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Eugenia J. Olguín · Gloria Sánchez-Galván
Teresa Pérez-Pérez · Arith Pérez-Orozco

Surface adsorption, intracellular accumulation and compartmentalization of Pb(II) in batch-operated lagoons with *Salvinia minima* as affected by environmental conditions, EDTA and nutrients

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Abstract The effects of environmental factors and nutrients on the various possible removal mechanisms (surface adsorption, intracellular accumulation and precipitation to sediments) and partitioning of lead among various compartments (plant biomass, water column and sediments) in *Salvinia minima* batch-operated lagoons, were evaluated. Surface adsorption was found to be the predominant mechanism for Pb(II) removal under all environmental conditions tested in the absence of nutrients (an average of 54.3%) and in a nutrient medium (modified Hutner 1/10 medium) free of EDTA and phosphates (54.41%) at "high" initial Pb(II) concentrations (in the range of 10.3 ± 0.13 to 15.2 ± 0.05 mg/L). Under these conditions, the bioconcentration factors (BCFs) were 2,431 ± 276 and 2,065 ± 35, respectively. Lead removal was very rapid during the first 4 h and reached 70% in the absence of nutrients at the "medium" light intensity and temperature (LIT) tested, 88% in nutrient medium free of EDTA and supplemented with synthetic wastewater (at the "lowest" LIT tested), and 85% in medium free of EDTA and phosphates. It was concluded that the mechanisms of lead removal by *S. minima*, and the compartmentalization of this metal in the microcosm of batch-operated lagoons, are primarily a function of the presence of certain nutrients and chelants, with secondary dependence on environmental conditions. In addition, the results indicate that the percentage of lead removed is only a gross parameter and that the complementary use of BCF and compartmentalization analysis is required to gain a full insight into the metal removal process.

Keywords *Salvinia minima* · Lead · Pb(II) · Surface adsorption · Intracellular accumulation

Introduction

It is widely recognized that heavy metals are a serious threat to the environment and to human health [11]. In this regard, there are at least two major issues: (1) heavy metals are highly toxic at very low concentrations, and (2) current legislation demands very low concentrations of metals as maximum permissible levels. Lead is one of the most toxic heavy metals, inducing neurotoxic effects at a concentration in the blood as low as 20 μg/dL [2]. Conventional technologies for the treatment of wastewater containing these toxic compounds are usually expensive and inefficient, especially for treating effluents containing less than 100 mg/L metals [29]. The alternative technology of phytoremediation of heavy metals has been shown to be competitive compared to more conventional methods [18], especially for the removal of low concentrations of these compounds, conditions under which toxic effects to plants are minimal or absent.

Within this context, the use of aquatic plants for heavy metal removal from wastewater and industrial effluents is a promising alternative. Aquatic phytoremediation may be implemented through the use of two different technological approaches. One such approach promotes the use of specific emergent aquatic plants, such as cattails (*Typha latifolia*), bullrush (*Scirpus lacustris*) and common reed (*Phragmites australis*), in so-called "constructed" wetlands [25]. A second approach utilizes specific species of vascular aquatic floating (VAF) plants in lagoons. The latter approach has the

E. J. Olguín (✉) · G. Sánchez-Galván · T. Pérez-Pérez
A. Pérez-Orozco
Environmental Biotechnology Unit,
Institute of Ecology,
Km 2.5 carretera antigua a Coatepec No 351 Congregación El Haya, Xalapa, Veracruz, 91070, Mexico
E-mail: eugenia@ecologia.edu.mx
Tel.: +52-228-8421848
Fax: +52-228-8187809

advantage that plant biomass can be easily harvested and treated for recovery of metals before being disposed of. VAF plants are more widely distributed in tropical and subtropical regions, where they may become weeds in eutrophied water bodies. Thus, implementation and management of lagoons with VAF plants in these regions, where environmental conditions favor high plant productivity, appears to be a viable solution for treating metal-containing effluents and concomitant recovery of metal wastes.

Some floating aquatic plants of the Lemnaceae family (duckweed) have already been investigated for the removal of heavy metals [15, 23, 30]. More recently, it has been shown that *Salvinia minima* (Baker), an aquatic fern, is a more efficient hyperaccumulator of Cd(II) and Pb(II) compared to Lemnaceae, showing a much higher bioconcentration factor (BCF) for a similar initial metal concentration [20]. In the case of Pb(II), the BCF found for *S. minima* was in the range of 2,822–3,304, depending on the test conditions, for an initial Pb(II) concentration of 3 mg/L. In contrast, the reported BCFs for *Lemna minor* and *Azolla pinnata* for an initial concentration of 4 mg/L were 138 and 140, respectively [15]. *Salvinia* has additional advantages for use in phytoremediation; it can grow in very different habitats since its lethal temperatures are −3 and 43°C [28], thus explaining its wide geographical distribution within tropical and subtropical regions of the World. Furthermore, it has been shown to outgrow Lemnaceaes in mixed culture [1]. Our research group has found that *S. minima* reached a productivity of 3.38 g dry weight (DW) m^{-2} day^{-1} compared to a productivity of 1.52 g DW m^{-2} day^{-1} by *Spirodela polyrhiza* when both VAF plants were cultivated in modified 1/10 Hutner medium under the same light intensity and temperature (LIT) (unpublished results). A recent investigation (our unpublished results) into its physico-chemical characteristics has shown that *S. minima* has a very high surface area (264 m^2/g DW) and a high content of carboxylic ligands (0.95 mmol H^+/g biomass), two properties that make it very useful for phytoremediation. Finally, it is easy to harvest, a factor that promotes a higher rate of metal removal per surface unit, and higher recovery of metals after suitable treatment. We have recently developed an electrochemical method using ammonium oxalate for lead desorption from the biomass of *S. minima* as a very efficient and useful means of producing good quality compost (unpublished results).

On the other hand, the implementation and management of lagoons with VAF plants on a large scale for removal of heavy metals requires, among other things, an understanding of the predominant mechanisms occurring during the metal removal process as well as the compartmentalization of the metal within the lagoon microcosm. Various possible removal mechanisms include: (1) adsorption to the plant surface, (2) intracellular accumulation, (3) precipitation into sediments or the water column, and (4) permanence in solution in the water column. However, research on these aspects regarding VAF plants is very scarce. Usually, only the kinetics of metal removal from solution is reported. Noraho and Gaur [19] applied the methodology established by Bates and coworkers [4] for estimating the adsorbed extracellular concentration of metal by washing the plant biomass with a 10 mM EDTA solution. Analysis of this washing solution gave the extracellular metal concentration, and analysis of the EDTA-washed plant material gave the intracellular metal concentration. Following exposure of *A. pinnata* and *Spirodella polyrhiza* to an aqueous solution containing 0.1 mM Cd, adsorption onto the extracellular surface of the plants was found to be the predominant mechanism of cadmium removal (71–86%). In previous work on *S. minima* by our group [20], surface adsorption was proposed as the predominant mechanism during the first few hours of cadmium removal from the water column, since the rate of removal was very rapid during that time period. In contrast with these findings, in a compartmentalization study with *Typha domingensis* and *L. minor* in a wetland microcosm containing a high level of organic matter and very low concentrations of cadmium (105 µg/L) and lead (396 µg/L), Debusk and coworkers [9] found that precipitation of the metals into the sediments as sulfides was the predominant fate of these pollutants.

It is clear from the above discussion that there is still a need for a more detailed understanding of the factors that determine the various mechanisms of metal removal in VAF plants, and the fate of these compounds in an aquatic microcosm. Thus, the aim of the present work was to define the effect of some environmental factors (LIT) and nutrients (EDTA, phosphates and organic compounds) on the removal mechanisms and on the partitioning of lead among various compartments in *S. minima* batch-operated ponds.

Materials and methods

Experiments under outdoor conditions

Preparation of inoculum

S. minima was cultivated and maintained as a monoplant culture, utilizing the chemically defined medium Hutner 1/10 modified according to Vermat and Hanif [27] at pH 6.0. The cultures were maintained in a greenhouse. The climatic conditions were those prevailing from October to November in the City of Xalapa, Veracruz (19°32′31′N and 95°54′35′W and an altitude of 1,580 m above sea level). Light intensity was measured using a Lux meter “Lutron”, model LX-101.

After 7 days of cultivation, plants were used in various experiments. In a first experiment, plants were rinsed with a solution of EDTA (1.3 mM) before being exposed to the metal solution. For the remainder of the experiments, healthy plants were used after being washed with tap water for 5 min. The initial density of the inoculum was 42.85 g DW/m^2.

Experimental conditions

Five different experimental media were used: (1) tap water, (2) modified Hutner medium 1/10 containing EDTA, (3) modified Hutner medium 1/10 containing EDTA plus synthetic wastewater (SWW), (4) EDTA-free modified Hutner medium 1/10, and (5) EDTA-free modified Hutner medium 1/10 supplemented with SWW. The latter contained 82 mg/L sucrose, 74 mg/L sodium acetate and 0.0198 µg/L propionic acid according to Vermat and Hanif [27]. The initial pH of the experimental units was adjusted to 6.0 in all cases using NaOH and HNO_3. The tap water used had the following characteristics: pH 6.95; hardness 29.8 mg $CaCO_3$/L and 0.0636 µg N-NH_4/L.

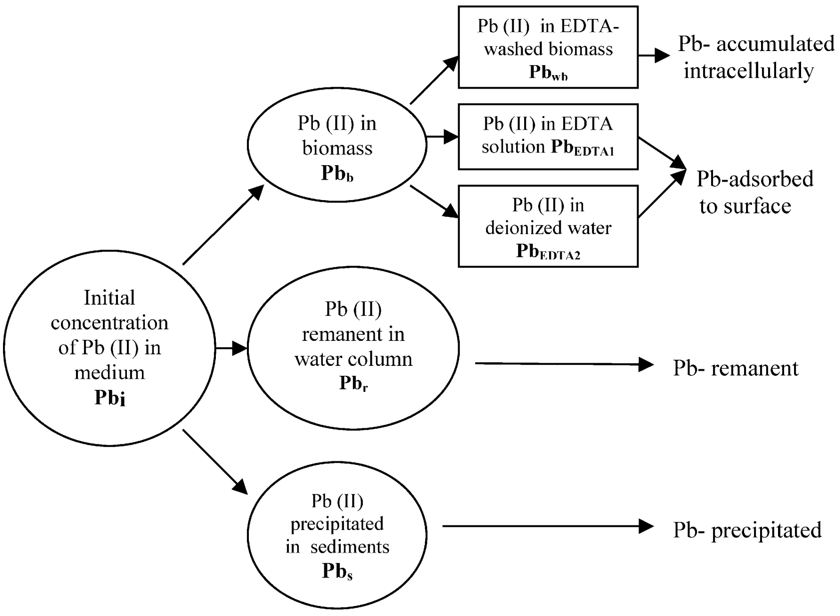
A metal stock solution was prepared with $Pb(NO_3)_2$ (analytical grade) at a concentration of 1,000 mg/L Pb(II). Appropriate additions of this stock solution were carried out to provide initial concentrations in the experimental units in the range of 1.4 ± 0.02 to 15.2 ± 0.05 mg Pb/L.

Experimental units were hard plastic containers (0.15 m ×0.19 m) containing 1.5 L experimental medium plus Pb(II) at specific concentrations. The column water had a depth of 0.06 m and the container a total surface area of 0.028 m^2. Three experimental units were used as replicates for each treatment and the data were reported as mean value ± standard deviation. The fresh weight of plant biomass was determined after removing excess liquid over a filter paper for 15 min. Dry weight of washed biomass was obtained after drying the biomass in an oven at 35°C to constant weight. The experimental units were maintained in a greenhouse and LIT were monitored at five different times during the day at 1000, 1200, 1400, 1600 and 1800 hours. Three different environmental conditions were used: (1) "high" LIT (290 µmol/m^2 and 23°C), (2) "medium" LIT (138 µmol/m^2 and 22°C), and (3) "low" LIT (55 µmol/m^2 and 19°C).

Analysis of compartmentalization

To evaluate the distribution of lead among various compartments of the experimental microcosms (the plant tissue, the water column, and the sediment in the ponds), a compartmentalization analysis was carried out as described in Fig. 1. Differentiation between adsorption to the plant surface and accumulation in the plant tissue was carried out as described by Noraho and Gaur [19]. Biomass samples were collected at the end of the each experiment (24 h) and washed with deionized water to remove residues from the medium. Plant biomass was then washed with 150 mL of a solution of EDTA (concentration equivalent to a molar ratio EDTA/Pb = 12) for 60 min in a shaker. The biomass was subsequently rinsed with deionized water. Samples from this washing were taken to quantify Pb adsorbed to the plant surface, while Pb accumulated in the plant tissue was quantified from the washed biomass.

Fig. 1 Analysis of lead compartmentalization in the various microcosms of batch-operated *Salvinia minima* lagoons



$$Pb_i = Pb_{wb} + (Pb_{EDTA1} + Pb_{EDTA2}) + Pb_r + Pb_s$$

Analytical methods

Samples from the experimental medium were taken at different times and filtered through Whatman paper (11 μm) to remove insoluble particles. Biomass samples (0.05 g) were treated overnight with 5 mL 65.9% HNO_3 (analytical grade). Subsequently, 6 mL nitric acid was added and samples were heated at 160°C in a AIM500-C digestion system until they were completely digested and their volume was reduced to 1 mL. Such samples were then diluted with 50 mL deionized water. Filtered samples from experimental medium and digested biomass were analyzed in an Atomic Absorption Spectrophotometer Buck Scientific 210 VGP using an air–acetylene flame.

Experiments under indoor "controlled" conditions

Inoculum preparation

S. minima was cultivated and maintained as a mono-plant culture in a temperature-controlled chamber at 25°C, utilizing modified Hutner 1/10 at pH 6.0. Light (intensity $= 142\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) was provided by cool white lamps (39 W) operating in cycles of 16 h light/8 h darkness.

Experimental conditions

The size of the experimental units and the density of the plant inoculum were similar to those described for outdoor experiments. The nutrient medium utilized was modified Hutner 1/10 medium free of EDTA and phosphates with a initial metal concentration of 12.9 ± 0.6 mg Pb/L at pH 6.0. Sampling and Pb determination in water column and plant biomass, and compartmentalization analysis were performed as described for experiments under outdoor conditions.

Evaluation of BCF

The BCF was calculated according to Zayed and coworkers [30]:

$$\text{BCF} = \frac{\text{Trace element concentration in plant tissue (mg/kg) at harvest}}{\text{Initial concentration of the element in the external nutrient solution (mg/L)}}$$

Statistical analysis

The effect of light intensity, temperature and nutrients, on the removal mechanism and on the partitioning of lead among various compartments in *S. minima* ponds, was analyzed using ANOVA test. The analysis was performed using the SIGMA STAT Program (version 3.0, 2003) at $P = 0.05$ level.

Results

Influence of LIT on Pb(II) removal and compartmentalization in the absence of nutrients

In order to establish the capacity of *S. minima* to remove Pb(II), and the compartmentalization of this compound in the experimental microcosm, a series of experiments was carried out using tap water as the experimental medium. The experimental units were exposed to three different LIT conditions under outdoor conditions. The removal of Pb(II) from the medium was very fast, reaching after 4 h a value of 55% of the initial concentration of metal in solution in experiments performed at the highest and lowest LIT conditions, and a value of 70% under the medium condition. In the following 6 h, the medium and lowest LIT conditions reached saturation of the removal capability (80–90% of the metal) whereas the highest LIT condition continued removal until 24 h, when a removal value of about 90% was reached. However, in terms of percentage of Pb removed, there were no significant differences among the three experimental conditions after 24 h of exposure to lead solution.

In addition, in order to understand the fate and compartmentalization of lead within the experimental microcosm, an analysis was made according to the methodology illustrated in Fig. 1. It was observed (Fig. 2b) that adsorption to the plant surface was the main mechanism encountered under the three experimental conditions, reaching 58% at the highest LIT, 54% at the medium LIT and 51% at the lowest LIT. However, these values were not statistically different.

The second most important mechanism for lead removal under these experimental conditions was accumulation into the cells (Fig. 2b), which was significantly higher ($P < 0.05$) at the highest LIT when compared with the other two conditions. The percentage of lead remaining in the water column was significantly higher ($P < 0.05$) at the lowest LIT, compared to the highest test condition (Fig. 2b).

Under the conditions described above, there were no significant differences in the value of the BCF among the three different environmental conditions tested (Table 1), with the average value being 2,431 ± 276.

Influence of light intensity, temperature, EDTA and nutrients on Pb(II) removal and compartmentalization

S. minima has been cultivated successfully in Hutner 1/10 modified synthetic medium, achieving a high productivity of 3.38 g DW $\text{m}^{-2}\ \text{day}^{-1}$ (our unpublished results). Thus, it seemed convenient to use this synthetic medium for evaluating the capacity of this aquatic fern

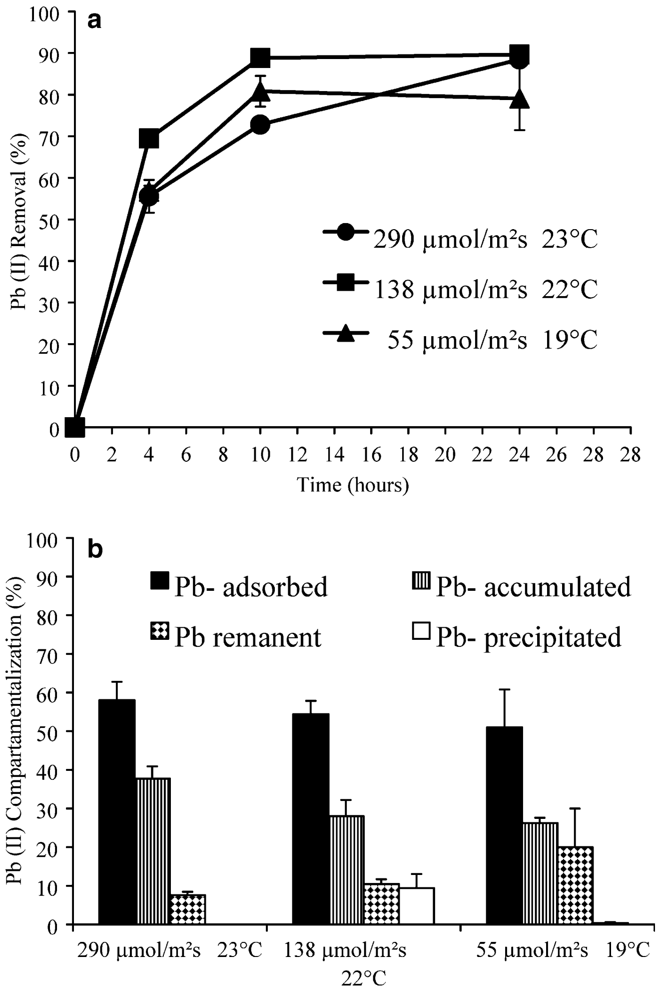


Fig. 2 Influence of light intensity and temperature (LIT) on **a** lead removal kinetics and **b** compartmentalization analysis in microcosms of batch-operated lagoons with *Salvinia minima*, using only tap water as experimental medium and at an average initial concentration of 11.4 ± 0.4 mg Pb/L

for lead removal in the presence of nutrients. However, since this medium contains EDTA, a well-known chelant, and phosphates, which are also known to precipitate lead, an evaluation of the effect of these two compounds on lead removal by *S. minima* was undertaken.

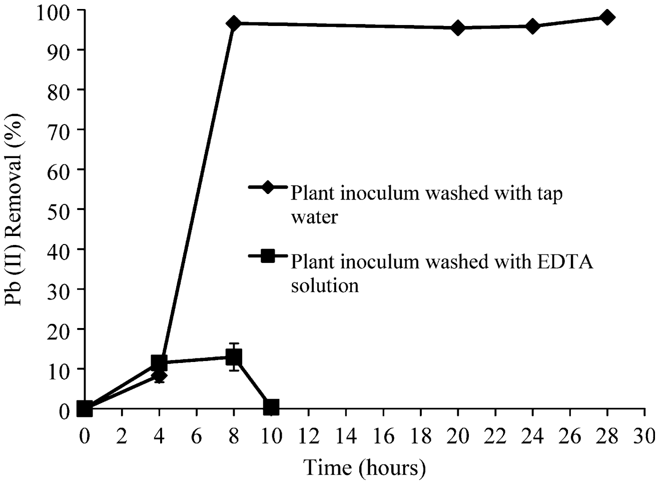


Fig. 3 Lead removal kinetics with two types of *S. minima* inocula: rinsed with 1.3 mM EDTA (*filled squares*) and rinsed with tap water (*filled diamonds*), in modified Hutner 1/10 medium + synthetic wastewater (SWW) with initial concentrations of 1.4 ± 0.02 and 2.6 ± 0.02 mg Pb/L, respectively

Healthy *S. minima* cultivated in modified Hutner medium 1/10 under outdoor conditions was used as inoculum for all Pb(II) removal experiments. A first experiment was performed by comparing two types of inocula. In one case, the plants were rinsed with a 1.3 mM EDTA solution before being exposed to 2.57 mg/L lead dissolved in Hutner medium with added SWW. The second type of inoculum consisted of plants rinsed only with tap water and exposed to an initial concentration of 1.4 ± 0.02. With plants rinsed with EDTA, the percentage of lead removed reached a value of 10% after 8 h of exposure, but removal then declined to zero by 10 h (Fig. 3). In addition, the plants showed unhealthy symptoms such as frond chlorosis at the end of the experimental period. In contrast, plants rinsed with tap water showed a high removal of lead (96.6 ± 0.05% after 8 h). Thus, treatment of the inoculum plants with EDTA was omitted in subsequent experiments.

When the experiments were performed at "high" LIT, it was observed that the EDTA contained in modified Hutner medium 1/10, inhibited almost completely the removal of lead (Fig. 4a). Furthermore, the compartmentalization analysis showed that 70.8 ± 5.9% of the lead remained in the water column, 27 ± 5% was precipitated, and only a small fraction (2.1 ± 0.3%) was adsorbed onto the plant surface and a very minor fraction (0.07%) accumulated inside the plant (Fig. 4b). When EDTA was not present in the synthetic medium, the lead removal percentage was 92.6 ± 2.5% after 10 h of exposure to this heavy metal (Fig. 4a). In this case,

Table 1 Bioconcentration factors (BCF) of Pb(II) by *Salvinia minima* in tap water under outdoor environmental conditions

Light intensity/temperature	Metal concentration in plant tissue (mg/kg)	Initial Pb concentration (mg/L)	BCF
290 μmol m^{-2} s^{-1},/23°C	29,794 ± 1,330	11.4 ± 0.4	2,606 ± 202
138 μmol m^{-2} s^{-1},/22°C	24,716 ± 1,538	11.8 ± 0.3	2,113 ± 229
55 μmol m^{-2} s^{-1},/19°C	27,486 ± 3,412	10.9 ± 2.1	2,573 ± 334

adsorption to the plant surface increased to 35.7 ± 10%, but there was still a large fraction (58.6 ± 10%) that was precipitated from the solution. This pattern was interpreted as indicating the presence of other compounds in the medium, probably phosphates, which were responsible for the observed high precipitation in the absence of EDTA. It is interesting to note that when the Hutner medium was free of EDTA but supplemented with a SWW containing sucrose, propionic acid and sodium acetate, the kinetics of lead removal was very similar to those observed for Hutner medium without EDTA (Fig. 4a), resulting in a 96.8 ± 2.2% removal after 10 h of exposure. However, in this case, adsorption to the plant surface was lower (28.2 ± 3.3%) and precipitation was higher (69.5 ± 4%). It appears that any, or all, of these organic compounds can form insoluble complexes with lead.

When a similar experiment was performed at "low" LIT, the lead was removed faster (Fig. 5a) compared to the previous experiments where the light intensity was "high". After only 4 h of exposure to the initial "high" lead concentration, the removal percentage was 87% for both media lacking EDTA. However, the fraction of lead adsorbed to the plant surface was significantly lower ($P < 0.05$) in the case of these two media (Fig. 5b), compared to experiments performed at higher light intensity. Furthermore, a larger fraction of lead was precipitated into the sediments. When EDTA was present in the Hutner medium, lead removal by *S. minima* after 24 h was insignificant (1.6 ± 0.2%), with most of the lead (62.1 ± 5%) remaining in the water column. The next largest fraction (35.5 ± 5%) was the precipitated fraction.

The value of the BCF was extremely low (56.5 ± 3.8 and 69 ± 6.8) when the medium contained EDTA under

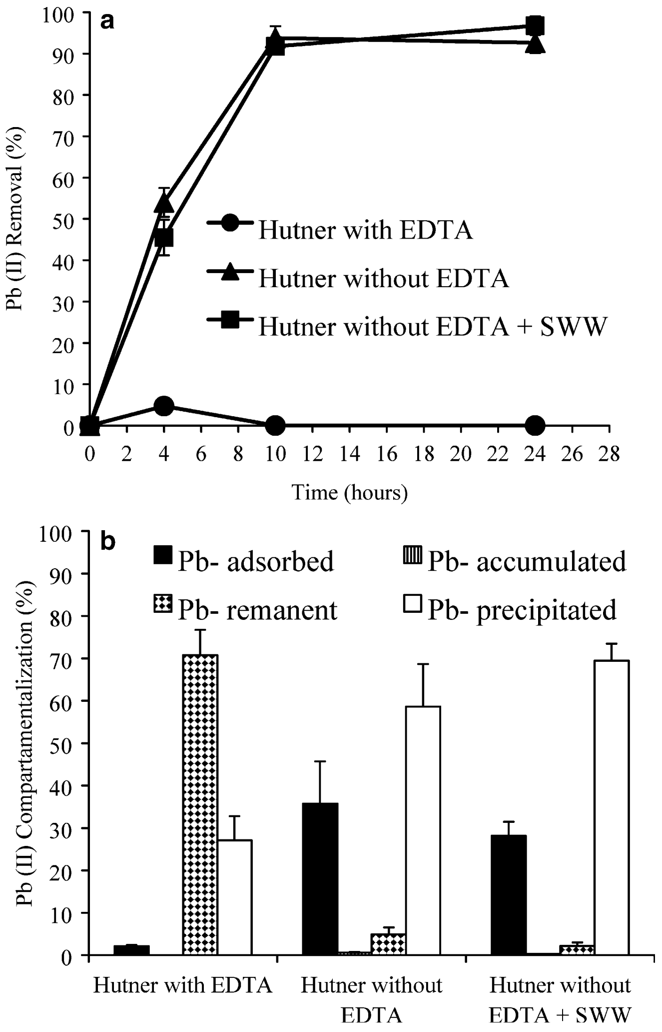


Fig. 4 Influence of EDTA and nutrients on **a** lead removal kinetics and **b** compartmentalization analysis in microcosms of batch-operated lagoons with *S. minima* using modified Hutner 1/10 nutrient medium and an average initial concentration of 12.1 ± 1.6 mg Pb/L under outdoor conditions (290 μmol m^{-2} s^{-1}, 23°C)

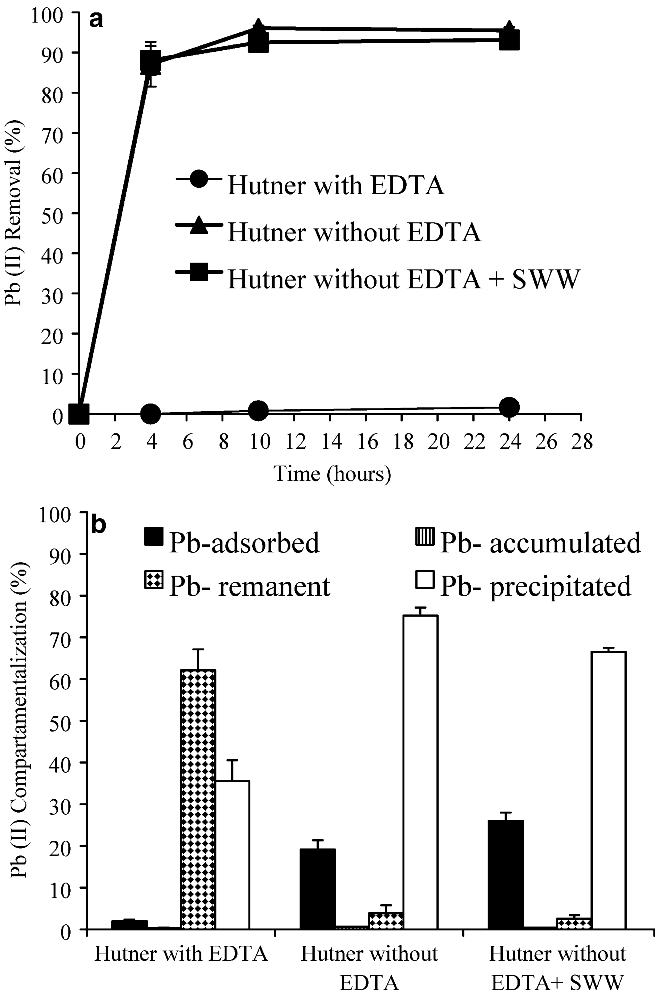


Fig. 5 Influence of EDTA and nutrients on **a** lead removal kinetics and **b** compartmentalization analysis in microcosms of batch-operated lagoons with *S. minima* using modified Hutner 1/10 nutrient medium and at average initial concentration of 13.2 ± 2.2 mg Pb/L under outdoor conditions (55 μmol m^{-2} s^{-1}, 19°C)

Table 2 BCFs of Pb(II) by *S. minima* in different nutrient media and environmental conditions

Light intensity/temperature	Medium	Metal concentration in plant tissue (mg/kg)	Initial Pb concentration (mg/L)	BCF
290 μmol m^{-2} s^{-1}, 23°C[a]	Hutner with EDTA	755.80 ± 72	13.4 ± 0.3	56.5 ± 3.80
	Hutner without EDTA	11,512 ± 220	10.3 ± 0.1	1,127 ± 30
	Hutner without EDTA + SWW	9,822 ± 769	12.7 ± 1.1	750 ± 11
55 μmol m^{-2} s^{-1}, 19°C[a]	Hutner with EDTA	1,051 ± 103	15.2 ± 0.05	69 ± 6.80
	Hutner without EDTA	8,780 ± 320	13.5 ± 0.7	649 ± 56
	Hutner without EDTA + SWW	11,351 ± 393	10.9 ± 1.1	1,096 ± 115
142 μmol m^{-2} s^{-1}, 25°C[b]	Hutner 1/10 without EDTA, phosphates	27,473 ± 758	12.9 ± 0.6	2,065 ± 35

[a] Outdoor conditions
[b] Indoor conditions

the two experimental conditions (Table 2), confirming that when this chelant is in solution, it may form a complex compound with lead, decreasing its availability for removal by *S. minima*. However, the BCF increased when EDTA was absent from the Hutner medium and when EDTA-free medium was supplemented with SWW for the two experimental conditions tested (Table 2).

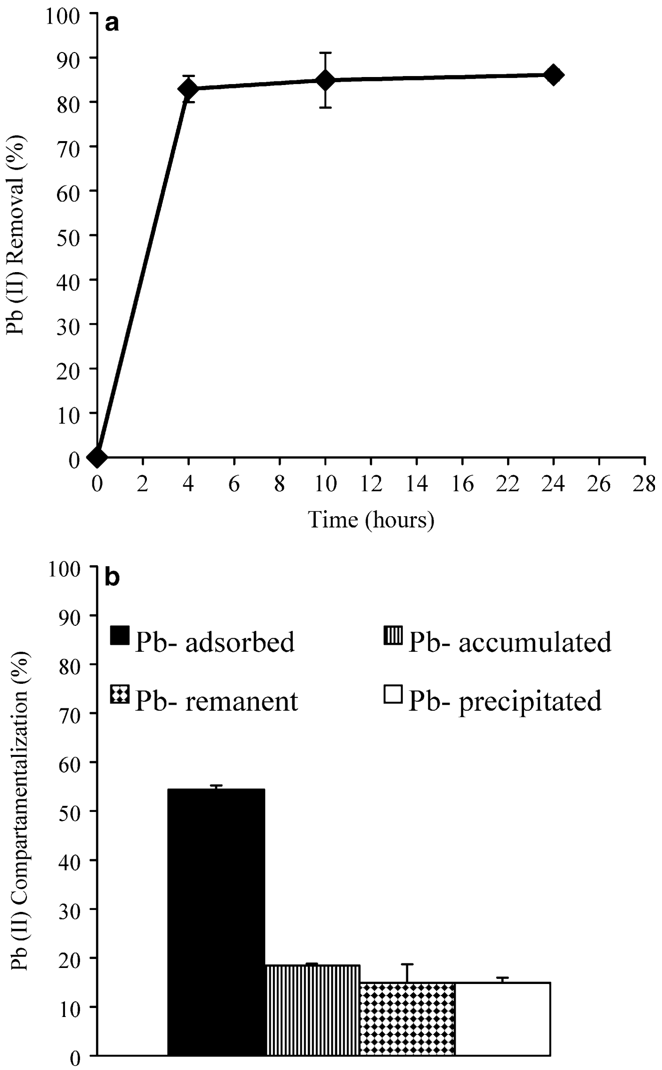


Fig. 6 **a** Lead removal kinetics and **b** compartmentalization analysis in microcosms of batch-operated lagoons with *S. minima* using modified Hutner 1/10 nutritive medium free of EDTA and phosphates and an average initial concentration of 12.7 ± 0.6 mg Pb/L under indoor "controlled" conditions (142 μmol m^{-2} s^{-1}, 25°C)

Taking into consideration the above results, a new experimental medium was designed by removing EDTA and phosphates from the modified Hutner 1/10 medium. *S. minima* was suspended in this new medium, and exposed to an initial concentration of 12.9 ± 0.6 mg Pb/L under controlled environmental conditions (142 μmol m^{-2} s^{-1} and 25°C). It was observed that the initial rate of Pb(II) removal was very fast, reaching 86% of removal at 4 h (Fig. 6a). In contrast with the previous experiments in which EDTA and phosphates were present, the predominant mechanism of lead removal in this medium was surface adsorption (Fig. 6b). The pH was monitored during the exposure of *S. minima* to lead and it was observed that only a slight alkalinization occurred after 10 h, the pH remaining rather constant at 6.7 after this period. The dissolved oxygen concentration was also monitored and, following a slight decrease during the first 4 h of lead removal, this also remained constant after this time (data not shown).

One more interesting point regarding this medium was that the BCF increased to 2,065 ± 35, and it was significantly higher ($P < 0.05$) than those observed in other media containing phosphates (Table 2).

Discussion

In experiments with *S. minima* in the absence of nutrients, very rapid removal of lead was observed during the first 4 h of exposure, regardless of the LIT conditions applied. Furthermore, 90% lead removal was achieved after 10 h exposure to an initial Pb(II) concentration of 11.8 ± 0.34 at the "medium" LIT tested, and 90% at both "medium" and "high" LIT after 24 h. These results are in agreement with those already reported by Olguín and coworkers [20] for lead and cadmium removal with *S. minima* in the absence of nutrients, under two very different experimental conditions (outdoor and indoor). They also confirm that lead removal from solution by *S. minima* is a lot more efficient than that achieved using Lemnaceaes. It has been reported that *L. minor* removed 90% of the initial lead concentration (5.0 mg/L) dissolved in an aqueous solution containing minor quantities of fertilizer, but after 3 weeks of exposure, with only 25% after 24 h of exposure [21].

Compartmentalization analysis showed that surface adsorption was the predominant mechanism of lead removal for all the environmental conditions tested in the absence of nutrients, reaching levels in the range of 51–58%, regardless of LIT. The work of Noraho and Gaur [19] on the removal of cadmium from an aqueous solution with *A. pinnata* and *S. polyrhiza*, also showed that surface adsorption was the predominant mechanism, reaching levels of 71–86%. Intracellular accumulation of lead in *S. minima* was the second most important mechanism, and was significantly higher at the highest LIT tested in the absence of nutrients (Fig. 2b). Thus, it seems likely that temperature, more than light intensity, is responsible for this effect, since previous reports have shown that higher temperatures increase the accumulation of lead in different plant species [5].

The BCFs found in this work for *S. minima* in a nutrient-free solution were in the range of 2,113 ± 229 to 2,606 ± 202. These values are similar to those we reported earlier for *S. minima* [14, 20]. Furthermore, it is important to mention that these values remained high, considering that the initial lead concentrations used in this work are approximately four times higher than those tested earlier (an average of 11.4 ± 0.4, versus an average of 3.5 mg/L).

Regarding the effect of some nutrients or compounds present in nutrient media, the effect of EDTA was relevant. When this chelant was added as a component of Hutner synthetic medium, it was very clear that its presence inhibited the lead removal process, at "high" initial metal concentrations (i.e., 12 mg/L) regardless of LIT. However, the presence of EDTA did not inhibit Pb(II) removal when the metal was at "low" initial concentrations (i.e., 2 mg/L). It has previously been recognized that addition of chelating agents to nutrient solutions usually decreases metal accumulation and phytotoxicity [24]. In contrast, addition of EDTA to Pb-contaminated soils increased the uptake and transport of lead-EDTA-chelate into stem and leaves by 100-fold [26].

Removal of lead by *S. minima* was faster during the first 4 h of exposure in EDTA-free medium, and in medium without EDTA supplemented with SWW, at "low" LIT compared to the trend observed at "high" LIT. The predominant mechanism of lead removal from these two nutritive media was precipitation, regardless of the environmental conditions. Thus, it seems likely that the presence of phosphates in both media was responsible for the occurrence of a large precipitation fraction. A similar situation has been reported previously [16], in which 60.4–82.6% of the Zn and 49.1–60.8% of the Cu were removed by precipitation as zinc phosphate and copper phosphate, respectively, in the microcosm of three aquatic plants (*Myriophylhum aquaticum*, *Ludwigina palustris* and *Mentha aquatic*).

When EDTA and phosphates were removed from the Hutner medium, a high percentage of lead removal (86%) by *S. minima* after 4 h of exposure to 12.9 ± 0.6 mg Pb/L was observed. In this case, the predominant mechanism was surface adsorption, and the BCF was 2,065 ± 35. This BCF value is similar to those found in the absence of nutrients (an average of 2,431 ± 276), confirming that phosphates were responsible for the predominance of precipitation when present in Hutner medium. It is worth noting that such BCF values are a lot higher than those previously reported for Lemnaceaes. The BCFs for *L. minor* and *A. pinnata* were 138 and 140, respectively, for an initial lead concentration of 4 mg/L [14]. In another work [30], a BCF of 60 was reported for "duckweed" exposed to an initial lead concentration of 1.0 mg/L. In relation to terrestrial higher plants, a BCF of 563 was reported for *Brassica juncea*, cultivated hydroponically and suspended in deionized water containing 2 mg/L lead during a so-called "rhizofiltration" treatment [10].

On the other hand, EDTA was found to inhibit the lead removal capability of *S. minima* when the whole plant was rinsed with an EDTA solution before being exposed to lead at a "low" initial concentration (2.6 mg/L). In contrast to this behavior, lead removal reached a maximum of 96.6% (after 8 h of exposure to a concentration of 1.4 ± 0.02 mg Pb/L), when the plants were rinsed only with tap water before being exposed to lead. This behavior suggests a blockage action of this strong cation chelant at the level of the plant surface. Furthermore, it may be possible that such blockage of lead uptake could be located at a specific membrane receptor site. It has been postulated that uptake of heavy metals takes place through secondary transporters such as channel proteins or H^+-coupled carrier proteins where negative membrane potential inside the plasma membrane drives the uptake of cations through secondary transporters [6]. Thus, it is postulated here that lead is absorbed into the cells of *S. minima* through the secondary transporters known as calcium channels. There are several arguments in favor of this hypothesis. In animal cells, lead enters through calcium channels, acting as an antagonist [3]. In plants, it has recently been reported that the first plant voltage-dependent Ca^{2+} channel, AtTPC1 (two-pore channel 1) of *Arabidopsis thaliana*, is the Al-sensitive ROS-responsive channel in plants, and that Al may be a novel channel blocker specific for this type of channel [17]. Further work is required in order to validate our hypothesis and provide evidence that lead enters the cells of *S. minima* through calcium channels.

Adsorption of lead onto the surface of the pseudo-roots or of the inferior part of the fronds (the part in contact with the water column), was the predominant mechanism found in the lead removal process by *S. minima* in the absence of nutrients and in Hutner medium free of EDTA and phosphates. Concerning the more specific aspects of this type of mechanism, a second hypothesis may be formulated, taking into consideration that *S. minima* biomass rinsed with EDTA before being exposed to a lead solution showed only a very small removal capacity. It is possible that EDTA forms complexes with some ions (such as Mg^{2+}, Ca^{2+} and K^{2+}) attached to ligands that are usually considered as the specific sites for adsorption of heavy metals (i.e., carboxylic groups). In this case, an ionic exchange mechanism could be responsible for lead adsorption. Further work is required to provide evidence of the occurrence of such an adsorption mechanism for the removal of Pb(II) by *S. minima*. However, it is likely to occur as there is direct or indirect evidence of its occurrence in the removal of other ions by plants and microalgae. Studies on the non viable biomass of *Potamogeton lucens* presented evidence that, for each Cu(II) ion sorbed onto the surface of this macrophyte, an equivalent of protons and/or other metal ions appeared in solution [22]. In the case of sorption of Cu(II), Ni(II) and Zn(II) onto *P. lucens*, *Salvinia herzogii* and *Eichhornia crassipes*, the data could be fitted to a Langmuir isotherm [22]. In the case of microalgae, early work performed by Darnall and coworkers [7] indicated that the algal cell wall can reversibly biosorb metals, functioning in a similar way to an ion-exchange resin. Thus, it is generally accepted that the biosorption mechanism can be considered as being dependent on the composition of the algal cell wall [12]. In fact, work done by our research group [13] showed that the chemical composition of *Spirulina* (*Arthrospira*) significantly affected the removal of Pb(II), Cd(II) and Cr(VI). The maximum adsorption capacities ($q_{\max}$) for Pb and Cd were highest (172.4 and 54.05 mg/g cells at pH 5.0 and 4.5, respectively) when cells exhibited higher polysaccharide content. It has also been observed in our research group (unpublished results) that an ion-exchange mechanism operates in the removal of Pb(II) by *Spirulina* enriched in polysaccharides. In addition, extensive work on this subject by the group of Volesky [8] has concluded that ion exchange is the dominant mechanism in brown algae.

Overall, we conclude that the mechanisms of lead removal by *S. minima* and the compartmentalization of this metal in the microcosm of batch-operated lagoons are primarily a function of the presence of certain nutrients and chelants and secondarily a function of environmental conditions. Surface adsorption was the predominant mechanism for all the environmental conditions tested in the absence of nutrients and in nutrient medium (modified Hutner 1/10 medium) free of EDTA and phosphates. In contrast, precipitation of lead into the sediments was the predominant lead removal mechanism in the presence of EDTA and phosphates. The second most important mechanism of lead removal in the absence of nutrients was intracellular accumulation. The BCF values were less than 40% when using EDTA-free Hutner medium, or EDTA-free Hutner medium supplemented with SWW, compared to those observed in the absence of nutrients, regardless of the light intensity. However, the BCF found in Hutner medium free of EDTA and phosphates was similar to those found in the absence of nutrients, indicating that phosphates have a high level of impact on the removal of lead. Finally, the work presented here provides evidence of the importance of additional understanding of the predominant mechanism of metal removal (surface adsorption, intracellular accumulation or precipitation into sediments) and the distribution of the toxic compounds within the different compartments of the experimental microcosm (plant biomass, the water column, or the sediment). There were significant differences between the fate of lead among the various compartments, even if the overall percentage of lead removal was similar for various experimental conditions, indicating that the latter is only a gross parameter. Thus, the kinetics of lead removal (lead removal percentage vs time) and the value of the BCF, should be complemented by additional analysis of compartmentalization, to gain full insight into the process of heavy metal removal by plants.

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