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Tolerance and hyperaccumulation of a mixture of heavy metals (Cu, Pb, Hg, and Zn) by four aquatic macrophytes

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ABSTRACT
In the present investigation, four macrophytes, namely Typha latifolia (L.), Lemna minor (L.), Eichhornia crassipes (Mart.) Solms-Laubach, and Myriophyllum aquaticum (Vell.) Verdc, were evaluated for their heavy metal (Cu, Pb, Hg, and Zn) hyperaccumulation potential under laboratory conditions. Tolerance analyses were performed for 7 days of exposure at five different treatments of the metals mixture (Cu^{+2}, Hg^{+2}, Pb^{+2}, and Zn^{+2}). The production of chlorophyll and carotenoids was determined at the end of each treatment. L. minor revealed to be sensitive, because it did not survive in all the tested concentrations after 72 hours of exposure. E. crassipes and M. aquaticum displayed the highest tolerance to the metals mixture. For the most tolerant species of aquatic macrophytes, The removal kinetics of E. crassipes and M. aquaticum was carried out, using the following mixture of metals: Cu (0.5 mg/L) and Hg, Pb, and Zn 0.25 mg/L. The obtained results revealed that E. crassipes can remove 99.80% of Cu, 97.88% of Pb, 99.53% of Hg, and 94.37% of Zn. M. aquaticum withdraws 95.2% of Cu, 94.28% of Pb, 99.19% of Hg, and 91.91% of Zn. The obtained results suggest that these two species of macrophytes could be used for the phytoremediation of this mixture of heavy metals from the polluted water bodies.

KEYWORDS
mixture of heavy metals; bioaccumulation; macrophytes

Introduction

Among the main water pollutants, heavy metals are dangerous because they are persistent and have the tendency to accumulate in sediments and in the tissues of living organisms. Metals such as Hg, Cd, Ni, Pb, Cu, Zn, and Cr are highly toxic either in its elemental form or as soluble. In addition, their presence in the atmosphere, soil, and water, even in trace, could cause serious health problems to animals and plants. (Jadia and Fulekar 2009). Heavy metals can form complexes with organic matter present in water and sediments, which increases their toxic effects on fish and crustaceans (Márquez *et al.* 2008). Also, it has been reported that through the trophic networks, the heavy metals tend to bioaccumulate and biomagnify (Rajeshkumar and Munuswamy 2011). In areas with high anthropogenic pressure, it has been found that heavy metals such as Cd, Cu, Pb, Cr, Zn, and Ni, at levels that exceed the maximum permissible limits, could cause serious problems to the organisms (Ali *et al.* 2013).

Concentrations of heavy metals in the environment increase with time, *e.g.*, the Belgian and Dutch countrysides that cover an area of 700 km^2 are diffusely contaminated with atmospheric deposition of Cd, Zn, and Pb (Meers *et al.* 2010). In China, the overexploitation of mines has caused the destruction of 2,880,000 ha of land (Xia 2004). In Mexico, the National Water Commission reported that based on the monitoring of chemical oxygen demand in 2006, 17.6% of water had acceptable quality, 11.3% of water was contaminated, and 5.4% of water was heavily contaminated. They also recognized that discharges from agriculture, deforestation, and poor waste management cause 70% of pollution in water bodies in the country (Comisión Nacional del Agua 2007).

Conventional techniques used to remove metals from wastewater include physicochemical methods such as precipitation, neutralization, ion exchange, filtration, reverse osmosis, electrochemical treatments, oxide/reduction, electrodialysis, activated carbon adsorption, and recovery by evaporation. Usually, these processes are effective in removing most of the metals from wastewater with concentrations from moderate to high. However, chemical processes produce a large amount of sludge, making the recovery of the metal difficult. Moreover, after the treatment of the effluent, the amounts of total dissolved solids are often unacceptably high. On the other hand, when these techniques are applied to highly diluted wastewater with metal ions at low concentrations, these methods are either inefficient or unprofitable. Also, these methods require highly skilled operational workers and have a lack of selectivity in the treatment process (Ali *et al.* 2013; Cañizares 2000; Guangyu and Thiruvenkatachari 2003; Guo *et al.* 2010).

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The urgent need to clean the waste water and fresh water contaminated with metals, has led to the development of more friendly alternatives using organisms to prevent or restore the damage caused by anthropogenic actions that contaminate different water bodies. In this regard, the bioremediation technologies that use both natural biological and genetically engineered systems to degrade, transform, or eliminate organic and inorganic hazardous substances in soil, water, and air have received increased importance in recent years. These technologies allow removing contaminant concentrations that are undetectable or are below the acceptable limits by the environmental regulations (Audet and Charest 2007; De Olivera 2004).

Within bioremediation, phytoremediation has been proposed as a promising, profitable, and environmentally friendly technology. In phytoremediation, the natural characteristics of plants or other species provided by genetic engineering are utilized to remove, transfer, stabilize, and degrade pollutants present in soil, sediment, and water, thereby cleaning up contaminated environments (Padmavathiamma and Li 2007).

Hyperaccumulator macrophytes are herbaceous or woody plants that can tolerate and accumulate high concentrations of heavy metals without any visible symptoms of damage. For being considered as hyperaccumulators, the plants must have the ability to accumulate of 0.1% to 1.0% of the metals concentration to which are exposed, determined in dry tissue. Plants have internal mechanisms of tolerance to metal toxicity, which makes them useful to humans as a tool in the new technologies of phytoremediation (Llugany *et al*. 2007).

Aquatic plants absorb elements through the roots and/or shoots; some species show a differential behavior in their ability to accumulate metals in the roots, stems, and leaves (Kumar *et al.* 2008). Studies in macrophytes of the genera *Eichhornia, Typha*, and *Myriophyllum*, showed accumulation of Cu and Zn in the root, whereas Pb was translocated to the leaves (Fawzy *et al.* 2012). Moreover, Arenas and collaborators (2011) determined that the macrophyte *Lemna minor* presented a mercury removal efficiency of 30% based on an initial concentration of 0.13 mg L^{-1} in 22 days of treatment. The aim of this study was to analyze the tolerance and hyperaccumulation of a mixture of metal ions (Cu^{+2}, Pb^{+2}, Hg^{+2}, and Zn^{+2}) by the aquatic macrophytes *Typha latifolia, Lemna minor, Eichhornia crassipes, and Myriophyllum aquaticum* to propose a system with selective bioaccumulation, enabling efficient bioremediation of polluted water bodies.

Materials and methods

Biological material

Macrophytes *L. minor, T. latifolia, E. crassipes*, and *M. aquaticum* were collected from the Ignacio Ramírez dam (19°27′4″N 99°48′7″W), in the municipe of Almoloya de Juárez, in the State of México. Macrophytes were collected manually; plastic containers were used to keep a certain amount of water to transport the species to the laboratory.

Cultivation of macrophytes

The macrophyte samples were first washed with running tap water followed by distilled water to remove extraneous matter. After washing, the plants were poured in plastic containers for their acclimation and cultivation, using 20 L of deionized water and 1 mL L^{-1} of Murashige y Skoog (MS) nutritional médium (Murashige and Skoog 1962). The temperature was maintained at 20 ± 2°C and pH was maintained between 6.5 and 7, in natural periods of light/dark.

Determination of tolerance to mixture of heavy metals

The experiments were carried out using eight test systems under similar conditions in which the plants were cultivated, using 3.5-L plastic containers with 1 L of solution containing 1 mL L^{-1} of nutritional medium at pH of 5.8 ± 2, and the temperature was set to 20 ± 2°C. The average biomass in each system was 76.78 ± 5 g for *E. crassipes*, 15.68 ± 3 g for *M. aquaticum*, 75.0 ± 2 g for *T. latifolia*, and 2.0 ± 0.2 g for *L. minor*. A mixture of Cu^{+2} ($CuSO_4$ $5H_2O$), Pb^{+2} ($Pb(NO_3)_2$), Hg^{+2} ($HgCl_2$), and Zn^{+2} ($ZnSO_4$ $7H_2O$) was added to the systems 1–6. The concentrations used in the experiments are presented in Table 1. The experiments 7 and 8 were used as control: experiment 7, control without metal (WM) and experiment 8, control without macrophytes (WP). Each treatment was performed for 7 days, observing the plants every day and recovering the water lost to evaporation and transpiration. All the tests were carried out in triplicate.

Determination of pigments

The production of carotenes and chlorophyll was used as an indicator of the health of plants. For a given exposed macrophyte, a sample of 0.344 g of biomass was taken, macerated with 5 mL of a 9:1 solution of ammonium nitrate and acetone at 80%, and refrigerated for 2 hours. Finally, 5 mL of 80% acetone was added to the sample. Then the sample was centrifuged at 3200 rpm for 20 minutes, and 2 mL of supernatant was taken and poured into 8 mL of 80% acetone. The solution mixture was analyzed for chlorophyll a (668 nm), chlorophyll b (640 nm), and carotenes pair (470 nm) in an UV/Vis GENESYS brand Model 10S Thermo spectrophotometer (EPA 1997). By using Equations (1)–(3), the amounts of pigments were calculated:

$$\mathrm{mg/mL\ (chlorophyll\ a)} = (12.7^{*}\mathrm{Abs}663) - (2.69^{*}\ \mathrm{Abs}\ 645) \tag{1}$$

$$\mathrm{mg\ /\ mL\ (chlorophyll\ b)} = (22.9\ ^{*}\mathrm{Abs}645) - (4.68^{*}\ \mathrm{Abs}\ 663) \tag{2}$$

$$\mu\mathrm{g\ /\ mL(carotenes)} = (3.775^{*}\mathrm{Abs}470) - (0.21^{*}\ \mathrm{chlorophyll\ b}) \tag{3}$$

Table 1. Concentrations (mg/L) of the mixtures of heavy metals at which the macrophytes were exposed.

	Treatment (mg/L)					
Metal	1	2	3	4	5	6
Cu^{2+}	0.5	2	5	10	20	100
Hg^{2+}	0.25	1	2.5	5	10	50
Pb^{2+}	0.25	1	2.5	5	10	50
Zn^{2+}	0.25	1	2.5	5	10	50

For measuring the concentration at which each macrophyte was tolerant to the mixture of metals, a one-way analysis of variance (ANOVA) with 95% confidence level was performed, followed by a multiple range test of least significant difference (LSD).

Kinetics of removal of heavy metals mixture

For the macrophytes most tolerant to the exposure to metals, the kinetics of removal of the mixture of four heavy metals was analyzed. The selected concentration for the experiments was an intermediate value between the lowest values at which the plants were tolerant (treatments 1 and 2 in Table 1). The experiments were performed under experimental conditions similar to those described for acclimation and tolerance.

The plants were exposed to concentrations of Cu—1.0 mg/L and Pb, Zn, and Hg—0.5 mg/L at different periods of time, 1, 3, 5, and 7 days of treatment, using one system as control. All the experiments were carried out in triplicate using 1 L of solution with the mixture of heavy metals, imposing a pH value between 5.8 and 6, under a temperature of 21 ± 2°C in natural periods of light/dark. The used biomass was 39.96 ± 0.15 g for *E. crassipes* and 21.89 ± 0.45 g for *M. aquaticum*. During the experiments, all the evaporated water was recovered, and the viability of the macrophytes was checked by bare eye.

Quantification of heavy metals

In the days of the determination of the kinetics, samples of water were taken in plastic bottles, which were washed, treated with nitric acid at 5%, and rinsed with deionized water. The samples were acidified with nitric acid, and preserved in cooling for later determination of heavy metals by atomic absorption spectrophotometry. On the other hand, the plant parts were separated and oven-dried at 65°C for 2 weeks. The dried plants were then smashed and powdered by an electric mill, IKA model MF 10 BS1, at 3500 rpm. 0.5 g of each powdered sample was predigested using 10 mL of nitric acid, J. T. Baker, for 24 hours. The mixture was placed in a microwave oven, Mars model Mars Xpress, for its full digestion. The final solution was filtered and graduated to 25 mL with deionized water. The determination of each metal was carried out in an atomic absorption spectrophotometer, Thermo, model S Series. Hydrides generator Thermo VP100 model was used for the determination of mercury. Calibration curves were performed using standard atomic absorption samples HYCEL grade for each of the quantified heavy metals (Cu 1, 2, 3, and 4 mg/L; Zn 0.2, 0.4, 0.8, and 1 mg/L; Pb 0.2, 0.6, 0.8, and 1 mg/L; and Hg 3, 6, 15, 30, and 60 μg/L).

Calculation of bioconcentration and translocation factors

With the obtained data from the heavy metals determination, the bioconcentration factors (BCFs) were calculated using Equation (4) proposed by Olivares and Peña (2009), in both the root and the aerial part of *E. crassipes* and *M. aquaticum*:

$$\mathrm{BCF} = C_{\mathrm{met}}\text{in vegetal tissue} \,/\, C_{\mathrm{met}}\text{from water} \qquad (4)$$

where C_{met} = metal concentration.

Translocation factors (TFs) to the aerial part of the macrophytes were calculated according to Equation (5) proposed by Zhang *et al.* (2006) and Olivares and Peña (2009):

$$\mathrm{TF} = C_{\mathrm{met}}\text{aerial part} \,/\, C_{\mathrm{met}}\text{root} \qquad (5)$$

where C_{met} = metal concentration.

Values of BCFs and TFs show whether macrophytes are tolerant (BCF and TF of 0.1–1), accumulator (BCF>1 and TF>1), or hyperaccumulator, if in addition the concentrations of metals in the plant exceed 0.1% by weight of dry plant (Baker and Brooks 1989).

Results and discussion

Determination of tolerance to the mixture of heavy metals

The concentration–response relation showed that for the highest concentrations (Table 1, treatment 6), macrophytes died after 24 hours of being exposed to the mixture of metals. In treatment 5, macrophytes survived until day 4, except *L. minor* that turned out to be the most sensitive species and no longer survived to any of the concentrations of metal mixture after 72 hours of exposure. For this reason, this species was excluded as a candidate in the treatment of the particular mixture of heavy metals studied in this work. However, some authors have found that *L. minor* is tolerant to high concentrations of heavy metals, *e.g.*, Armendariz *et al.* (2008) reported that *L. minor* can tolerate up to 100 mg/L of Cu^{+2}. On the other hand, *L. minor* has demonstrated to be an alternative for removing concentrations of 0.13 mg/L of Hg from water in 22 days of treatment, with an efficiency of 30% (Arenas *et al.* 2011).

Basile *et al.* (2012) determined that *L. minor* can be subjected to treatment for 7 days in the presence of Zn 10^{-4} M and can accumulate up to 58,800 μg/g of metal in tissue; for Pb at the same concentration (10^{-4} M), the plant can accumulate 22,533 μg/g of metal in tissue. For this reason, this macrophyte was proposed as a good candidate for remediation of wastewater. Keith *et al.* (2007) exposed *L. minor* to aqueous solutions of Cu 125, Cr 220, and As 205 mg/L in independent form and in mixtures. Their reported results showed that the plant can remove 60% of Cu, Cr, and As when they are mixed, but when Cu (60 mg/L) is the single species in solution the plant can remove up to 85% of Cu, in a period of 7 days. In the present work, the most probable reason why the macrophyte did not survive at lower concentration of heavy metals than those reported is because of the nature of the tested mixture, which can lead to synergism increasing its toxicity considerably.

T. latifolia was found to be tolerant only to the lowest tested concentration, since it presented significant differences with respect to the control plants, both in the production of chlorophyll (ANOVA $p < 0.05$) and in the chlorophylls/carotenoids ratio (ANOVA $p < 0.05$) starting in treatment 2. Some authors have shown that this plant is a good accumulator of heavy metals, *e.g.*, when *T. latifolia* was exposed to water from a stream of the mining industry with concentrations of 23.60 and 0.309 mg/L of Zn and Cd, respectively, it was able to remove 70% of Zn and 90% of

Cd after 7 days of treatment (Ruiz *et al.* 2010). Mays and Edwards (2001) showed that *T. latifolia* is an accumulator of Cd, Cu, Ni, and Zn, present in natural and artificial wetlands, where acidic water is poured from a mine.

E. crassipes showed no significant differences in the production of chlorophyll when exposed to four treatments with respect to the control experiment, but evidenced to be sensitive in chlorophylls/carotenoids ratio, obtaining significant differences (ANOVA $p < 0.05$) from the concentration of treatment 1. Kumar and Tripathi (2009) found that *E. crassipes* could survive in concentrations up to 20 mg/L Zn^{+2} for 11 days without morphological signs of toxicity.

It was observed that for *M. aquaticum*, in terms of the most sensitive biomarker, the chlorophylls/carotenoids ratio, no significant differences (ANOVA $p > 0.05$) were found in any of the treatments compared to the control. It has been reported that this macrophyte can survive in water of the river Xanaes in Cordoba, Argentina, which presents concentrations of Mn, Zn, and Fe ranging from 100 to 300 mg/L for each of the metals (Harguinteguy *et al.* 2013).

Kinetics of removal of heavy metals mixture

E. crassipes and *M. aquaticum* were tolerant to the concentrations of treatments 1 and 2, as indicated in Table 1; therefore, an intermediate concentration was considered for the experiments of removal kinetics (Cu 1.0 and Pb, Zn, and Hg 0.5 mg/L). In Figure 1a, the removal percentages per day are displayed for each of the metals in the mixture for *E. crassipes;* it can be observed that on day 5, the removal of the metals reached its maximum value. The highest percentage of removal was for copper (99.8%), followed by mercury (99.6%), lead (97.9%), and zinc (94.37%); for copper and zinc, on day 7, a decrease in removal rate was observed, suggesting that there is a desorption of the metals due to the saturation of root of the plants. For *M. aquaticum*, in Figure 1b, it is noticeable that the highest percentage of removal of metals is reached on day 7. Mercury (98.2%) has the highest efficiency; followed by copper (95.2%), lead (94.3%), and zinc (86.5%); for the latter metal, on day 5, 91.9% of removal is observed. The removal percentages were calculated based on the obtained concentration of metals in the control system without plants, after 7 days of observation under the same experimental conditions, in order to include the amount of metals eliminated for reasons unrelated to the treatment.

In both the macrophytes, the removal of copper occurs from 24 hours to be in contact with the solution. Moreover, it is noticeable that E. crassipes has a better capacity to remove the metal because it has a root more abundant with higher contact surface that M. aquaticum. In this work, the percentage of removal of zinc for *E. crassipes* was better than that reported by Hadad *et al.* (2011); in that study, the macrophyte was treated with zinc solution at a concentration of 1 mg/L obtaining 70% of removal. Both the macrophytes were efficient in removing the mixture of metals especially for mercury, similar to the results reported by Kamal *et al.* (2004), in the treatment of solution mercury with concentration of 0.5 mg/L by *M. aquaticum*, obtaining a 99.8% removal of metal after 21 days of treatment.

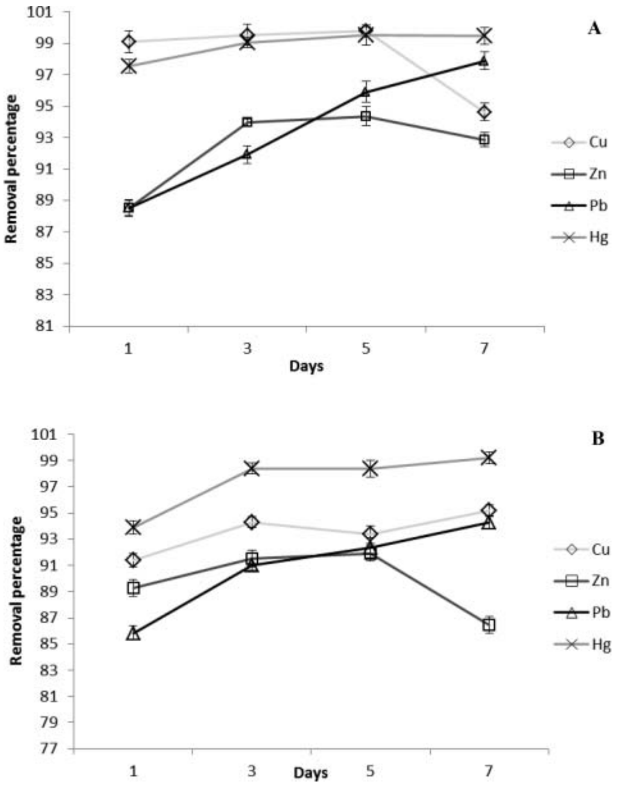

Figure 1. Average percentage of removal of metals from the mixture in water for *Eichhornia crassipes* (Mart.) Solms-Laubach (a) and *Myriophyllum aquaticum* (Vell.) Verdc (b) at different days. The bars indicate the standard error.

Translocation

The obtained results in the determination of the mobility of metals in plants were consistent with those of the analysis in the removal kinetics. As shown in Figures 2a, b and 3a, b, the control plants contained a significant amount of copper and zinc (3.02–178.6 and 0.6–1435 mg/kg in dry tissue weight, respectively), since they are essential elements for growth mainly the root. *E. crassipes* presented the highest concentration of metals compared with *M. aquaticum*.

The heavy metals, lead and mercury, were found as a source of pollution in the control plants, to concentrations of 1.2 to 18.15 and 1.78 to 3.64 mg/kg in dry tissue weight, respectively. Lead was mainly found in the root of *M. aquaticum*, and mercury was distributed in homogeneous form in the whole plant of *E. crassipes*. These concentrations were taken into account during the calculation of the TFs.

In both the macrophytes, the mixture of metals is mainly accumulated in the root. In *E. crassipes*, the concentrations of all the metals were higher in roots than in the aerial part, obtaining superior TFs for mercury and zinc as shown in Table 2. For *M. aquaticum* also, high levels of copper, lead, and mercury were found in the root, but in the case of zinc, on days 1 and 7, the concentrations were greater in root, showing an opposite behavior on days 3 and 5 during which the metal was more concentrated in the aerial part. TFs show that for *M. aquaticum* translocates greater amounts of copper and lead but lower for mercury and zinc, and it is also noticeable that *E. crassipes* translocate less amounts of all the metals.

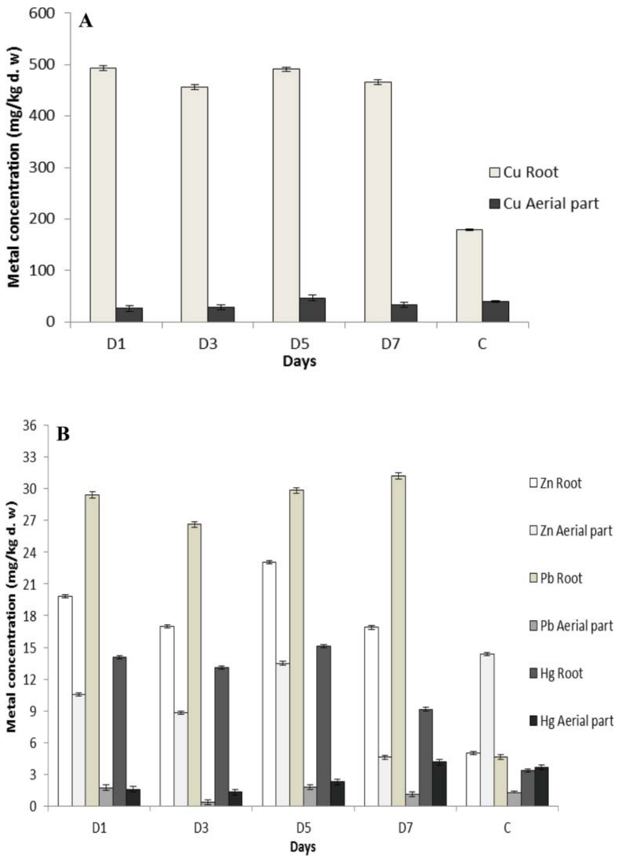

Figure 2. Concentration of Cu (a) and Zn, Pb, and Hg (b) in *Eichhornia crassipes* (Mart.) Solms-Laubach biomass over time. The bars indicate the standard error.

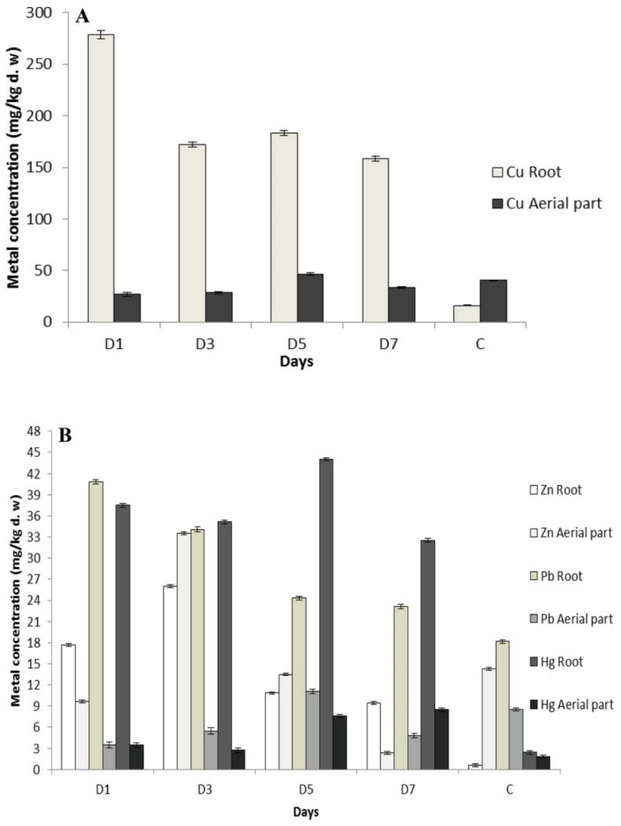

Figure 3. Concentration of Cu (a) and Zn, Pb, and Hg (b) in *Myriophyllum aquaticum* (Vell.) Verdc biomass over time. The bars indicate the standard error.

Table 2. Translocation factor from the root to the aerial part of metals in the macrophytes.

	Translocation factor	
Metal	*Eichhornia crassipes* (Mart.) Solms-Laubach	*Myriophyllum aquaticum* (Vell.) Verdc
Cu	0.051	0.299
Zn	0.272	0.253
Pb	0.034	0.208
Hg	0.455	0.260

Hyperaccumulation

In Table 3, a BCF> 1 was found for copper in both the aerial part and the roots of *E. crassipes* and *M. aquaticum* when the plants are exposed to a Cu concentration of 1.0 mg/L. In the roots, BCF values calculated for *E. crassipes* have the order of Cu > Pb > Hg > Zn, whereas in the aerial part, the obtained order was Cu > Hg > Zn > Pb. The BCF value of 0.2342 in the aerial part of *E. crassipes* for zinc is similar to that found by Hadad *et al.* (2011), *i.e.*, 0.275. However, the value found in the root (0.5552) is approximately eight times lower than that reported by the same authors (4.059). These differences may be due to the fact that they used a solution of a single heavy metal with twice a concentration as that of zinc (1.0 mg/L) compared with the solution used in this work (0.5 mg/L).

On the other hand, BCF values found for *M. aquaticum* have the order of Cu > Hg > Pb > Zn in both the root and the aerial part as shown in Table 3. The percentage of metals in the dry biomass has shown that the most retained heavy metal is copper, followed by zinc, lead and at the end mercury. Nevertheless, *E. crassipes* showed the highest capacity for retaining all the metals studied in the present work, as shown in Table 4.

To consider a macrophyte as a hyperaccumulator, it must satisfy the following conditions: BCF $\geq$ 1 and TF $\geq$ 1 for the studied metal, and also a metal percentage in dry plant tissue $\geq$ 0.1% (Baker and Brooks 1989; Llugany *et al.* 2007). The results obtained in this work showed that both the macrophytes have TFs less than 1. The values of the BCF were 18.75 (Cu) and 4.1 (Pb) and 1.0 or less for Zn and Hg with *E. crassipes* and in the case of *M. aquaticum*, 8.67 (Cu), 2.0 (Pb) and 2.61 (Hg). Similar to the percentage of the metal in dry biomass, the obtained values are lesser than the recommended ones, except for mercury in *E. crassipes*. These data suggest that the macrophytes

Table 3. BCF of the mixture of heavy metals (Cu, Zn, Pb, and Hg) in the aerial and root parts of macrophytes.

			BCF	
Metal (mg/L)	Aerial part (mg/kg)	Root (mg/kg)	Aerial part	Root
Eichhornia crassipes (Mart.) Solms-Laubach				
Cu 1.0	46.74	490.68	1.3978	17.3574
Zn 0.5	4.6003	16.9097	0.2342	0.5552
Pb 0.5	1.0914	31.2175	0.2258	4.1935
Hg 0.5	0.5834	1.2801	0.4214	0.5930
Myriophyllum aquaticum (Vell.) Verdc				
Cu 1.0	47.475	158.45	1.1897	7.4886
Zn 0.5	2.38	9.405	0.0347	0.2592
Pb 0.5	4.83	23.115	0.1968	1.8031
Hg 0.5	1.185	4.553	0.3175	2.3006

Table 4. Percentage of Cu, Zn, Pb, and Hg in dry tissue for macrophytes.

Metal	% of metal in dry tissue	
	Eichhornia crassipes (Mart.) Solms-Laubach	*Myriophyllum aquaticum* (Vell.) Verdc
Cu	21.458	6.778
Zn	0.672	0.387
Pb	1.009	0.919
Hg	0.058	0.188

are not hyperaccumulators for the mixture of heavy metals proposed in the present work. Nevertheless, even with the relatively low values of the factors, these species of macrophytes could be considered as viable alternatives in the treatment of water polluted with this particular mixture of heavy metals.

Conclusions

The macrophytes *L. minor* and *T. latifolia* are sensitive to the mixture of metals tested in this study, even to the lowest investigated concentration (0.5 Cu and 0.25 Zn, Pb, and Hg in mg/L). *E. crassipes* and *M. aquaticum* are tolerant to the mixture of metals at concentrations of 10 mg/L for Cu and 5 mg/L for Zn, Pb, and Hg, making these species good candidates for bioremediation of polluted water bodies that contain this particular mixture of heavy metals. E. crassipes shows higher removal capacity of heavy metals that M. aquaticum, probably for its larger surface contact. The percentage of remotion after 5 days of exposure for *E. crassipes* was 97.9% (±3.5), and for *M. aquaticum*, it was 94.9% (±3.0), starting with a concentration of 1 mg/L of Cu and 0.5 mg/L of the other metals.

The macrophytes *E. crassipes* and *M. aquaticum* exposed to the mixture of metals Cu, Zn, Pb, and Hg present values <1 for the TF. Moreover, the percentage of metals in dry tissue is lower than 0.1%; for these reasons, these macrophytes cannot be considered as hyperaccumulators.

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