



Evaluation of autochthonous free-floating macrophytes for use in the phytoremediation of industrial wastewater contaminated with heavy metals.

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Keywords:	industrial wastewater, heavy metals, lead, phytoremediation, <i>Salvinia biloba</i> Raddi

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6 **use in the phytoremediation of industrial wastewater**
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9 **contaminated with heavy metals**
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ABSTRACT

Plant-based systems for treatment of contaminated environments (phytoremediation) have been proved to be highly efficient in removing pollutants, especially heavy metals. The range of plants used in phytoremediation processes is generally low and mainly restricted to emerging ones. 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 be propitious and sustainable, it is essential that plants used were not only efficient in pollutants removal, but also abundant at region, easily accessible, and do not require special culture conditions. In this work, we evaluate the capacity of four different autochthonous aquatic macrophytes obtained from the Paraná river (Argentina) to adapt and reproduce at «indoor» conditions. From different pre-selected species, only *Salvinia biloba* Raddi showed a wide capacity of adaptation and reproduction at greenhouse conditions. Moreover, *S. biloba* lead (Pb^{2+}) removal ability 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 found to be 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: industrial wastewater; heavy metals; lead; phytoremediation; *Salvinia biloba* Raddi.

1. Introduction

In the last decades, natural waters contamination by the release of large volumes of aqueous effluents containing heavy metals has been increased according to anthropogenic activity and industrialization. Heavy metals are a serious threat to the environment and to human health because most of these ions are inert to chemical or biological degradation, highly toxic at very low concentrations, and can be transferred through the trophic chain assuming a serious risk to living beings [1].

Lead (Pb) is omnipresent and one of the oldest 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 at the environment [2]. Lead is present in the wastewaters of petrochemical, painting and coating, founding, metal electroplating, mining, plumbing and battery industries. In more cases, lead is discharged into the environment without adequate treatment [3]. Lead poisoning has been recognized as a major public health risk, particularly at 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 $\mu\text{g/dL}$ [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 metals concentrations as maximum permissible levels [5]. The use of aquatic phytoremediation for the removal of pollutants from wastewater and industrial effluents

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3 is a sustainable and promising alternative rather than classical physicochemical
4 procedures [6]. This eco-friendly technology is based on plant ability to adsorb,
5 accumulate, and tolerate high concentrations of pollutants, including heavy metals
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10 [5, 7]. Phytoremediation-based technologies have been shown to be competitive
11 compared to more conventional methods, especially for the removal of low metal
12 concentrations wherein toxic effects to plants are minimal or absent. One of its major
13 advantages is that plants can be used as low-cost extract pumps to purify contaminated
14 water being an appropriate solution to remediate large areas or to complete
15 decontamination in longer periods of time [8]. Its success depends on the
16 physicochemical properties of the surface of the utilized plants, and some others
17 biological features such as productivity, geographical distribution and tolerance to toxic
18 compounds [9].
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29 Aquatic phytoremediation has been extensively implemented using helophytes or
30 emergent plants in constructed wetlands [10]. However, a second approach may utilize
31 vascular aquatic free-floating plants in lagoons. In such sense, several macrophytes have
32 been used for heavy metal remediation from water samples with the additional
33 advantage that plant biomass can be easily harvested and treated for metals recovery
34 before its final disposal [11]. Vascular floating plants are widely distributed in tropical
35 and subtropical regions, thus the implementation and management of lagoons for
36 treating metal-containing effluent at these regions appears to be a viable solution since
37 environmental conditions favor high plant productivity [12].
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49 In Argentina, the ecosystem surrounding the Paraná River is characterized by an
50 extensive wetland area with abundant and diverse aquatic vegetation. Macrophyte
51 species of these regions that have high efficiency in heavy metal uptake could be used
52 for the removal of ions in artificial systems or as bioindicators. Therefore, the aim of the
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3 present work was to assess the potential of several autochthonous free-floating
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5 macrophytes obtained from the Paraná River to be used in the phytoremediation of
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7 industrial wastewater contaminated with lead.
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10 11 **2. Materials and Methods**

12 13 *2.1. Collection and characterization of macrophytes*

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15 Specimens of *Hydrocotyle ranunculoides*, *Eichhornia crassipes*, *Lemma minor*, and
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17 *Salvinia* sp. were collected from a shallow lake at the middle Paraná river (32°52' 37" S
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19 - 60° 40' 36" O), with no known pollution load. The collected plants were transported to
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21 the laboratory at ambient temperature in plastic recipients containing river water. Only
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23 mature plants with uniform size were selected for experimental purposes. Taxonomic
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25 classification was carried out by the *Instituto de Botánica del Nordeste* (IBONE-
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27 CONICET, Universidad Nacional del Nordeste, Corrientes, Argentina) based on
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29 morphological characteristics. *Salvinia* sp. specimens were classified as *Salvinia biloba*
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31 Raddi (synonym name *Salvinia herzogii* de la Sota) according to the criteria establish by
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33 [13].
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40 41 *2.2. Plant material cultivation and selection*

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43 All plants collected were cultivated and maintained in greenhouse conditions receiving
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45 natural light to allow acclimation. Temperature ranged from 24 to 28 °C. Glass aquaria
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47 of 10 L capacity were used. Fresh plants and water from the sampled natural
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49 environment were placed in every aquarium. No further nutrients or special
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51 requirements were added to the medium. The specimens that showed a higher rate of
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53 growth in such conditions were considered for experimental purposes.
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2.3. Stock solution of Pb(II)

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

Plants selected as best adapted to growth in un-supplemented conditions were rinsed gently with deionized water and placed in glass containers 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 are 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 light intensity for a photoperiod 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 \pm 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

Pb²⁺ 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 HNO₃ (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 Pb²⁺ 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 Pb²⁺ elimination from water samples. Pseudo-first order or Lagergren model [15] (and pseudo-second order [16] rate equations were used for evaluating the kinetics of Pb²⁺ adsorption to *S. biloba* biomass. The Langmuir and the Freundlich models were chosen to describe the adsorption equilibrium of Pb²⁺ [3].

2.6. Compartmentalization analysis

To assess the distribution of Pb²⁺ among compartments (remainder at water column, adsorbed at plant surface and accumulated in 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/Pb²⁺≥15) for 60 min in an orbital shaker at 180 rpm. Samples from this washing were taken to quantify Pb²⁺

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3 adsorbed on the plant surface. The EDTA-washed biomass was then rinsed with
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5 deionized water and dried at 90 °C to constant weight. Samples of 0.05 g of this dried-
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7 biomass were treated overnight with 10 mL 65% HNO₃ analytical grade (Cicarelli,
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9 Santa Fe, Argentina). Subsequently, samples were heated at 120 °C in a digestion
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11 system for 2 h. Aliquots of those samples were diluted with deionized water to quantify
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13 Pb²⁺ accumulated into the plant tissue. All the samples were analyzed in an atomic
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15 absorption spectrophotometer Varian AA240FS (Varian Inc., Palo Alto, CA, EEUU) at
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17 207 nm using an air-acetylene flame [17].
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20 21 22 23 *2.7. Calculation of the bioconcentration factor*

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25 The bioconcentration factor (BCF) was calculated as an indicator of plant lead removal
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27 efficiency according to [18] as follows:
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$$30 \quad \text{BCF} = \frac{\text{Pb}^{2+} \text{ in total biomass (mg/Kg dry biomass)}}{\text{Pb}^{2+} \text{ initial concentration in solution (mg/L)}}$$

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36 37 *2.8. Statistical analysis*

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39 Linear regression, non-linear regression and variance analyses (ANOVA) were carried
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41 out using the SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA) at
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43 $p=0.05$ level.
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47 **3. Results and Discussion**

48 49 *3.1. Plants selection for metal remediation studies*

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51 For an aquatic system based on phytoremediation strategies be propitious for
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53 contaminants elimination, it is essential that macrophytes used at system design do not
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55 require special culture conditions. Properties such as high biomass productivity and
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3 high sorption capacity are essential for use at these technologies. In this study, from the
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5 different autochthonous aquatic macrophytes collected from Paraná River analyzed,
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7 only *S. biloba* showed a remarkable capacity to grow and reproduce at greenhouse
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9 conditions. Among various species, *Salvinia* holds a distinctive position due to several
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11 advantages including high yield and tolerance to a wide range of temperatures. It is
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13 widely spread in tropical zones but it is also found in subtropical and temperate zones.
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15 In Argentina, it grows abundantly in wetlands. Specimens from this genus can be easily
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17 and inexpensively handled. The capability of *Salvinia* for mass-scale use in
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19 phytoremediation must be highlighted since different species, including *S. minima*, *S.*
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21 *auriculata*, *S. natans*, and *S. rotundifolia*, have demonstrated high potential to remove
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23 heavy metals from wastewaters [19]. Based on this background and our observations,
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25 the free-floating aquatic plant *S. biloba* was selected for further experiences.
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32 3.2. Batch sorption kinetics

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34 In order to establish the capacity of *S. biloba* to remove Pb^{2+} a series of batch
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36 experiments were carried out using deionized water as the experimental medium and
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38 three different metal concentrations (4.8 ± 0.3 , 9.1 ± 0.4 and 19.6 ± 0.5 mg/L). In all cases,
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40 Pb^{2+} in water decreased over time and most of this reduction took place during the first
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42 24 h (Fig. 1). As can be seen in Table 1, metal elimination by *S. biloba* reached more
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44 than 95% removal at 24 h for 4.8 ± 0.3 and 9.1 ± 0.4 mg/L of initial Pb^{2+} concentration,
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46 but this percentage slightly declined at 19.6 ± 0.5 mg/L. After a contact period of 7 days
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48 with the highest initial Pb^{2+} concentration, approximately 98.7% removal was observed
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50 [20]. It is interesting to note that, in all cases, remnant Pb^{2+} at water samples were below
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52 limit of admissible lead discharge in industrial waste liquids (<0.50 ppm), establish by
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54 resolution No. 1089/82 (Santa Fe, Argentina). In addition, plants general appearance
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was good during all bioassay and no growth differences or evidence of necrosis was observed along the experiment.

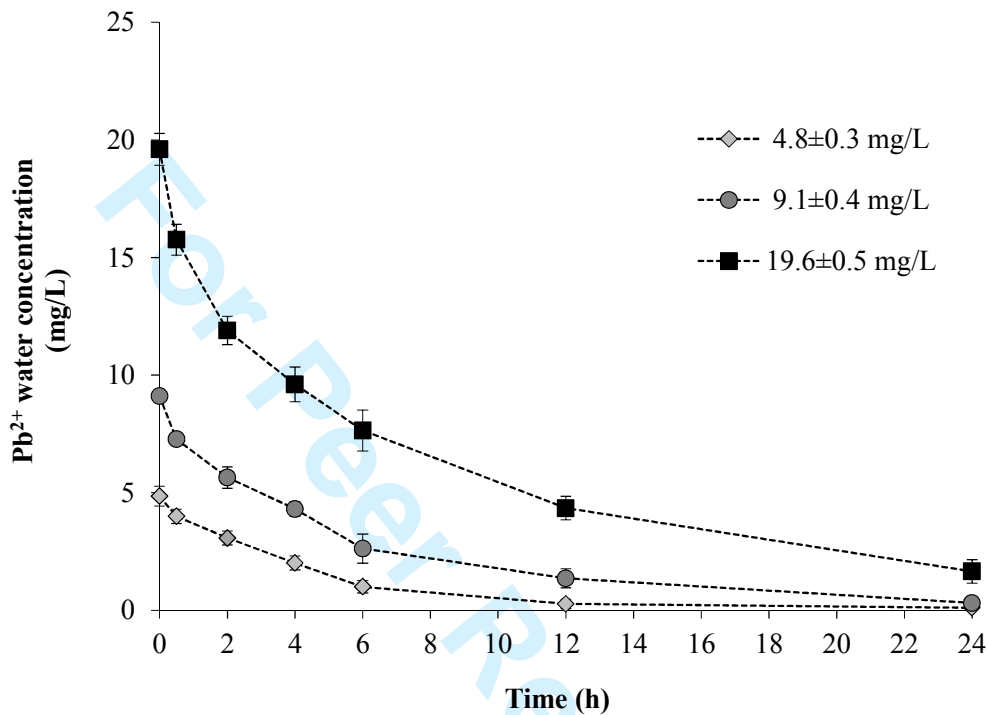


Fig. 1. Lead (Pb^{2+}) removal kinetics from water column by *S. biloba*.

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, the two lowest Pb^{2+} concentrations reached saturation of the removal capability (~85-95% of the metal) whereas the highest Pb^{2+} condition continued removal until 24 h, when about 90% metal elimination was reached. In addition, Pb^{2+} elimination from water column fits to a first order reaction model, indicating that the rate of this process depends of the initial concentration of the metal. That is, as Pb^{2+} concentration increases, its removal rate (K value) decreases (Table 2).

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 ($p < 0.05$).

Table 2. Rate constants of first (K_1) and second (K_2) order kinetic models obtained for Pb^{2+} elimination from 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

Data depicted in Figure 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.

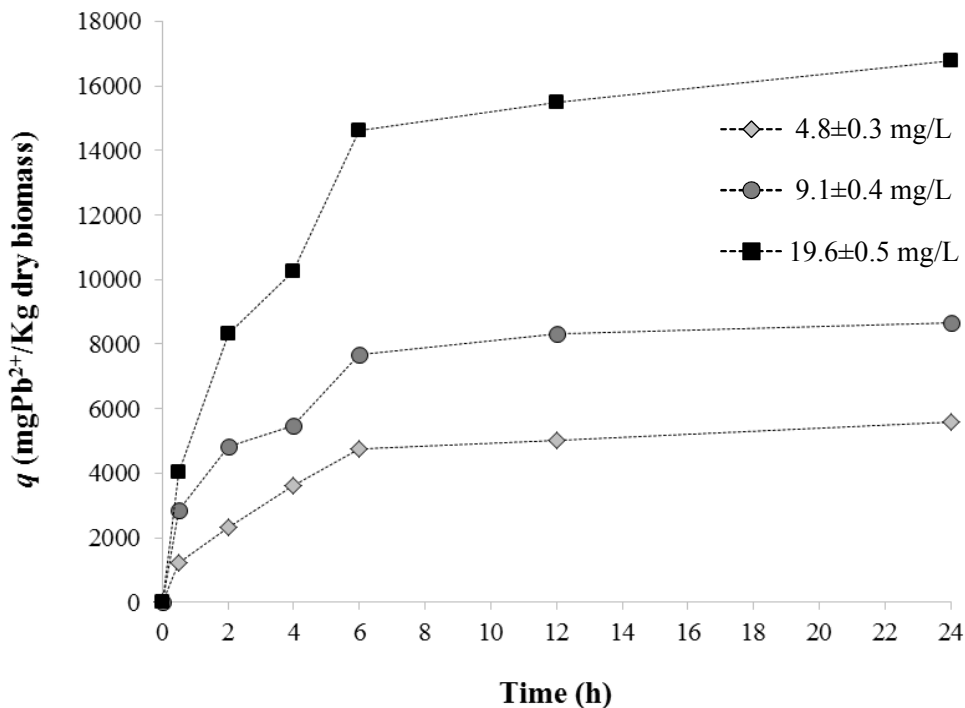


Fig. 2. Lead (Pb^{2+}) adsorption kinetics of *S. biloba* at different initial concentrations.

Moreover, the non-linear kinetics observed in Figure 2 suggests that *S. biloba* utilizes different mechanisms for removing Pb^{2+} . 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 because of the saturation of these sites. Similar patterns were 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, 22, 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

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3 groups are likely to occur [24]. Surface sorption is a combination of physical and
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5 chemical processes such as chelation, ion exchange and chemical precipitation of metal
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7 ions [25]. Biological processes (intracellular uptake and traslocation to the aerial parts)
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9 and root-mediated precipitation of metal (particularly pronounced at high metal
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11 concentration) are probably responsible for the slower components of *S. biloba* lead
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13 removal mechanism [21].

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16 Our results demonstrate that metal concentration and exposure time directly affect
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18 pollutant removal efficiency. Removal of Pb^{2+} using *S. biloba* proved to be fast enough
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20 to reach a state of equilibrium between the first 12 to 24 h of exposure to all metal
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22 concentration evaluated. When desired to control the operation cycles of an effluent
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24 treatment system that utilize aquatic plants as part of the bioremediation mechanism, it
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26 is important to know the time required to reach certain degree of contaminant removal.
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28 In this way, it is possible to adapt the periodicity of the discharges that impact the
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30 system, establishing a minimum of resting time that allows the system to recover part of
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32 its phytoremediation capacity.
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36 The batch kinetic results were also tested in two models in order to evaluate the best fit
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38 sorption reaction mechanism: pseudo-first order equation on the basis of adsorbent
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40 uptake capacity [15] and pseudo-second order reaction model based on the solid-phase
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42 sorption [16]. Kinetic parameters obtained from both proposed models at different metal
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44 initial concentrations are presented in Table 3. Metal adsorption by *S. biloba* biomass
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46 was satisfactorily adjusted to a pseudo-second order kinetic model for all Pb^{2+}
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48 concentrations tested, with high coefficients of determination (Table 3). The adsorption
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50 capacity at equilibrium (q_e) and the initial adsorption rate (h) significantly increased as
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52 metal content in solution increased. These results indicate that increasing metal
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54 concentration in water samples causes more interactions between the metal and the
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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 when 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*.

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

Pseudo-second order kinetic model have 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 ionic exchange reaction type. 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 solution during the adsorption of Pb^{2+} , Cd^{2+} , Zn^{2+} , or Ni^{2+} by different aquatic macrophytes [28, 29, 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 being the most common the Langmuir and Freundlich models. The Langmuir isotherm model assumes that a finite number of active sites exist at the adsorbent uniformly distributed over its 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 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 surface of the plant. These results are compatible with the presence of different functional groups responsible for metals adsorption in *Salvinia*. A successful metal phytoremediation partially depends on the surface characteristics of the plant. *Salvinia* biomass has a great specific surface area that is rich in carbohydrates and carboxyl groups, which may explain its high metal removal capacity [3, 14, 19]. 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.

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

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 K_F (8.31) and n (1.57) values for Pb^{2+} adsorption by *S. minima* from water samples lower than those obtained in the present work. So, our results suggest that *S. biloba* may be a better choice than *S. minima* for the phytoremediation of waters contaminated with Pb^{2+} .

3.4. Analysis of compartmentalization

To understand the fate of lead within biomass microcosm, a compartmentalization analysis was carried out according to the methodology proposed by [14]. The major quantity of Pb^{2+} removed from the water column was adsorbed onto plant roots regardless of the initial metal concentration and exposure time (Fig. 3), 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 into cells. However, metal accumulation was found in a lower proportion. After 24 h, Pb^{2+} accumulated at plant cells represented near 25% of the total

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3 initial concentration. Moreover, within the range of concentration tested, the Pb^{2+}
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5 intracellular accumulation rates fitted a Michaelis-Menten model (*data not shown*) in
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7 accordance with previous observations reported by [3].
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10 11 12 **INSERT Fig. 3**

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16 The percentage of lead remaining in the water column was less than 10% for each metal
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18 concentration evaluated. Noticeably, [20] reported that after 30 days the amount of
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20 metal accumulated in plant biomass increased with the concomitant decrease in the
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22 adsorbed metal at roots. This observation involves the metal translocation from roots
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24 into the aerial part (leaves) suggesting the presence of membrane carrier proteins at
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26 plant surface. In this connection, [34] reported that Pb^{2+} ions induce the production of
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28 phytochelatins in *S. minima* and so these proteins are involved in detoxification
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30 processes as part of a metal-tolerance mechanism developed by the plant.
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36 *3.5. Evaluation of BCF*

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38 Ambient metal concentration in aquatic media is one of the critical factors that
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40 influences metal uptake efficiency in aquatic plants [35]. It has been suggested that BCF
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42 can be used as blueprint of metal uptake efficiency of aquatic macrophytes in
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44 phytoremediation trials [36, 37]. The BCF values for Pb^{2+} calculated after 24 h for
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46 *S. biloba* were 1255, 1235 and 1202 for initial Pb^{2+} concentrations of 4.8 ± 0.3 mg/L,
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48 9.1 ± 0.4 mg/L and 19.6 ± 0.5 mg/L, respectively. Interestingly, these values did not
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50 significantly change against increasing Pb^{2+} concentrations in water sample. In general,
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52 high BCF values denote the accumulative loads of different metals in plant biomass,
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54 which can be used as indicator parameter to select a plant or a treatment set-up for
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3 designing a phytoremediation system. Comparatively, the value of BCF for Pb^{2+} was
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5 higher than the BCF values reported by several authors for Cu^{2+} , Cd^{2+} and Cr(III)
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7 suggesting that *S. biloba* can remove Pb^{2+} with high accumulative efficiency [21, 38,
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9 39]. The physiological need of metals in plant and uptake kinetics directly or indirectly
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11 affects the accumulative process for certain species of metals. BCF values over 1000
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13 indicate suitability of a plant (*i.e.* hyper-accumulative plant) for phytoremediation.
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15 On the basis of our results, *S. biloba* can be considered as hyper-accumulator for Pb^{2+}
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17 under the conditions set at the present study, suggesting the appropriateness of this
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19 water fern to be used for heavy metals removal from wastewaters.
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25 **4. Conclusions**

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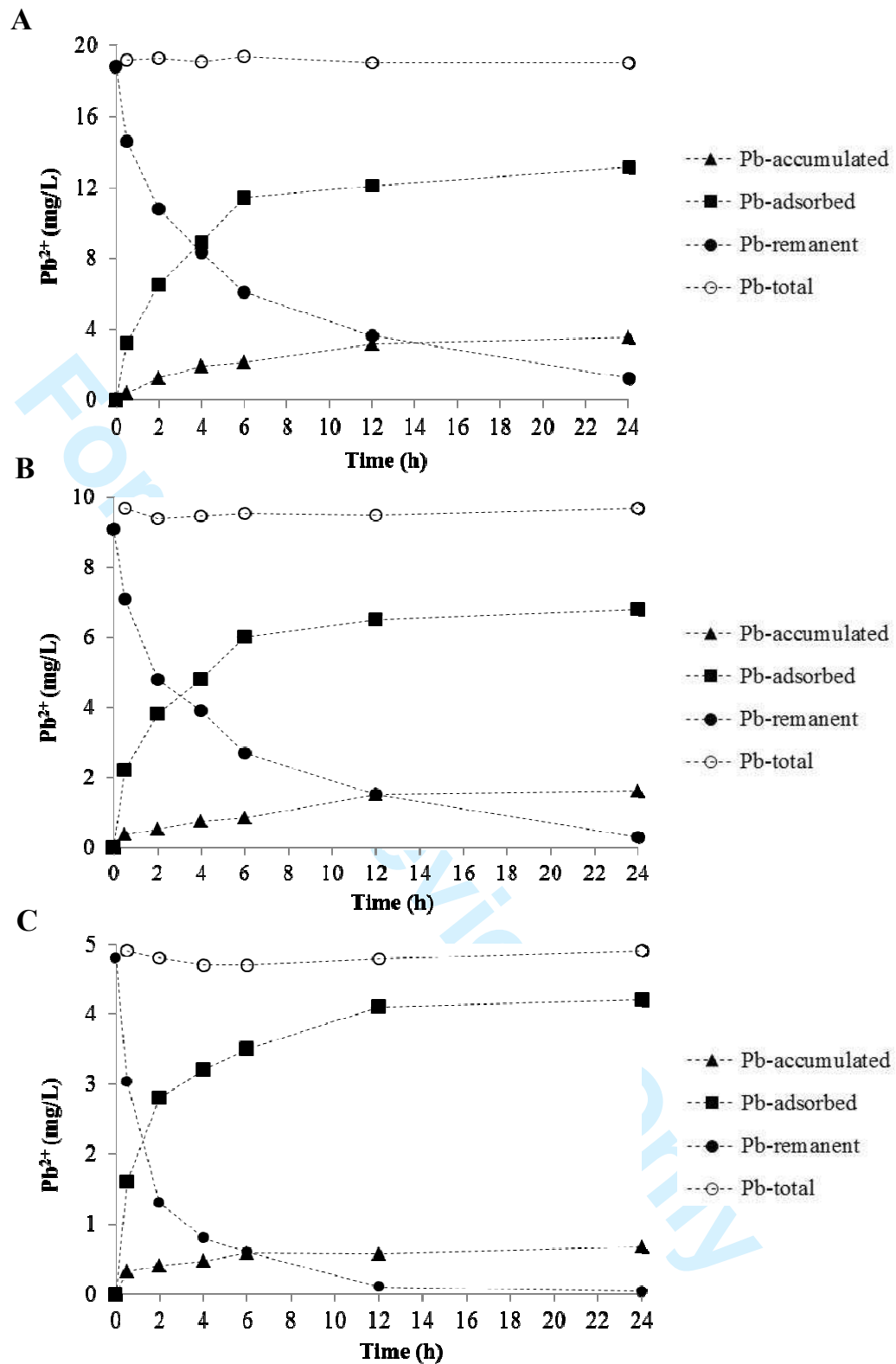


Fig. 3. Compartmentalization analysis in microcosms of batch-operated systems with *S. biloba* using deionized water as experimental medium at three different initial Pb²⁺ concentration: A) 19.6±0.5 mg/L, B) 9.1±0.4 mg/L, and C) 4.8±0.3 mg/L.

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