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THEMATIC ISSUE

Bioaccumulation of heavy metals in *Limnobium laevigatum* and *Ludwigia peploides*: their phytoremediation potential in water contaminated with heavy metals

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Abstract
Contamination with heavy metals in surface and groundwater is a threat to human health and ecosystems. Due to this, the need arises to remediate water polluted through ecological and profitable technologies, such as phytoremediation. The objective of the work was to evaluate the concentration of lead (Pb) and zinc (Zn) in the floating macrophytes *Limnobium laevigatum* and *Ludwigia peploides*, after being exposed to contaminated water experimentally. In this way to be able to determine if these plants have mechanisms that allow them to accumulate the metals in the roots and to perform the translocation of these to different vegetative organs, *L. laevigatum* and *L. peploides* were placed in solutions contaminated with Pb ($[\mathrm{Pb}] = 5$ mg/l) and Zn ($[\mathrm{Zn}] = 20$ mg/l). The concentrations of metals in water, root and leaf samples were evaluated as a function of time (0, 1, 2 and 4 days). The determination of the metals was performed by the atomic absorption spectrophotometry technique. After 4 days of exposure to Pb and Zn, the plants showed high metal removal efficiencies of water, more to 70% in all cases. Pb was accumulated fundamentally by roots, while Zn was accumulated more in the leaves. In addition, the bioconcentration and translocation factors for each metal were calculated.

Keywords Water pollution · Heavy metals · Macrophytes · Phytoremediation

Introduction

The contamination of the aquatic environment with trace metal has attracted considerable public attention during the last decades (Peng et al. 2008; Mishra and Tripathi 2008). Anthropogenic sources can contribute significantly to environmental pollution problems, mainly due to poor management of industrial discharges and their inefficient treatment. When released into the environment, heavy metals can reach surface and underground watercourses through different transport mechanisms. Once there, these can be deposited in sediments, or be absorbed and adsorbed by plants and animals. Unlike organic pollutants, the heavy metals are essentially nonbiodegradable. Consequently, these elements accumulate in the body tissues of living organisms and their concentrations increase as they pass from lower trophic levels to higher trophic levels (Ali et al. 2013). Aquatic ecosystems are directly or indirectly end destinations of these elements, and they often present high pollutant concentrations that may be deleterious for organisms therein (Megateli et al. 2009).

It is important to develop and use methods to remove heavy metals in effluents before dumping them into surface waters. There are several methods to remove heavy metals from water: ion exchange, reverse osmosis, electrolysis, precipitation and adsorption. However, these methods can be very expensive, especially for large volumes, low metal concentration and high levels of cleanliness required (Juned and Ahmaruzzaman 2016; Gunatilake 2015; Nilanjana et al. 2008; Miretzky et al. 2004). Due to this, the search for ecological and profitable alternatives for the elimination of toxic metals has increased. In this direction, the accumulation capacity of aquatic plants is a particularly interesting feature, which allows them to be used in phytoremediation processes.

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Phytoremediation basically refers to the use of plants to reduce, remove, degrade, or immobilize harmful chemicals (inorganic and organic). This can reduce risk from contaminated soil, sludges, sediments, and water through contaminant removal, degradation, or containment (Zavoda et al. 2001; Kamal et al. 2004). Green plants have an enormous ability to uptake pollutants from the environment and achieve their detoxification by various mechanisms, such as Phytoaccumulation, Phytostabilization, Phytodegradation, Phytotransformation, Phytovolatilization and Rhizofiltration (Sinha et al. 2007; Ali et al. 2013). Phytotechnologies are an effective and valid alternatives to remediate contaminated water bodies, not only under experimental conditions but also under natural conditions. Phytoremediation offers a cost-effective, non-intrusive and safe alternative respect to conventional cleanup techniques. Aquatic phytoremediation systems have been perfected, diversified, increasingly accepted and used to reduce water pollution (Vithanage et al. 2011; Veselý et al. 2011; Tangahu et al. 2011; Kamal et al. 2004; Rezania et al. 2016; Radzali et al. 2015).

Tandil is a town located in the southeast of Buenos Aires Province, Argentina (37°19′00″S, 59°08′00″W). The industrial activity is mainly based on food production (including slaughterhouses) and manufacture of metal products (metalworking, turnery, foundry, and manufacture of heaters and radiators). There are several streams that cross the city, but the Langueyú Stream receives elimination of industries, being especially contaminated with different compounds (organic waste, hydrocarbons, phenols, heavy metals) (Albornoz et al. 2016; Banda Noriega and Díaz 2010). It is important to determine if local plants can accumulate metals in aboveground and belowground tissues to assess if they could be used in phytoremediation processes in the region.

The objective of the present study is to evaluate the accumulation of Pb and Zn in two floating macrophytes (*Limnobium laevigatum* and *Ludwigia peploides*) after being exposed to experimentally contaminated water, under natural conditions of illumination and temperature, and different study times.

Materials and methods

Ex situ experiments

Two aquatic plant species were used in the present study: *L. laevigatum* (Fig. 1a) and *L. peploides* (Fig. 1b). The *L. laevigatum* is an aquatic plant with broad leaves floating on the surface, small stems and submerged roots; *L. peploides* is also a floating macrophyte, with small leaves, and large and submerged stems and roots. For the assay, approximately 300 g plants of each species were collected in the San Gabriel stream (Fig. 2). This place was considered as a control site, without contamination, due to the sparse anthropogenic activity and the low concentrations of metals found there, according to preliminary measurements (Pb concentration < 25 mg/kg and Zinc concentration < 100 mg/kg in bottom sediment). The macrophytes were taken to the laboratory and washed thoroughly with tap water to remove adhering particles. For acclimatization, the plants were placed in four plastic containers filled with tap water (two containers of 10 l for each species) and left outdoors for 7 days. The containers were kept illuminated with natural light (approximately 16–8 h light/dark) and with continuous aeration.

After acclimatization, the plants were placed in aquariums with 1 l of Pb and Zn salts solution. For each metal (Pb and Zn) and each study time (T1: 1 day, T2: 2 days and T3: 4 days), three aquariums were used, each of which contained six plants of similar biomass and height (average wet weight in container: 15 g of *L. laevigatum* and 20 g of *L. peploides*). The concentrations used were 5 mg/l Pb and 20 mg/l Zn. These concentrations were chosen based on the values found in contaminated surface and subterranean waters in the

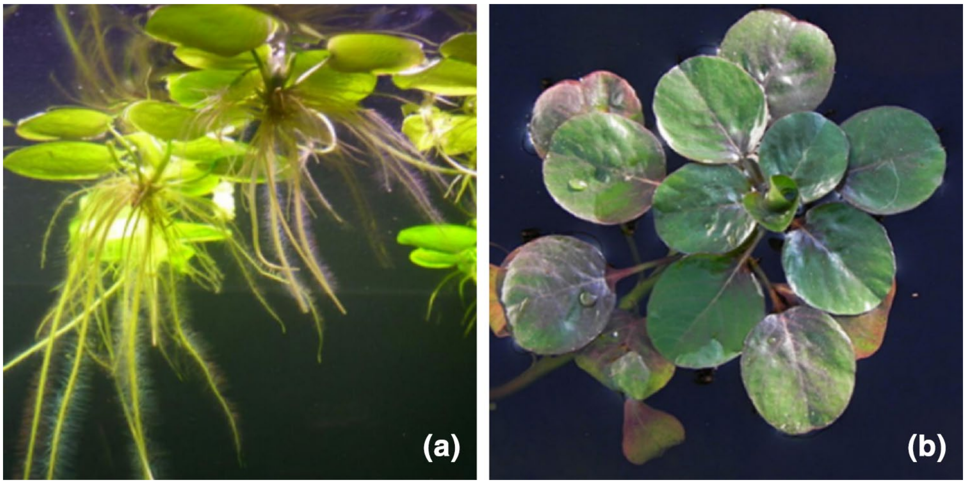

Fig. 1 **a** *Limnobium laevigatum*; **b** *Ludwigia peploides*

Fig. 2 Streams of Tandil city, Argentina (37°19′00″S, 59°08′00″W). It details the flow direction of the streams, the stream sections that cross the city of underground way and the place where the plants were collected to realize the assay (San Gabriel stream)

region (Hernandez et al. 2002), and preliminary measurements made in Langueyú stream, where Zn concentrations in sediment and water were three to four times higher than Pb. During the experimental period, the temperature ranged from 15 to 23 °C.

Sample collection and heavy metal determination

Water samples (100 ml) were taken at the beginning and the end of the study. In addition, at 0, 1, 2 and 4 days of exposure, the entire plants were removed from the solution and samples of roots and leaves of each species were separated. The stems were discarded because they are not necessary to determine of the translocation factor (TF). Plant samples (previously washed with distilled water) were dried in a heater (80 °C) up to constant weight. Subsequently, roots and leaves were homogenized. Then, 0.5 g samples were taken in triplicate and mineralized through acid digestion with a mixture of HNO_3 and $HClO_4$ (3:1) for 6 h at room temperature, and 12 h at 95 °C. Samples were centrifuged at 1200*g* for 15 min. After extracting the supernatant, the samples were diluted to 10 ml with deionized water to measure metals with the analytical instruments. Pb and Zn content in samples were determined by atomic absorption spectroscopy (AAS, GBC 906, Australia) (Dean and Rains 1975; Price 1979). Material reference was used for the calibration and quality assurance of the analytical determinations: Pb (Merck, Germany) and Zn (Biopack, Argentina). Reagent blanks were used. The detection limits were Pb 0,2 mg/l and Zn 0.04 mg/l. Recovery percentages for both metals were > 90%.

Removal of heavy metal

The removal percentage of metal is used to calculate the efficiency of phytoremediation in contaminated soil and water. It was calculated with the following algorithm (Pandey et al. 2008):

$$\%\text{Removal} = \left[(C_\mathrm{i} - C_\mathrm{f})/C_\mathrm{i}\right] \times 100, \tag{1}$$

where C_i is the initial metal concentration in the water and C_f is the final metal concentration in the water.

Bioconcentration and translocation factors

The bioconcentration factor (BCF) was calculated as the ratio of the trace element concentration in the plant tissues to the concentration of the element in the water (Kastratović et al. 2015). The BCF formula is given as follows:

$$\text{BCF} = C_\mathrm{p}/C_\mathrm{w}\ , \tag{2}$$

where BCF is the bioconcentration factor and is dimensionless; C_p represents the trace element concentration in plant tissues (mg/kg) and C_w represents the trace element concentration in the water (mg/l).

A larger ratio implies better phytoaccumulation capability. In this work, was calculated the bioconcentration factor in leaves (BCFl) and the bioconcentration factor in roots (BCFr) as the ratio between the concentration of metals in the leaves and roots (respectively), and concentration of trace elements in water.

The translocation factor (TF) was calculated dividing the metal concentrations in leaves tissues by that accumulated in the root tissues as follows (Deng et al. 2004):

$$\text{TF} = C_\mathrm{l}/C_\mathrm{r}, \tag{3}$$

where C_l is the metal concentration in the leaves tissues (mg/kg) and C_r is the metal concentration in the roots (mg/kg). A larger value of TF implies higher translocation capability.

Statistical analyses

Statistical analyses were performed using the software R commander. The results were evaluated through parametric analysis of variance (ANOVA). The variance homogeneity was checked by Levene test. Logarithmic transformations performed by the post hoc Tukey and Kruskal–Wallis analysis were applied in cases where the data were not normally distributed. The significance level was $p < 0.05$.

Results and discussion

Metal removal efficiencies

Percentages of Pb and Zn removal from the water on the fourth day of the exposure are shown in Fig. 3. *L. laevigatum* presented higher Pb removal values than *L. peploides* (96 and 71%, respectively). For Zn, *L. laevigatum* showed removal values greater than 98% and *L. peploides* greater than 94%. In both plants, the removal percentages were higher for Zn than for Pb. The values obtained were higher than 70% in all cases. Similar results have been obtained by Mishra and Tripathi (2008) in *Eichhornia crassipes* and *Pistia stratiotes* (Sagar, India), with removal percentages of heavy metals (Fe, Cu, Zn, Cd and Cr) between 77 and 95% in 12 days incubation period; Megateli et al. (2009) observed Zn removal efficiencies between 77 and 85% in *Lemna gibba* (Annaba, Algeria) after 10 days of exposure; in the case of Pb, the percent metal accumulation in *Najas indicates* (Unnao, India) at 20 mg/l was about 92% at day 4 (Singh et al. 2010), and in *Lemna minor* (Mersin, Turkey) at 10 mg/l was 69% on 7 days (Uysal and Taner 2009); Miretzky et al. (2004) observed a removal of Pb and Zn > 95% in *Spirodela intermedia* (Chascomúz, Argentina) after being exposed to 1 mg/l for 15 days.

Accumulation of heavy metals in macrophytes

The variation of the Pb concentration in roots and leaves of each studied plants vs. exposition time (days) is shown in Fig. 4a (*L. laevigatum*) and Fig. 4b (*L. peploides*). In roots of both species, there is a pronounced increase of Pb concentration during the first 24 h, and then it continues to increase but in a milder way reaching a maximum mean level at 48 h. The fast metal uptake in root suggests that adsorption in the cell walls is probably the process responsible (Hadad et al. 2011). The variation of Pb concentration in the root respect to the time not showed significant difference for *L. peploides*. However, in the roots of *L. laevigatum*, the mean concentration at the second day of exposure (2603 ± 115 mg/kg) was significantly different from that at the fourth day (1868 ± 143 mg/kg) ($p \ll 0.001$). A similar effect was observed in *P. stratiotes* (Veselý et al. 2011).

In leaves of the two macrophytes, the concentration of Pb increases throughout the study time, although more slowly

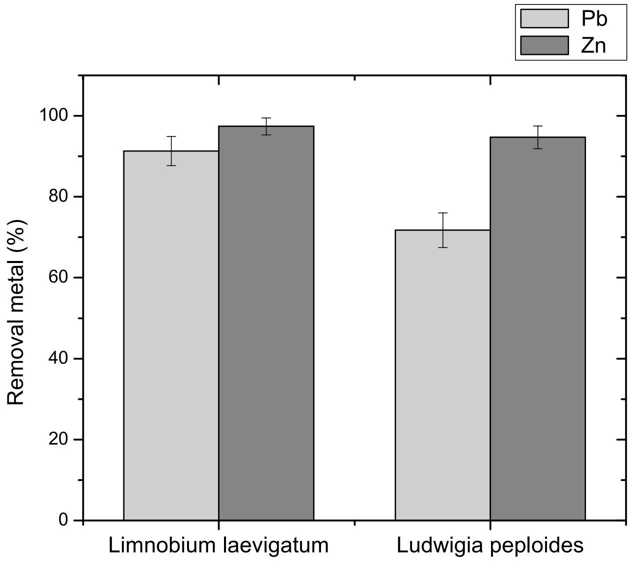

Fig. 3 The bars represent the percentage of Pb (light gray) and Zn (dark gray) removal from the water at the fourth day of the exposure of *Limnobium laevigatum* (left) and *Ludwigia peploides* (right)

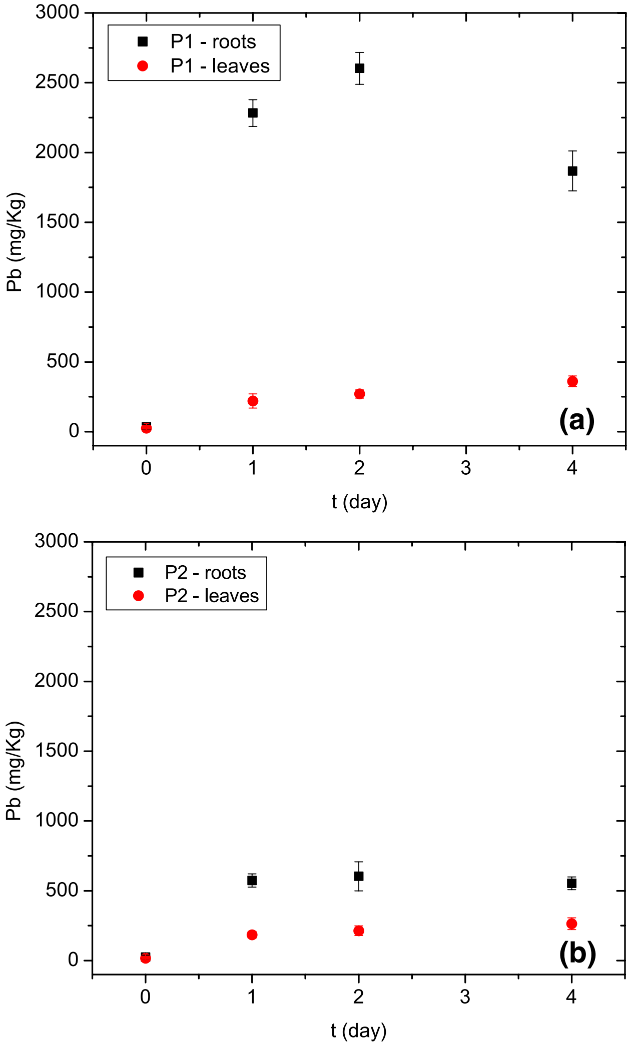

Fig. 4 **a** Concentration of Pb (mg/kg) in roots (R) and leaves (L) of *Limnobium laevigatum* as a function of time (days); **b** concentration of Pb (mg/kg) in roots (R) and leaves (L) of *Ludwigia peploides* as a function of time (days)

than in roots. The final Pb mean concentrations in leaves were 361 ± 38 mg/kg in *L. laevigatum* and 264 ± 42 mg/kg in *L. peploides*.

The concentration of Pb in roots of *L. laevigatum* and *L. peploides* was between 2 and 9 times higher than the concentration in leaves. This agrees with observed in other studies (Liao and Chang 2004; Albornoz et al. 2016). In addition, in roots as in leaves of the exposed plants, the concentrations of Pb were higher than in the plants not exposed for the different study times (***$p < 0.001$). This ability to accumulate Pb was also observed in *Najas indica* (2983 ± 95 mg/kg) after being exposed to 10 mg/l Pb for 4 days (Singh et al. 2010); another study showed that *Salvinia auriculata* and *Salvinia minima* accumulated high concentrations of Pb (7061 ± 1853 and 4491 ± 825 mg/kg, respectively) after being exposed to 5 mg/l for 28 days (Veselý et al. 2011).

The concentrations of Zn in roots and leaves of the two macrophytes studied are shown in Fig. 5a (*L. laevigatum*) and Fig. 4b (*L. peploides*). In *L. laevigatum* roots, the concentration of Zn increases from 145 ± 37 to 612 ± 58 mg/kg in the first 24 h of exposure, and at the fourth day it decreases to 203 ± 29 mg/Kg. In contrast, in the roots of *L. peploides*, the Zn concentration increases from 204 ± 46 mg/kg to a final concentration of 940 ± 119 mg/kg. In leaves, on the other hand, the concentration of Zn after a day of exposition did not present a significant variation with respect to the initial values. That is, the significant increase occurs after 24 h of exposure, and continues to rise continuously in both plants, reaching values of 1402 ± 69 mg/kg in *L. laevigatum* and 739 ± 55 mg/kg in *L. peploides* at the fourth day of exposure.

The Zn concentrations found in leaves of *L. laevigatum* and *L. peploides* were similar to those observed by Mishra and Tripathi (2008) in the *P. stratiotes, Spirodela polyrrhiza* and *E. crassipes*, after having been exposed to a solution of 5 mg/l Zn for 15 days (980, 1500 and 5520 mg/kg, respectively); also high concentrations were observed in *Spirodela intermedia* (479 mg/kg), after 15 days with a Zn concentration of 4 mg/l (Miretzky et al. 2004).

Moreover, at the fourth day of the study, the concentrations of Zn in the roots were similar or lower than in the leaves. This coincides with that observed in *Lemna minor* and in *Potamogeton pectinatus* (Kastratovic et al. 2015; Demirezen and Aksoy 2004). However it is not always so: Cheng et al. (2002) found that *Cyperus alternifolius* roots accumulated about 8–70 times more Zn than did the leaves; in *Acorus gramineus*, Zn concentration was four or more times higher in root than in leaves (Soda et al. 2012).

Bioconcentration and translocation factors

During the period of 4 days, the plants absorbed metals from water. Thus, Pb and Zn concentration in water decreased at the end of study time. Two indicators were calculated to evaluate the efficiency of phytoremediation with *L. laevigatum* and *L. peploides*: the bioaccumulation factor (BCF) and translocation factor (TF). The bioaccumulation factor (in different plant organs) and the translocation factor can be useful tools to choose between different phytoremediation techniques.

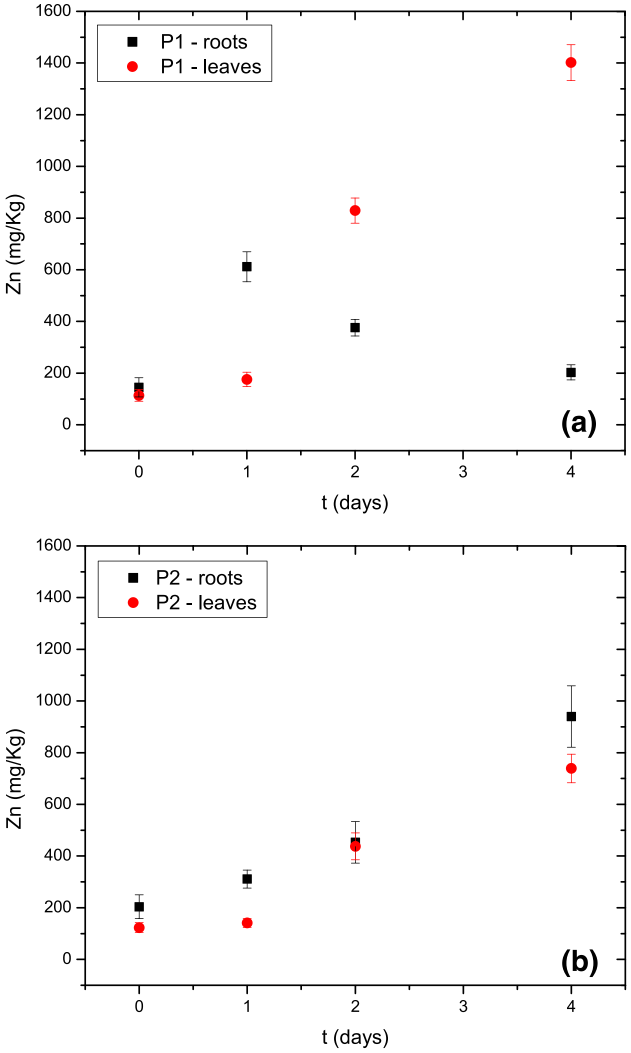

Fig. 5 **a** Concentration of Zn (mg/kg) in roots (R) and leaves (L) of *Limnobium laevigatum* as a function of time (days); **b** concentration of Zn (mg/kg) in roots (R) and leaves (L) of *Ludwigia peploides* as a function of time (days)

The BCF measures the ability of a plant to bioconcentrate an element in its tissues taking into account the concentration of that element in the water (environment). A plant is considered a good accumulator of metals when it possesses the ability to bioconcentrate the element in its tissues. According to Zhu et al. (1999), the plant is a good accumulator of metals when BCF is greater than 1000. Therefore, in this work, the BCF in roots (BCFr) and leaves (BCFl) was considered for to evaluate the capacity of *L. laevigatum* and *L. peploides* as phytoremediators of Pb and Zn.

Table 1 shows the bioconcentration values in roots and leaves of Pb and Zn resulting after 4 days of exposure. *L. laevigatum* obtained high BCF of Pb, being greater than 1000 in roots and close to this value in leaves, reason why according to the criterion mentioned before, it could be considered a good accumulator of the metal. In contrast, *L. peploides* obtained values of BCF < 1000, both in roots and leaves. Similar results were observed in *Najas indica* by Singh et al. (2010).

Regarding the Zn, *L. laevigatum* presented a value of BCF greater than 1000 only in leaves. *L. peploides* obtained high BCF values; however, these values were lower than 1000 in both roots and leaves (940 and 739, respectively). BCF values of Zn greater than 1000 have also been observed in the following species: *Ceratophyllum demersum* (Umebese and Motajo 2008), *E. crassipes* (Hammad 2011) and *Acorus gramineus* (Soda et al. 2012).

The translocation factor is the ratio between the concentration of trace element accumulated in leaves' tissues and that accumulated in roots' tissues. A higher ratio implies a greater translocation capacity from the roots to the leaves. TF values greater than 1 means that the plant has important translocation mechanisms of the metal towards the leaves (Albornoz et al. 2016; Soda et al. 2012). Stoltz and Greger (2002) classified plant species with TF < 1 as 'shoot metal excluders' that are suitable for phytostabilization of metal contaminated soils. Some metals are accumulated in roots, probably due to some physiological barriers against metal transport to the aerial parts, while others are easily transported in plants (Lu et al. 2004). However, it should be considered that in the floating macrophytes, in addition of the translocation of the root, the leaves can also absorb metals from the water (Kastratovic et al. 2015).

The TF of the plants studied in this work are also presented in Table 1. The TF values of Pb were low for the two macrophytes. This is because both plants accumulated Pb mainly in the roots, being probably excluding of this metal. Similar results have been obtained by Albornoz et al. (2016) in *Festuca arundinacea*.

Table 1 Bioaccumulation factor in roots (BCFr) and leaves (BCFl), and translocation factor (TF) in *Limnobium laevigatum* and *Ludwigia peploides*

	BCFr	BCFl	TF
	Limnobium laevigatum		
Pb	4670	903	0,2
Zn	677	4673	6,9
	Ludwigia peploides		
Pb	425	203	0,5
Zn	940	739	0,8

Limnobium laevigatum presented a TF value of Zn greater than 6, which would indicate that it has mechanisms to translocate the metal to the leaves. *L. peploides*, presented a FT lower to 1 but close to this value. According to this, the translocation from roots to leaves in these plants is more effective for the Zn than for the Pb. This may be because the mobility of Zn is usually larger than for other metals (Barry and Clark 1978; Kähkönen et al. 1997). An example is shown by Hammad (2011) in *E. crassipes*, where Zn accumulation was highest than for Cu and Ni, although the initial concentrations of Cu and Ni exceeded Zn in the water environment. This could be due to the metal needed for metabolism can be more easily absorbed by the surrounding environment and transported to the green parts of plants (Kastratovic et al. 2015; LASAT 2000). Zn is an essential micronutrient for plants, however, Pb is not an essential metal, and therefore is usually attached to the roots and is translocated to the outbreak only to a lesser extent (Fritioff and Greger 2003).

Conclusions

Limnobium laevigatum and *L. peploides* were tested in the present study of bioaccumulation of heavy metals. These aquatic macrophytes were exposed to heavy metals for 4 days, and the end of the experiment, both suggested good potential to remove Pb and Zn from water.

In the two plants studied, the Pb concentration occurred mainly in the roots, reaching the maximum concentrations at second day of exposure and showed a decrease towards fourth day, more pronounced in *L. laevigatum*. For Zn, the accumulation was important in roots of *L. peploides* and leaves of the both plants. Although the plants could accumulate high concentrations of Pb and Zn in their tissues, the values of BFC and TF were important for Zn. With respect to Pb, the *L. Laevigatum* was better accumulator than the *L. peploides*.

The absorption process was different for two macrophytes. However, both had high percentages of metals removal in a short period of time. Because of these reasons, both plants could be efficient for the removal of metals in aquatic systems.

Limnobium Laevigatum and *L. peploides* showed an important ability to accumulate metals. Both are representative plants of the region and are strongly adapted to climatic conditions. However, Pb concentration in roots decreased towards the end of the study time. Because of this, it is necessary to perform new assays with longer exposure times to analyze the long-term metal compartment, and confirm the potential of these plants to be applied in phytoremediation technologies in the city.

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