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Short communication

Nickel, lead and zinc accumulation and performance in relation to their use in phytoremediation of macrophytes *Myriophyllum aquaticum* and *Egeria densa*

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ABSTRACT

In order to study the accumulation of nickel, lead and zinc for their use in the phytoremediation of aquatic environments, the aquatic plants *Myriophyllum aquaticum* and *Egeria densa* were exposed to increase the concentrations of these metals (Ni: 0.05–10 mg L^{-1}, Pb: 0.05–15 mg L^{-1}, Zn: 0.15–20 mg L^{-1}) for 7 days. The accumulation of Ni, Pb and Zn in plants was determined and their effects on physiological parameters (chlorophyll *a* concentration and degradation, lipid peroxidation measured as malondialdehyde) were evaluated in the leaves for both species. *M. aquaticum* showed a higher accumulation capacity of Pb and Zn than *E. densa*, particularly at the highest concentrations of exposure to these metals. Nevertheless, the physiological changes observed in these species, especially in *M. aquaticum*, at high metal concentrations and accumulations, did not represent a risk in relation to their survival during the study period. Therefore, taking into account their accumulation capacity and tolerance to heavy metals, *M. aquaticum* is more suitable for phytoremediation in aquatic environmental contaminated with Ni, Pb or Zn than *E. densa*.

1. Introduction

The contamination of aquatic environments is a major problem worldwide, especially in countries with emerging and developing economies. This threatens human health through both water and food consumption, as plants and animals from polluted environments that are consumed as food, are in contact with pollutants in the aquatic ecosystem. Due to heavy metal transfer and accumulation occurring in different biotic and abiotic compartments, they are among the most common pollutants (Guo et al., 2015; Yusof et al., 2001). Although their contribution to the aquatic ecosystem may come from natural sources such as those of lithogenic or geochemical origin, the greatest contribution of heavy metals, especially those considered to be toxic to aquatic ecosystems, is from anthropogenic discharges often associated with inadequate management of wastewater through industrial effluent discharge, runoff from agricultural and urban land, or mining. The entry of these pollutants in aquatic systems can affect water quality and thereby increase both the ecological and human health risks (Yi et al., 2011). The restoration of ecosystems, which have been disturbed by human activities, using of aquatic species in the development of new ecosystems for the wastewater treatment is a necessary practice in the last decade (Mitsch, 2012; Odum and Odum, 2003 Ray et al., 2015).

Heavy metals in aquatic systems can be easily absorbed and accumulated in aquatic plants (Jackson, 1998). Although the exposure to high concentrations of these metals may be toxic, nickel (Ni) and zinc (Zn) are the essential micronutrients for plants (Broadley et al., 2007; Seregin and Kozhevnikova, 2006). However, an excess Ni and Zn may be toxic to plants, with lead (Pb) not being an essential element and toxic for organisms even at micro concentrations. These phytotoxic effects include chlorophyll degradation which has been attributed to changes in the synthesis of this pigment (Prasad and Strzałka, 1999), as well as increasing degradation (Somashekaraiah et al., 1992), in general measured as malondialdehide (MDA).

High concentrations of Ni, Pb and Zn have been detected in rivers in the central region of Argentina, possibly due to the runoff of urban soils, fertilizer application or effluents discharged into rivers (Harguinteguy et al., 2014 Monferrán et al., 2011). In some cases, the concentration of these metals exceeded the guidance levels for water quality standard for the protection of aquatic life in

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surface freshwater established by national legislation (República Argentina, 1991, Ley Nacional 24051). Furthermore, in a previous study, it was observed that the macrophyte, *Myriophyllum aquaticum*, showed a high accumulation of heavy metals in a polluted aquatic ecosystem (Harguinteguy et al., 2013).

The submerged macrophytes of the genus Myriophyllum and Egeria, which are common in the Southern Hemisphere, have been used in biomonitoring studies of water pollution (Cardwell et al., 2002; Harguinteguy et al., 2013; Schreiber et al., 2013) and as organisms for the bioaccumulation and bioabsorption of heavy metal aquatic pollutants in plant tissues (Ngayila et al., 2007; Pietrobelli et al., 2009).

In order to study the accumulation capacity of Ni, Pb and Zn, the two species of aquatic plants, *M. aquaticum* and *E. densa*, were exposed to increasing concentrations of these metals under hydroponic conditions. At the same time, some physiological parameters (chlorophyll *a* concentration and degradation, lipid peroxidation) were evaluated in both species in order to determine the effects of heavy metal concentrations on their physiology and to detect their specific tolerance, with the aim of employing these species in the phytoremediation of aquatic environments polluted by these metals.

2. Materials and methods

2.1. Plant material and treatment conditions

The aquatic plants *M. aquaticum* and *E. densa* were collected from a site in the Anizacate River (64°30′01″ W; 31°42′51″ S). This site is located 10 km upstream of La Bolsa town, which was chosen to be the reference site because it still maintains predominantly pristine forest features (Schreiber et al., 2013). The collected plants were thoroughly washed "*in situ*" with river water, before being placed in polyethylene bags and then in cooling chambers to keep them at a stable temperature during transport to the laboratory. Once there, the plants were washed with distilled water in plastic tanks to remove any remaining material adhering to their surface, and then they were maintained under hydroponic conditions in tanks of 100 L capacity in a medium prepared with distilled water in a nutrient solution (0.25%, v/v: Tripathi et al., 2003) comprising 5.8 g L^{-1} of KH_2PO_4, 8.5 g L^{-1} of KNO_3 and 5.3 g L^{-1} NH_4NO_3 (Franzaring et al., 2007).

Light was provided by day light lamps, with an irradiance of 100 μE m^2 s^{-1}, at a light:dark cycle of 14:10 h, and the temperature was kept between 20 and 22 °C and the pH between 7 and 8. The plants were maintained for a period of 2–3 weeks for acclimatization using a constant aeration system. Once acclimated in these greenhouse conditions, the macrophytes were placed in beakers of 3.5 L (five plants per beaker, 15–20 g wet weight per liter and a species per beaker) for exposure tests, and maintained under the same experimental conditions and constant aeration system during the treatment period.

2.2. Experimental design

To evaluate the accumulation capacity, each species was exposed to six increasing concentrations of each metal (Ni, Pb and Zn) for 7 days (Singh et al., 2010). The lowest concentration of each metal was selected by taking into account the value stated in the guide levels of water quality standards for protection of aquatic life in surface freshwater established by national legislation (República Argentina, 1991; National Law 24051). For treatments with the highest concentration of each metal, these were selected by taking into account the maximum values of the permissible limits for the discharge of wastewater in surface water [Dirección Provincial de Agua y Saneamiento (DiPAS) de la provincia de Córdoba, Argentina. Decreto 415/99] (Supplementary material – Table A1). In all the cases, control samples ($n = 3$) were used. After harvesting, the plants were washed with distilled water, dissected using a lyophilizer chamber (Rificor® Model L-A-B4) and kept in the dark until analysis of the heavy metals and physiological parameters.

2.3. Heavy metal and physiological parameter determinations

Samples of the dried plant material (1 g) were carbonized in an oven at 450 °C for 4 h, and the ashes were digested using a mixture of HCl (20%) and concentrated HNO_3 (3:1) (v/v) (Nekrasova et al., 2011). For metal determinations, solid residues were removed by centrifugation, and the samples were diluted with ultrapure water to a final volume of 10 mL. Finally, the contents of Ni, Pb and Zn were analyzed using GFAAS (graphite furnace atomic absorption spectrometer, AAnalyst 600, PerkinElmer, USA). Digestion blanks were prepared and analyzed in the same manner, with the results being expressed in mg kg^{-1} DW (dry weight).

For the physiological parameter determinations in plants, the procedures followed for the quantification of chlorophyll *a* (Chl-*a*), phaeophityn *a* (Phaeoph-*a*), and malondialdehyde (MDA) have been previously described by Pignata et al. (2002).

2.4. Statistical analysis

Statistical analysis of results was performed using the InfoStat Version 1.1 software. The data obtained were analyzed by analysis of variance (ANOVA) at a significance level of $p < 0.01$, to investigate differences between treatments and between species for each of the metals and the physiological parameters analyzed, which was followed by an a posteriori test of Duncan's multiple range ($p < 0.05$). Assumptions of analysis of variance were previously checked analytically and graphically for each of the measured variables. Variables that did not meet the assumptions of the model were transformed to Log_{10} prior to statistical analysis.

3. Results

3.1. Nickel, lead and zinc accumulation in M. aquaticum and E. densa

Nickel, Pb and Zn accumulation in both *M. aquaticum* and *E. densa* were dependent on the metal concentration in the exposure medium after 7 days (Fig. 1). The maximum levels of accumulation of Ni (Fig. 1a) were found to 10 mg L^{-1} of Ni^{2+} in *M. aquaticum* and *E. densa*. No significant differences ($p > 0.01$) were found in the mean accumulation of Ni between the species.

The maximum Pb accumulation levels (Fig. 1b) were recorded in 15 mg L^{-1} of Pb^{2+} in both species, with the mean concentration of Pb in *M. aquaticum* (3798.9 ± 1032.5 mg kg^{-1}) being significantly different ($p < 0.01$) than that observed in *E. densa* (2302.5 ± 882.1 mg kg^{-1}).

The maximum accumulations of Zn (Fig. 1c) were observed to be 20 mg L^{-1} of Zn^{2+} in both species, with the mean accumulation of Zn in *M. aquaticum* (2348.4 ± 713.2 mg kg^{-1}) being significantly different ($p < 0.01$) than that observed in *E. densa* (1083.6 ± 568.1 mg kg^{-1}).

3.2. Nickel, lead and zinc effects on physiological parameters in M. aquaticum and E. densa

Increased concentration of Ni, Pb and Zn was observed in both *M. aquaticum* and *E. densa*. The variation in chlorophyll *a* was unclear in relation to Ni^{2+} (Table 1), but a significant decrease in this pigment was observed with increasing concentrations of $Pb^{2+}$and Zn^{2+} (Tables 2 and 3). Moreover, the chlorophyll *a* degradation (Pheo-*a*/Chl-*a* ratio) did not show any changes with

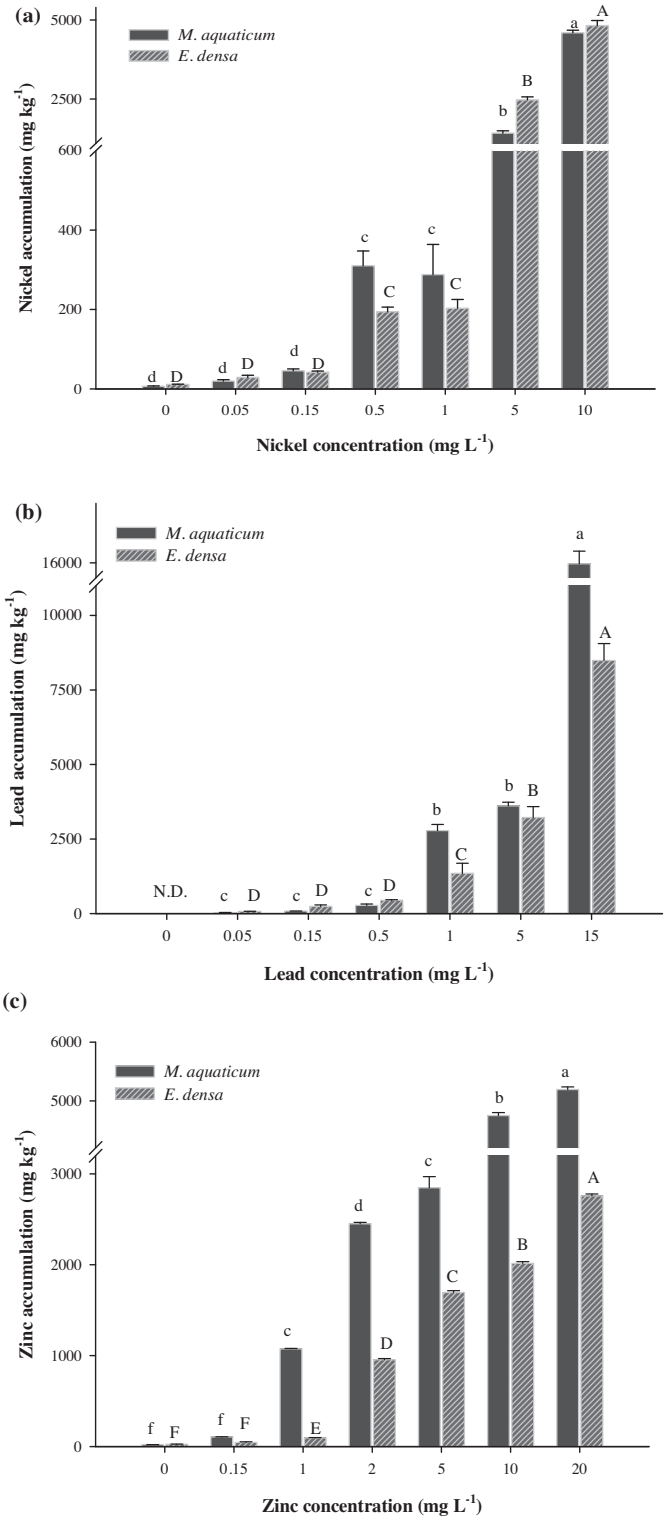

Fig. 1. Means and standard deviation (vertical bar) of Ni (a) Pb (b) and Zn (c) accumulation in *M. aquaticum* and *E. densa* exposed to different concentrations of these metals for 7 days. ANOVA followed by Duncan's multiple range test was used to determine significant differences between treatments within each species. Means with the same letters are not significantly different ($p > 0.05$) according to Duncan's test, (small letters indicate *M. aquaticum* and capital letters show *E. densa*).

increasing Ni^{2+} or Pb^{2+} in any treatment in either species, or with increasing of Zn^{2+} in any treatment in *M. aquaticum*.

In *M. aquaticum*, the highest concentrations of lipid peroxidation (MDA) were observed at 1 mg L^{-1} of Ni^{2+} and in *E. densa* at 10 mg L^{-1} of Ni^{2+} (Table 1). Regarding the Pb^{2+} effect on MDA content (Table 2), this parameter showed a significant reduction in both species at the highest concentration of this metal in the exposure medium. With respect to Zn^{2+} (Table 3) in *M. aquaticum*, the lowest MDA concentration observed was 1 mg L^{-1}, and in *E. densa*, this parameter showed a significant reduction at the highest concentration of this metal in the exposure medium. No significant differences ($p > 0.01$) were found in the mean MDA concentrations between species.

4. Discussion

M. aquaticum and *E. densa* showed similar accumulations of Ni, with both species revealing comparable physiological responses to increasing concentrations of this metal in the exposure medium. Nevertheless, the accumulation of Ni was lower than that described by Maleva et al. (2009) in *Elodea canadensis* grown at similar concentrations of Ni^{2+}.

The maximum accumulation of Pb was higher in *M. aquaticum* than *E. densa*, and higher than those cited by Wang et al. (2011) in *Vallisneria natans* in solutions higher than 15 mg L^{-1} of Pb^{2+}. In the same way, in our study, the Pb accumulation levels in *M. aquaticum* and in *E. densa* were higher than those reported by Singh et al. (2010) in *Najas indica* after 7 days of exposition.

The Zn accumulations in *M. aquaticum* and *E. densa* after 7 days of exposure to Zn^{2+} were higher than the ones cited by Nyquist and Greger (2007) in *E. canadensis* in a culture medium with a similar concentration of this metal after a period of 15 days, whereas the accumulation reported by Umebese and Motajo (2008) in *Ceratophyllum demersum* was similar to the one observed in *M. aquaticum* in this study. In the present study, the species *M. aquaticum* showed a higher accumulation capacity of Zn than *E. densa*.

With respect to the sensibility or tolerance of *M. aquaticum* and *E. densa* to expose at high concentrations of Ni (Table 1), Pb (Table 2) and Zn (Table 3), similar responses to Ni in relation to chlorophyll *a* degradation were observed, which were similar to those reported in *E. canadensis* growing in a culture medium with Ni^{2+} (Maleva et al., 2009) and in *Hydrilla verticillata* (Sinha and Pandey, 2003). The results showing an MDA increase produced by Ni in *E. densa* were comparable to those reported for *H. verticillata* (Sinha and Pandey, 2003).

In relation to Pb toxicity in *E. densa*, chlorophyll *a* revealed a significant reduction with increasing concentration of Pb^{2+}. Similar results were observed in *N. indica* by Singh et al. (2010), who reported a decrease in photosynthetic pigments with increased Pb concentration after 7 days of exposure. The chlorophyll content reduction in *E. densa* may be attributed to decreased chlorophyll synthesis, due to the inhibition of δ-aminolevulinic acid dehydratase (ALAD) caused by Pb (Sharma and Dubey, 2005). *M. aquaticum* did not reveal any decrease in chlorophyll *a* concentrations with increasing Pb concentration, which could explain the higher accumulation of Pb in this species when exposed to higher Pb^{2+} concentrations under hydroponic conditions, with this result possibly showing tolerance of *M. aquaticum* to Pb because no significant differences in the degradation of chlorophyll *a* were observed (Pheo-*a*/Chl-*a* ratio).

Regarding lipid peroxidation, in both *M. aquaticum* and in *E. densa* the MDA showed a decrease at the highest concentrations of Pb after 7 days of exposure. Different results were observed in *N. indica* by Singh et al. (2010), who both reported a rise in lipid peroxidation products (MDA) with increasing of Pb concentrations after 7 days of exposure.

A reduction in Chl-*a* was observed in *M. aquaticum* with an increase in the Zn concentration in the medium. However, the Chl-*a* degradation (Pheo-*a*/Chl-*a* ratio) did not show any changes with

Table 1
Physiological parameters in *M. aquaticum* and *E. densa* for increasing concentrations of Ni^{2+} (mg L^{-1}) under hydroponic conditions (mean values and standard deviation, $n = 3$). ANOVA followed by Duncan's multiple range test was used to determine significant differences between treatments. Means with the same letters are not significantly different ($p > 0.05$) according to Duncan's test.

Treat. Ni^{2+} (mg L^{-1})	*M. aquaticum*			*E. densa*		
	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*
0	5.74 ± 0.13 d	0.155 ± 0.035 cd	1.101 ± 0.031	8.07 ± 0.51 a	0.176 ± 0.030 d	1.076 ± 0.037
0.05	6.22 ± 0.27 c	0.177 ± 0.048 c	1.084 ± 0.031	7.30 ± 0.72 b	0.175 ± 0.037 d	1.069 ± 0.090
0.15	6.48 ± 0.26 c	0.251 ± 0.021 a	1.042 ± 0.053	7.11 ± 0.54 b	0.147 ± 0.071 e	1.083 ± 0.057
0.5	7.68 ± 0.10 a	0.150 ± 0.018 d	1.048 ± 0.061	8.01 ± 0.21 ab	0.200 ± 0.019 c	1.057 ± 0.081
1	5.45 ± 0.23 d	0.252 ± 0.037 a	1.059 ± 0.081	7.92 ± 0.14 ab	0.206 ± 0.074 c	1.060 ± 0.061
5	6.21 ± 0.20 c	0.150 ± 0.016 d	1.045 ± 0.031	4.92 ± 0.04 c	0.241 ± 0.046 b	1.037 ± 0.084
10	6.96 ± 0.18 b	0.230 ± 0.058 b	1.076 ± 0.041	7.21 ± 0.72 b	0.290 ± 0.058 a	1.031 ± 0.072
ANOVA	***	***	n.s.	***	***	n.s.

Note: n.s., not significant; Chl, chlorophyll; MDA, malondialdehyde; Pheo, pheophytin.
*** $p < 0.01$

Table 2
Physiological parameters in *M. aquaticum* and *E. densa* for increasing concentrations of Pb^{2+} (mg L^{-1}) under hydroponic conditions (mean values and standard deviation, $n = 3$). ANOVA followed by Duncan's multiple range test was used to determine significant differences between treatments. Means with the same letters are not significantly different ($p > 0.05$) according to Duncan's test.

Treat. Pb^{2+} (mg L^{-1})	*M. aquaticum*			*E. densa*		
	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*
0	5.74 ± 0.13 f	0.155 ± 0.035 d	1.101 ± 0.031	8.07 ± 0.51 ab	0.176 ± 0.030 bc	1.076 ± 0.037
0.05	7.17 ± 0.08 d	0.179 ± 0.019 c	1.081 ± 0.005	8.53 ± 0.11 a	0.119 ± 0.015 f	1.070 ± 0.021
0.15	8.33 ± 0.06 c	0.205 ± 0.040 b	1.096 ± 0.018	6.99 ± 0.42 b	0.189 ± 0.042 b	1.059 ± 0.032
0.5	8.52 ± 0.07 b	0.134 ± 0.033 e	1.057 ± 0.014	7.35 ± 0.61 b	0.170 ± 0.039 c	1.070 ± 0.017
1	10.21 ± 0.14 a	0.150 ± 0.041 d	1.036 ± 0.007	6.30 ± 0.48 c	0.189 ± 0.012 b	1.068 ± 0.035
5	6.24 ± 0.04 e	0.264 ± 0.010 a	1.049 ± 0.015	5.92 ± 0.07 c	0.250 ± 0.028 a	1.116 ± 0.031
15	7.23 ± 0.03 d	0.187 ± 0.025 c	1.047 ± 0.011	3.98 ± 0.12 d	0.159 ± 0.042 d	1.079 ± 0.041
ANOVA	***	***	n.s.	***	***	n.s.

Note: n.s., not significant; Chl, chlorophyll; MDA, malondialdehyde; Pheo, pheophytin.
*** $p < 0.01$

Table 3
Physiological parameters in *M. aquaticum* and *E. densa* for increasing concentrations of Zn^{2+} (mg L^{-1}) under hydroponic conditions (mean values and standard deviation, $n = 3$). ANOVA followed by Duncan's multiple range test was used to determine significant differences between treatments. Means with the same letters are not significantly different ($p > 0.05$) according to Duncan's test.

Treat. Zn^{2+} (mg L^{-1})	*M. aquaticum*			*E. densa*		
	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*	Chl-*a* (mg g^{-1})	MDA (μmol g^{-1})	Pheo-*a*/Chl-*a*
0	2.30 ± 0.12 a	0.033 ± 0.006 c	1.223 ± 0.078	4.61 ± 0.11 a	0.077 ± 0.009 c	1.118 ± 0.014 bc
0.15	1.74 ± 0.22 b	0.032 ± 0.005 c	1.330 ± 0.171	4.51 ± 0.09 b	0.049 ± 0.008 d	1.108 ± 0.023 c
1	1.60 ± 0.01 b	0.072 ± 0.010 ab	1.253 ± 0.026	2.06 ± 0.03 c	0.144 ± 0.009 a	1.132 ± 0.025 bc
2	1.17 ± 0.05 c	0.069 ± 0.024 ab	1.317 ± 0.047	1.36 ± 0.04 e	0.128 ± 0.011 b	1.157 ± 0.023 ab
5	1.14 ± 0.02 c	0.054 ± 0.011 bc	1.246 ± 0.035	1.54 ± 0.03 d	0.117 ± 0.011 b	1.152 ± 0.009 bc
10	1.12 ± 0.00 c	0.086 ± 0.012 a	1.292 ± 0.042	1.40 ± 0.05 e	0.082 ± 0.008 c	1.154 ± 0.001 b
20	1.03 ± 0.09 c	0.070 ± 0.013 ab	1.255 ± 0.123	1.55 ± 0.04 d	0.072 ± 0.008 c	1.195 ± 0.044 a
ANOVA	***	***	n.s.	***	***	***

Note: n.s., not significant; Chl, chlorophyll; MDA, malondialdehyde; Pheo, pheophytin.
*** $p < 0.01$

increasing Zn concentration. This may indicate an inhibitory effect of Zn on the biosynthesis of chlorophyll *a* in this species to high concentrations of this metal. However, Zn^{2+} at these concentrations does not produce degradation of Chl-*a*. The results are in agreement with Prasad and Strzałka (1999), who reported an inhibition of the chlorophyll pigment synthesis attributed to the impact of high levels of Zn as well as structural and functional damage. In *E. densa*, on the other hand, it was observed that increasing Zn had a deleterious effect on chlorophyll degradation. Also, a Zn effect on chlorophyll degradation was reported in *Lemna gibba* by Megateli et al. (2009). The MDA results showed an increase at Zn^{2+} concentrations at the 1.0 mg L^{-1} treatment in *M. aquaticum* and *E. densa*. Similar results were also observed in *Potamogeton pectinatus* (Tripathi et al., 2003). In fact, Zn is an essential micronutrient that plays an important role in many biological processes, including protein synthesis, and it is also involved in the processes of oxidative stress response (Li et al., 2006). However, high levels of bioaccumulation of this metal may cause damage to the photosynthetic apparatus, as was observed in chlorophyll *a* concentrations in *E. densa* in this study.

5. Conclusions

In general terms, the accumulation capacity for Pb and Zn in *M. aquaticum* was higher than in *E. densa*, especially, at the highest levels of exposure to these metals.

Nickel, Pb and Zn caused physiological changes due to their greater toxicity at high concentrations in *E. densa* more than *M. aquaticum*. Nevertheless the damage at the level of the photosynthetic system and the lipid peroxidation values measured as MDA in both species did not represent a risk for their survival at high concentration of these metals.

Taking into account its better accumulation capacity and tolerance to heavy metals, *M. aquaticum* is a species suitable for its use in phytoremediation at the aquatic environments contaminated with Ni, Pb and Zn. *E. densa* could also be used in phytoremediation, provided that concentrations of these metals in the aquatic environment are not too high given its lower tolerance to them.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2015.05.039.

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