

Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina)

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Abstract

Heavy metal removal from water has been approached by using different technologies. Phytotechnologies, with an increasing development during the last two decades, involve using plants for metal removal. Three autochthonous floating macrophytes, common in pampean shallow lakes (Argentina), *Pista stratiotes*, *Spirodela intermedia* and *Lemna minor* were used in laboratory experiences for the simultaneously removal of several heavy metals (Fe, Cu, Zn, Mn, Cr and Pb) resulting from anthropogenic activity, in order to simulate a naturally polluted environment. The experiences were performed for different concentrations of metals along 15 days. High metal removal percentages were obtained for the 3 species and metals. *L. minor* did not survive the conditions of the experiment. High correlation between the final water and the macrophytes metal concentration was obtained, deviations were due to PbCrO₄ precipitation. The rate of metal uptake was dependent on the metal concentration for the 3 species studied.

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1. Introduction

Heavy metals, industrial pollutants, in contrast with organic materials cannot be degraded and therefore accumulate in water, soil, bottom sediments and living organisms. Water contamination with heavy metals is a very important problem in the current world. Occurrence of toxic metals in pond, ditch and river water affect the lives of local people that depend upon these water sources for their daily requirements (Rai et al.,

2002). Consumption of such aquatic food stuff enriched with toxic metals may cause serious health hazards through food-chain magnification (Khan et al., 2000).

A number of methods are available to remove toxic metals from water: ion exchange, reverse osmosis, electrolysis, precipitation and adsorption. The latter is by far the most versatile and widely used. However, the methods present different efficiencies for different metals and they can be very expensive especially if large volumes, low metal concentration and high standards of cleaning are required. The main processes by which heavy metals are removed from aquatic environment are physical, biological and biochemical and they take place in water, biota and suspended solids. The predominance

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of one of them will depend on the composition of the system, the pH, the redox condition, and the pollutant nature.

It is a well known fact that aquatic plants accumulate metals that they take from the environment and concentrate on the trophic chains with accumulative effect (Outridge and Noller, 1991; Tremp and Kohler, 1995). The final metal concentration in plants is usually significantly larger than in the watercolumn and this fact has led investigators to be interested in the toxicity of these metals for the plants, in the plant tolerance (Ernst et al., 1992; van Steveninck et al., 1992); in the role of the aquatic plants in the biochemical cycles (Jackson et al., 1994; St-Cyr et al., 1994); in their use as biological filters for polluted waters (Brix and Schierup, 1989; Dunbabin and Bowmer, 1992; Ellis et al., 1994) and in their use as biomonitors of environment metal levels (Whitton, 1985; Phillips, 1994; Market, 1995; Whitton and Kelly, 1995; Mal et al., 2002). Some investigators have studied the metal uptake kinetics, but always in laboratory scale (Lee and Hardy, 1987; Thornton and Macklon, 1989; Brune et al., 1994). The extent of metal adsorption and its distribution in plants seems to have important consequences in the capacity and rate of metal removal, in the metal residence time and in the eventual metal release to the environment (Ellis et al., 1994).

Phytotechnologies are an effective and valid alternative for the remediation of contaminated water bodies, not only under experimental conditions but also under natural conditions. Aquatic macrophytes have been used during the last two decades for water metal removal competing with other secondary treatments, being the principal mechanism for metal uptake adsorption through roots (Denny and Wilkins, 1987). For aquatic macrophytes that possess roots but do not have a close physical association with sediments, the water is undoubtedly the principal source of elements. The uptake of trace metals through the root systems and subsequent release of metals during decomposition of plant material and transmission of these metals to organisms of higher trophic levels represent a pathway of cycling of trace metals in aquatic ecosystems.

The aquatic floating macrophytes *Pistia stratiotes* L. (water lettuce), *Spirodela intermedia* W. Koch (duckweed) and *Lemna minor* L. (duckweed) present a high growth rate and have been used for the removal of Cd, Cr and Pb from watercolumn (Nasu and Kugimoto, 1981; Sridahar, 1986; Bassi et al., 1990; Jain et al., 1990; Taraldzen and Norberg-King, 1990; Wang, 1990; Maine et al., 2001; Cardwell et al., 2002). *S. intermedia* and *L. minor* present the additional advantage of growing under varied climatic conditions. Also they diminish algal production as they compete for nutrients and extend all over the water surface, restricting the light penetration and consequently photosynthesis (Hammouda et al., 1990). The above studies refer, in general, to the

removal of only one or two metals, few of them refer to the simultaneous removal of various metals, and the synergy and antagonism between the metal removing is scarcely evaluated.

In previous studies (Miretzky et al., 1998) surficial waters of the Sistema de Encadenadas de Chascomús, pampean shallow lakes, were characterized, and high concentrations of heavy metals were observed, likely due to industrial activities in Chascomús city. In particular Fe, Zn, Mn, Cu, Pb concentrations were above the values recommended by Argentinean law for the protection of the aquatic life.

The objective of the present study is to select among the autochthonous floating macrophytes those of high potential for removing different metals simultaneously from the water column by means of laboratory experiences and to analyse the removal process rate.

2. Study area

The Salado River lower drainage basin, Chaco-Pampa plain, Argentina (34°20' to 35°40'S and 57°40' to 61°10'W), constitutes an interesting system for environmental studies as it presents essential characteristics of wetlands, hydrophytic vegetation, hydric soils and wetland hydrology. The Chaco-Pampa plain socio-economic importance derives from its major agricultural, cattle and farming development.

The region has a wet climate and a mean temperature between 13 and 16 °C. Mean annual precipitation (1961–1990) was between 850 and 950 mm. Although precipitation is high in the summer, the precipitation-evaporation balance is usually negative in this season. The region is characterized by a very gentle slope (0.3 mkm⁻¹, Sala, 1975) and extremely low morphologic potential that makes the runoff difficult during flooding periods.

Pampean lakes are shallow alkaline ecosystems, with circulation pattern corresponding to polymictic lakes. This obeys to the nearly continuous vertical mixing that promotes a high concentration of suspended particulate matter and low transparency. The water column is almost always thermally homogeneous, saturated with dissolved oxygen and with a high content of dissolved organic matter, mainly humic substances (Conzonno and Fernández, 1988), with aquatic macrophytes at different development stages. Nutrient concentrations, total phosphorus (mean value 0.250 mg l⁻¹, Conzonno and Fernández, 1987, 1988) and total nitrogen (mean value 1.56 mg l⁻¹, Conzonno and Fernández, 1995) as well as organic matter, phytoplankton biomass (chlorophyll *a* mean value 25.3 µg l⁻¹, Conzonno and Fernández, 1995), presence of aquatic macrophytes, algae blooms and fish community let to consider these ecosystems as eutrophic (Ringuet, 1962; Quirós, 1999).

Vegetation in the catchment area is mainly herbaceous, stable and cosmopolitan, denominated “pastizal” and used for cattle production (Barbagallo, 1983).

3. Materials and methods

3.1. Experimental set-up

Floating macrophytes from Chascomús area were collected, *P. stratiotes* from Lake Chascomús, *S. intermedia* from Vitel stream and *L. minor* from Lake Vitel. Macrophytes were washed with miliQ water to eliminate remains of lake sediments and were placed in 10 l plastic reactors with the corresponding lake water receiving 8 h of fluorescent light per day. The following experiences (in duplicate) were performed:

Experience I (control). *P. stratiotes* 50 g (fresh weight) in 5 l of Lake Chascomús water.

Experience II. *P. stratiotes* 50 g (fresh weight) in 5 l of Lake Chascomús water. Fe, Mn, Cu, Zn, Pb and Cr were added simultaneously so that the concentration in the plastic reactor of each metal added was 1 mg l⁻¹.

Experience III. *P. stratiotes* 50 g (fresh weight) in 5 l of Lake Chascomús water. Fe, Mn, Cu, Zn, Pb and Cr were added simultaneously so that the concentration in the plastic reactor of each metal added was 2 mg l⁻¹.

Experience IV. *P. stratiotes* 50 g (fresh weight) in 5 l of Lake Chascomús water. Fe, Mn, Cu, Zn, Pb and Cr were added simultaneously so that the concentration in the plastic reactor of each metal added was 4 mg l⁻¹.

Experience V (control). *S. intermedia* 50 g (fresh weight) in 5 l of Vitel stream water.

Experience VI. *S. intermedia* 50 g (fresh weight) in 5 l of Vitel stream water. Fe, Mn, Cu, Zn, Pb and Cr were added simultaneously so that the concentration in the plastic reactor of each metal added was 1 mg l⁻¹.

Experience VII (control). *L. minor* 50 g (fresh weight) in 5 l of Lake Vitel water.

Experience VIII. *L. minor* 50 g (fresh weight) in 5 l of Vitel stream water. Fe, Mn, Cu, Zn, Pb and Cr were added simultaneously so that the concentration in the plastic reactor of each metal added was 1 mg l⁻¹.

3.2. Data analysis

Duplicate water samples (10 ml each one) from all reactors were taken at 0, 1, 2, 4, 6, 24 h and 2, 4, 8

and 15 days and Fe, Mn, Cu, Zn, Pb and Cr concentration was determined in all of them. Also, Fe, Mn, Cu, Zn, Pb and Cr concentration was determined in Lake Chascomús, Lake Vitel and Vitel stream water used to perform the experiences.

The entire macrophytes specimens of the 3 species were thoroughly cleaned with miliQ water, dried at 60 °C to constant weight, then pulverized in a Moulinex mill and digested with H₂SO₄–H₂O₂. The content of Fe, Mn, Cu, Zn, Cr and Pb in the digested plants, before and after performing the experiments was determined.

Metal concentration was determined by ICP-emission (Perkin–Elmer, Optima 2000) according to norms APHA, 1993. Determinations were performed in duplicate being the relative error <1.0% for all of them.

Statistical analysis of data and simple regression analysis was performed by the use of Spearman correlation (Sokal and Rohlf, 1995; Zar, 1999). All data were tested for normality and homogeneity of variance by the Kolmogorov–Smirnov (Lilliefors) test and the Levene statistic, respectively ($p < 0.05$). The software used in statistical analysis was Statistica 5.1 (Statsoft® 1999).

4. Results

Heavy metal mean concentration (Pb, Cu, Cr, Zn, Fe, Mn) in Lake Chascomús, Lake Vitel and Vitel stream watercolumn is observed in Table 1. It is important to note the high Fe mean concentration value (2.84 mg l⁻¹) in Lake Chascomús.

The variation of the Fe, Zn, Mn, Cu, Cr and Pb water concentration vs. time in the reactors with *P. Stratiotes* for concentrations of added metal 1, 2 and 4 mg l⁻¹ is shown in Fig. 1(a)–(f). All metals were added simultaneously with the purpose of simulating a natural polluted environment from diverse industries. The metal removed percentages for *P. Stratiotes* when added metal concentration is 1, 2 and 4 mg l⁻¹ are detailed in Table 2. It is important to note the immediate diminish of Pb and Cr initial concentration to minimum values when the amount added is 4 mg l⁻¹ (Fig. 1(e) and (f)).

The variation of the Fe, Zn, Mn, Cu, Cr and Pb water concentration vs. time in the reactors with *S. intermedia* and *L. minor* respectively when the concentrations of added metal is 1 mg l⁻¹ is shown in Fig. 2(a), (b) Fig. 3(a) and (b). Metal removal percentages by *S. intermedia* and *L. minor* are shown in Table 3.

Table 1
Water chemical composition

	Fe (mg l ⁻¹)	Zn (mg l ⁻¹)	Mn (mg l ⁻¹)	Cu (mg l ⁻¹)	Cr (mg l ⁻¹)	Pb (mg l ⁻¹)
Vitel stream	0.214	0.007	0.036	0.089	0.014	0.069
Lake Vitel	0.245	0.023	0.021	0.032	0.014	0.032
Lake Chascomús	2.84	0.079	0.065	0.039	0.015	0.029

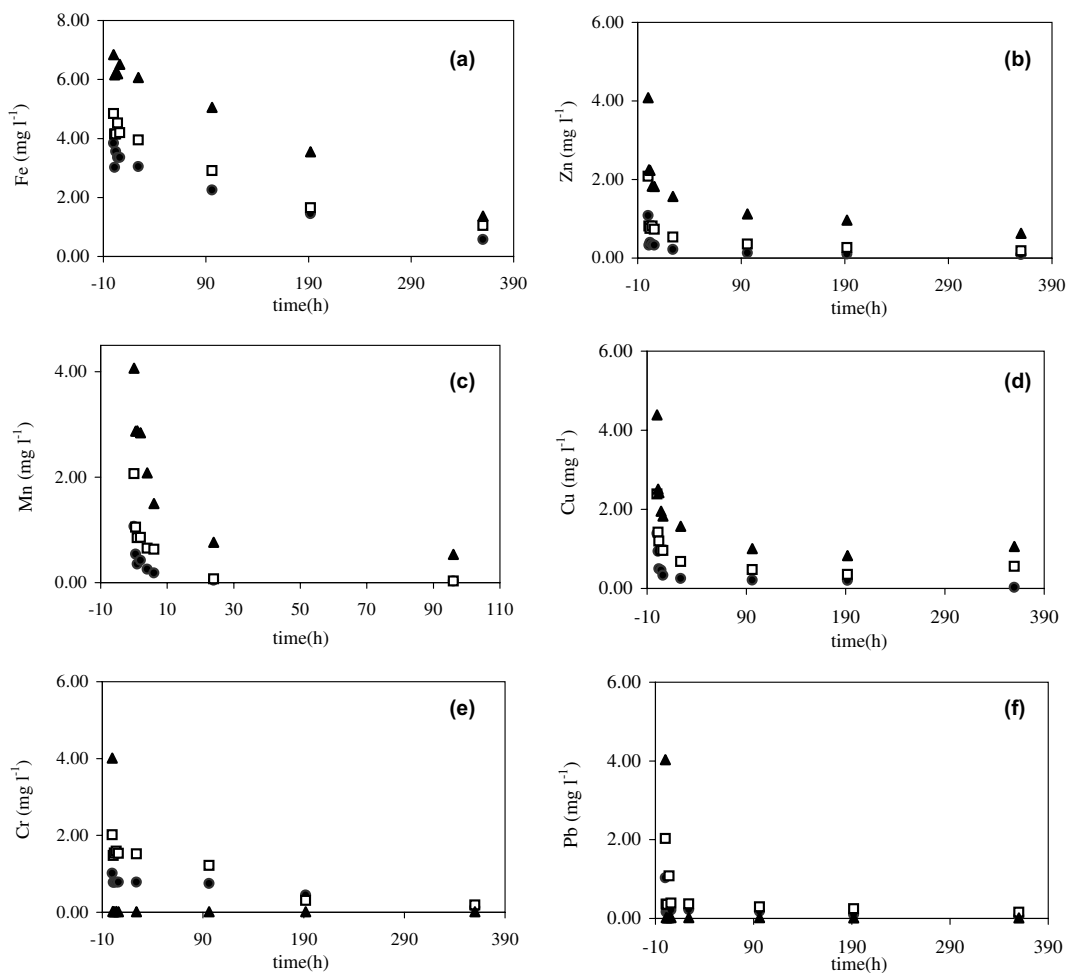


Fig. 1. *Pistia Stratiotes*. Metal concentration (mg l^{-1}) vs. time (a) Fe, (b) Zn, (c) Mn, (d) Cu, (e) Cr, (f) Pb. (\blacktriangle , 4 mg l^{-1} ; \square , 2 mg l^{-1} ; \bullet , 1 mg l^{-1} metal added.)

Table 2

Pistia stratiotes: % metal removed from water

	% Zn	% Fe	% Mn	% Cu	% Cr	% Pb
1 mg l^{-1}	91.1	85.0	97.56	97.3	87.68	94.14
2 mg l^{-1}	90.6	78.3	98.45	72.2	90.47	92.24
4 mg l^{-1}	84.3	80.0	86.81	73.5	99.63	99.74

The initial and final (after 15 days experiment) *P. Stratiotes* and *S. intermedia* metal content (mg metal/g dry macrophyte) was determined (Table 4).

Spearman's correlations were used to correlate the amount of metal removed from water to the amount of metal accumulated by *P. stratiotes* for Cu, Pb, Zn, Fe, Mn and Cr $1, 2$ and 4 mg l^{-1} concentration added. The results show a significant high correlation for metal concentration 1 and 2 metal mg l^{-1} ($r^2 = -0.98$; $p < 0.01$;

$t = -10.95$ and $r^2 = -0.93$; $p < 0.01$; $t = -5.02$, respectively). Whereas, when added metal concentration was 4 mg l^{-1} , the correlation was not significant ($p > 0.05$). When Pb and Cr were eliminated prior to the correlation analysis, the results for $1, 2$ and 4 mg l^{-1} were: $r^2 = -0.98$; $p < 0.01$; $t = -7.07$; $r^2 = -0.93$; $p < 0.05$; $t = -3.81$ and $r^2 = -0.91$; $p < 0.05$; $t = -3.25$, respectively.

Spearman's correlation for *S. intermedia* was significant only for Cu, Mn and Zn ($r^2 = -0.97$; $p < 0.05$;

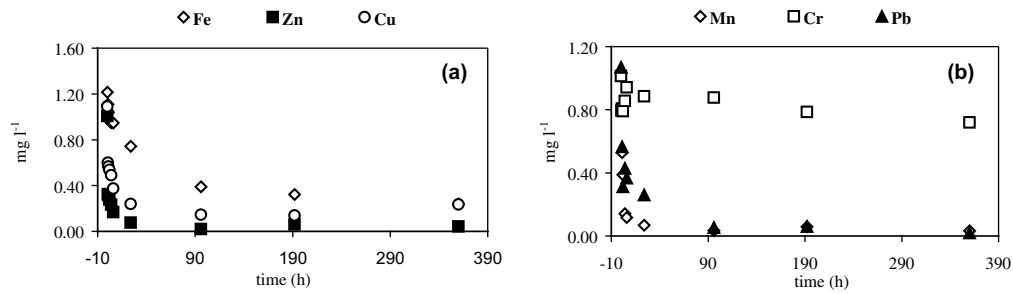


Fig. 2. *Spirodela intermedia*. Metal concentration (mg l^{-1}) vs. time (a) Fe Zn Cu, (b) Mn Cr Pb.

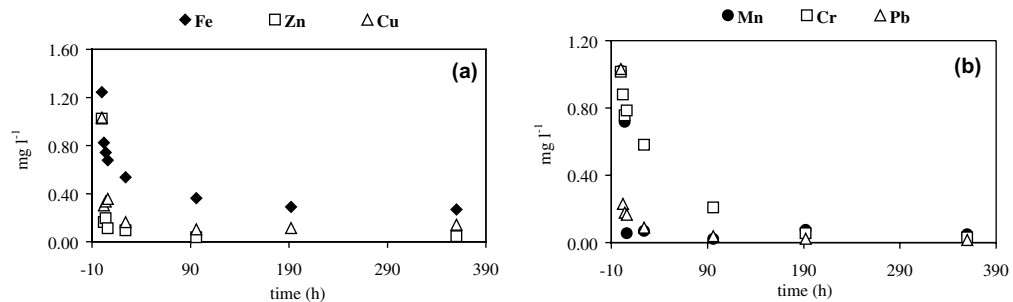


Fig. 3. *Lemna minor*. Metal concentration (mg l^{-1}) vs. time (a) Fe Zn Cu, (b) Mn Cr Pb.

Table 3
% metal removed from water

	<i>Spirodela intermedia</i> (%)	<i>Lemna minor</i> (%)
Fe	80.23	78.47
Zn	95.73	97.56
Mn	96.91	95.20
Cu	91.70	90.41
Cr	33.88	96.94
Pb	98.22	98.55

$t = -4.05$). Spearman’s correlation for *L. minor* is not shown because the species did not survive the end of the experiment. Concentration factors (CF) defined as

$(\text{Metal})_{\text{plant}}/(\text{Metal})_{\text{aq}}$ were calculated (Table 5). *P. stratiotes* CF_{Fe} remained the same for Fe concentration 1, 2 and 4 mg l^{-1} whereas CF_{Zn} CF_{Mn} CF_{Cu} increased with metal concentration. CF_{Zn} CF_{Fe} CF_{Mn} were higher for *S. intermedia* than for *P. stratiotes*.

The relation of the removal rate with the metal concentration was analysed. The removal rate was assumed to be proportional to metal concentration

$$v = -dc/dt = kc,$$

$$\ln C = \ln C^0 - kt.$$

In the above equation, the rate coefficient ($-k$) is the slope of the line ($k, \text{ l/g}; C$ and $C^0, \text{ mM}$). Simple regression analysis results are shown in Tables 6 and 7.

Table 4
Initial and final metal concentration (mg g^{-1}) in *Pistia stratiotes* and *Spirodela intermedia*

	<i>Pistia stratiotes</i>				<i>Spirodela intermedia</i>	
	Initial mg g^{-1}	Final (1 mg l^{-1}) mg g^{-1}	Final (2 mg l^{-1}) mg g^{-1}	Final (4 mg l^{-1}) mg g^{-1}	Initial mg g^{-1}	Final (1 mg l^{-1}) mg g^{-1}
Fe	7.969	9.694	9.881	9.869	4.113	4.288
Zn	0.096	0.518	0.889	1.214	0.031	0.479
Mn	1.089	1.319	1.425	1.750	5.038	5.513
Cu	0.019	0.349	0.655	0.965	0.010	0.431
Cr	0.012	0.189	0.400	0.013	0.005	0.099
Pb	0.013	0.203	0.227	0.019	0.012	0.078

Table 5
Concentration factors $CF = (\text{Metal})_{\text{plant}}/(\text{Metal})_{\text{aq}}$

Metal	<i>Pistia stratiotes</i>			<i>Spirodela intermedia</i>
	1 mg l ⁻¹ CF (lg ⁻¹)	2 mg l ⁻¹ CF (lg ⁻¹)	4 mg l ⁻¹ CF (lg ⁻¹)	1 mg l ⁻¹ CF (lg ⁻¹)
Fe	3.41	3.48	3.47	20.04
Zn	6.55	11.26	15.37	68.48
Mn	20.29	21.92	26.92	153.13
Cu	8.94	16.79	24.74	4.84
Cr	12.63	26.67	0.87	7.04
Pb	7.00	7.82	0.65	1.13

Table 6
Pistia stratiotes: regression analysis

	<i>F</i>	<i>p</i>	<i>k</i> (min ⁻¹)	ln <i>C</i> ⁰	<i>r</i> ²
Fe 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 305.9	<0.01**	-0.005	-2.77	0.99
Fe 2 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 186.9	<0.01**	-0.004	-2.55	0.98
Fe 4 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 640.8	<0.01**	-0.004	-2.13	0.97
Zn 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 22.48	<0.01**	-0.217	-4.56	0.44
Zn 2 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 53.27	<0.01**	-0.189	-3.84	0.45
Zn 4 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 62.92	<0.01**	-0.179	-2.97	0.72
Mn 1 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 10.44	<0.05*	-0.109	-4.65	0.85
Mn 2 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 18.10	<0.01**	-0.121	-3.80	0.94
Mn 4 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 13.04	<0.05*	-0.061	-2.90	0.88
Cu 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 34.01	<0.05*	-0.222	-4.03	0.84
Cu 2 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 25.05	<0.01**	-0.208	-3.44	0.85
Cu 4 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 7.81	<0.05*	-0.178	-2.85	0.77
Cr 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 66.89	<0.01**	-0.004	-4.12	0.88
Cr 2 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 81.94	<0.01**	-0.007	-3.40	0.77
Pb 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 64.88	<0.01**	-0.158	-6.22	0.26
Pb 2 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 7.40	ns			

Table 7
Spirodela intermedia y *Lemna minor*: regression analysis

	<i>F</i>	<i>p</i>	<i>k</i> (min ⁻¹)	ln <i>C</i> ⁰	<i>r</i> ²
<i>Spirodela intermedia</i>					
Fe 1 mg l ⁻¹	<i>F</i> _(0.05;1;7) = 45.25	<0.01**	-0.111	-3.85	0.93
Zn 1 mg l ⁻¹	<i>F</i> _(0.05;1;7) = 5.43	<0.05*	-0.534	-4.60	0.66
Mn 1 mg l ⁻¹	<i>F</i> _(0.05;1;7) = 6.32	<0.05*	-0.481	-4.04	0.69
Cu 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 17.98	<0.01**	-0.303	-4.11	0.87
Cr 1 mg l ⁻¹	<i>F</i> _(0.05;1;7) = 7.72	<0.05*			
Pb 1 mg l ⁻¹	<i>F</i> _(0.05;1;6) = 36.60	<0.01**	-0.617	-5.27	0.93
<i>Lemna minor</i>					
Fe 1 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 18.22	<0.01**	-0.096	-3.90	0.85
Zn 1 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 14.37	<0.05*	-0.322	-4.61	0.72
Mn 1 mg l ⁻¹	<i>F</i> _(0.05;1;4) = 3.26	ns			
Cu 1 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 87.81	<0.05*	-0.155	-4.51	0.49
Cr 1 mg l ⁻¹	<i>F</i> _(0.05;1;7) = 108.49	<0.01**	-0.046	-3.97	0.81
Pb 1 mg l ⁻¹	<i>F</i> _(0.05;1;5) = 27.37	<0.01**	-0.288	-5.71	0.74

5. Discussion

Hydrochemical analysis of Lake Chascomús, Lake Vitel and Vitel stream showed that Pb, Cu, Cr, and Zn (only in Lake Chascomús) mean concentration values exceeded the recommended values for the protection of the aquatic life (1.0, 2.0, 2.0 and 30 g l^{-1} , respectively; Niveles Guía para la protección de la biota acuática, 2001(Recommended)).

The percentages removed by *P. stratiotes* were very high (ca >85% Pb, Cr, Mn and Zn) *P. stratiotes* was able to remove the metals almost completely in the first 24 h of exposure. Fe showed the minor removal percentages, but it should be kept in mind that Fe concentration in aquatic environment was high (Lake Chascomús 2.84 mg l^{-1}) and that macrophytes had already assimilated it previous to the sampling.

The immediate diminish of Pb and Cr initial concentration to minimum values when the amount added was 4 mg l^{-1} , suggest PbCrO_4 precipitation implying that high Cr and Pb removal percentages are observed not only due to macrophyte removal process but also to PbCrO_4 precipitation. When 4 mg l^{-1} of polluting metals are added the ionic product overcomes K_{ps} (1.8×10^{-14} at 25 °C). According to the stoichiometry of the precipitation reaction and to the reagent amounts added, Pb is the restrictive reagent, being Cr in excess removed by macrophytes.

Although the removal percentages by *S. intermedia* and *L. minor* were very high, the Fe percentage is the minor, probably due to the fact that macrophytes had already assimilated it previous to the sampling. It is interesting to note that *S. intermedia* removes Cr only in a low percentage (33%). It is known that in many plant species the mobility of Cr is low due to the fact that there are barriers or lack of transport mechanism suitable for Cr transport from roots to shoots (Kähkönen and Manninen, 1998). In *Spirodela polyrhiza*, the Cr presence decreases growth rate inhibiting photosynthesis (Appenroth et al., 2001).

When the initial and final plant metal content was determined, it was observed that Cu, Zn, Cr and Pb final concentration was more than 10 times the initial for *S. intermedia* and *P. stratiotes*, but Fe and Mn showed a smaller increase, probably because the macrophytes had already removed Fe and Mn from the aquatic environment. *L. minor* did not survive the end of the experiment, probably due to the low dissolved oxygen concentration in the reactor. *L. minor* extends as a mantle, covering the total surface and impeding the free exchange with the atmospheric oxygen. Although there are very few studies on bioassays using heavy metals and aquatic macrophytes, Cd concentrations above 10 mM and Cu concentrations above 50 μM promote pigment degradation and photosynthesis inhibition in *Lemna trisulca* L (Prasad et al., 2001). These concentrations

are higher than those used to perform our experiments. Fjällborg and Dave, 2003 have reported that $\text{EC}_{50} = 3800 \text{ mg Cu/kg } L. \text{ minor}$.

The correlation of the amount of metal removed from water with the amount of metal accumulated by *P. stratiotes* for Cu, Pb, Zn, Fe, Mn and Cr 1, 2 mg l^{-1} was significant, instead was not significant when 4 mg l^{-1} of metals was added. When Pb and Cr were eliminated prior to the correlation analysis, the results were significant, implying that when Pb and Cr concentration is 4 mg l^{-1} , the main process of water removal is PbCrO_4 precipitation instead of macrophytes metal uptake.

The analysis of the CF obtained showed that *P. Stratiotes* had already accumulated Fe from the environment and was unable to remove further amounts, whereas Zn, Cu and Mn amounts removed were larger at higher metal concentrations. *S. intermedia* seem to be more efficient than *P. Stratiotes* in removing Fe, Zn and Mn from the contaminated environment.

The results of the regression analysis confirm that the metal removal rate by the 3 macrophytes studied is proportional to metal concentration at least in the first hours, as all the regressions were highly significant ($p < 0.01$) or significant ($p < 0.05$) and the r^2 values were high. The *P. stratiotes* rate coefficient (k) value obtained for 1, 2 and 4 mg l^{-1} metal concentration was higher for Mn, Cu, Zn and Pb (1 mg l^{-1}) than for Fe, suggesting a faster process. The same tendency is observed for *S. intermedia* and *L. minor*, probably due to the fact that the species had already accumulated Fe from the aquatic environment. *S. intermedia* shows the highest rate coefficients among the 3 species studied, resulting the most appropriated for metal removal.

6. Conclusions

The simultaneous removal of Fe, Cu, Zn, Mn, Cr and Pb by *P. stratiotes*, *S. intermedia* and *L. minor* was analysed. The macrophytes resulted highly effective in the uptake of heavy metals, even when prior to sampling they had already accumulated Fe and Mn from the aquatic environment (*P. stratiotes*: Fe 7.97 mg g^{-1} , Mn 1.09 mg g^{-1} ; *S. intermedia*: Fe 4.11 mg g^{-1} , Mn 5.04 mg g^{-1}).

The high correlation between the final metal concentration in the water and the metal concentration in macrophytes ratifies that the main process of metal removal from water is macrophytes uptake. *S. intermedia* showed the highest rate coefficients and concentration factors, resulting the most appropriated for metal removal. The main deviations were observed with Cr and Pb, and were due to the competition with the PbCrO_4 precipitation process. The fact that they have been able to remove all metals simultaneously constitutes a strong indication of their potential for advanced

treatment of polluted waters. However, the economic potential of the studied macrophytes may not be fully realized until they can be economically reduced to a dried, compact commodity.

The rate of the metal uptake process was dependent on the metal concentration for the three species studied in the first hours of the experiment. After the first hours, the rate of sorption gradually recedes and becomes negligible. It is therefore suggested that in treatment plants, resultant plant growth might be harvested regularly, so that the water purification becomes a continuous process. Studies with regard to aquatic macrophytes combinations to be used in treatment ponds and the period of macrophyte replacement should be seriously undertaken for developing a more efficient, natural and economic integrated macrophyte based system, most advantageous for heavy metal removal.

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