalization analysis in microcosms of batch-operated systems with *S. biloba* using deionized water as an experimental medium at three different initial Pb^{2+} concentrations: A) 19.6 ± 0.5 mg/L, B) 9.1 ± 0.4 mg/L, and C) 4.8 ± 0.3 mg/L.

rates fitted a Michaelis-Menten model (*data not shown*) in accordance with previous observations reported by [3].

The percentage of lead remaining in the water column was less than 10% for each metal concentration evaluated. Noticeably, [20] reported that after 30 days the amount of metal accumulated in plant biomass increased with the concomitant decrease in the adsorbed metal at roots. This observation involves the metal translocation from roots into the aerial part (leaves) suggesting the presence of membrane carrier proteins at plant surface. In this connection, [36] reported that Pb^{2+} ions induce the production of phytochelatins in *S. minima* and so these proteins are involved in detoxification processes as part of a metal-tolerance mechanism developed by the plant.

3.5. Evaluation of BCF

Ambient metal concentration in aquatic media is one of the critical factors that influence metal uptake efficiency in aquatic plants [37]. It has been suggested that BCF can

be used as a blueprint of metal uptake efficiency of aquatic macrophytes in phytoremediation trials [38,39]. The BCF values for Pb^{2+} calculated after 24 h for *S. biloba* were 1255, 1235 and 1202 for initial Pb^{2+} concentrations of 4.8 ± 0.3 mg/L, 9.1 ± 0.4 mg/L and 19.6 ± 0.5 mg/L, respectively. Interestingly, these values did not significantly change against increasing Pb^{2+} concentrations in the water sample. In general, high BCF values denote the accumulative loads of different metals in plant biomass, which can be used as an indicator parameter to select a plant or a treatment set-up for designing a phytoremediation system. Comparatively, the value of BCF for Pb^{2+} was higher than the BCF values reported by several authors for Cu^{2+}, Cd^{2+} and Cr(III), suggesting that *S. biloba* can remove Pb^{2+} with high accumulative efficiency [21,40,41]. The physiological need for metals in plant and uptake kinetics directly or indirectly affects the accumulative process for certain species of metals. BCF values over 1000 indicate suitability of a plant (*i.e.* hyper-accumulative plant) for phytoremediation. On the basis of our results, *S. biloba* can be considered as a hyper-accumulator for Pb^{2+} under the conditions set at the present study, which suggests that this water fern is appropriate for the removal of heavy metals from wastewaters.

4. Conclusions

The aim of the present study was to analyze autochthonous aquatic macrophytes obtained from Paraná river (Argentina) in order to evaluate the metal removal capacity for phytoremediation purposes. Four different aquatic plants were studied but only *S. biloba* was found to be efficient for growth at non-supplemented ambient conditions. Moreover, *S. biloba* proved to be highly effective for lead removal from water samples suggesting that this water fern could be used for designing cost-effective plant-based biotechnologies for heavy metal removal from the environment. Additionally, insight into *S. biloba* Pb^{2+} removal mechanisms was also discussed using a compartmentalization analysis. Our results demonstrated that surface adsorption was the main mechanism used by this plant to remove the metal in the first 24 h whereas lead accumulation occurs in a lower proportion. However, large-scale performance and long-term efficiency of *S. biloba* should be further analyzed in order to develop a bioremediation system for industrial scale wastewater management.

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