



Manganese accumulation and its effects on three tropical aquatic macrophytes: *Azolla caroliniana*, *Salvinia minima* and *Spirodela polyrhiza*

Acúmulo e efeitos do manganês em três macrófitas aquáticas tropicais: *Azolla caroliniana*, *Salvinia minima* e *Spirodela polyrhiza*

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Abstract

The phytoremediation technique, which consists of using plants to remove ions, has been increasingly chosen over past decades due to its low-cost technology to mitigate contaminated areas. The aim of this study was to evaluate the potential of the aquatic macrophytes, *Azolla caroliniana* Willd, *Salvinia minima* Baker and *Spirodela polyrhiza* (L.) Schleiden, to accumulate manganese (Mn), an element which, at high concentrations, may be toxic to human populations. The three species accumulated Mn in their tissues and the absorption was independent of the metal concentration in the solution. *Spirodela polyrhiza* accumulated Mn at higher concentrations of the ion (17.062 mg g⁻¹ MS), followed by *S. minima* (4.283 mg g⁻¹ MS) and *A. caroliniana* (1.341 mg g⁻¹ MS). Manganese excess reduced total chlorophyll content in all three species. Carotenoid content was reduced in *A. caroliniana* (27.02 %) and *S. polyrhiza* (25.34 %). Growth was only significantly reduced (21.34%) in *S. polyrhiza*. The species *A. caroliniana* and *S. minima* were able to tolerate excess Mn, but were inefficient regarding the accumulation of high concentrations of the metal. High accumulated Mn content in the tissues of *S. polyrhiza* suggests that the species is able to accumulate this element. Therefore, it has potential for use in phytoremediation and provides a new resource for exploring the Mn accumulation mechanism.

Key words: aquatic plants; growth; toxicity; phytoremediation.

Resumo

A fitorremediação que consiste na utilização de plantas para remoção de íons, tem aumentado nas últimas décadas, tendo em vista a busca por tecnologias de baixo custo para mitigar áreas contaminadas. O objetivo do presente trabalho foi avaliar o potencial das macrófitas aquáticas: *Azolla caroliniana* Willd, *Salvinia minima* Baker (Salviniaceae) e *Spirodela polyrhiza* (L.) Schleiden (Araceae) em acumular manganês (Mn), um elemento que em concentração elevada pode ser tóxico para população humana. As três espécies acumularam Mn em seus tecidos e a absorção foi dependente da concentração do metal em solução. *Spirodela polyrhiza* acumulou as concentrações mais elevadas de Mn (17,062 mg g⁻¹ MS), seguida por *S. minima* (4,283 mg g⁻¹ MS) e *A. caroliniana* (1,341 mg g⁻¹ MS). O excesso de Mn causou redução do conteúdo de clorofila total nas três espécies. O conteúdo de carotenóides diminuiu em *A. caroliniana* (27,02 %) e *S. polyrhiza* (25,34 %). Apenas em *S. polyrhiza* o crescimento foi reduzido significativamente (21,34%). As espécies *A. caroliniana* e *S. minima* toleraram excessos de Mn, mas foram ineficientes no acúmulo de concentrações elevadas do metal. O elevado conteúdo de Mn acumulado nos tecidos de *S. polyrhiza* sugere que a espécie possui capacidade para acumular este elemento. Portanto, apresenta potencial para ser utilizada na fitorremediação e oferece um novo recurso para explorar os mecanismos de acúmulo do Mn.

Palavras-chave: plantas aquáticas, crescimento, toxicidade, fitorremediação.

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Introduction

The presence of heavy metals in aquatic ecosystems causes serious impact on the biological components. As a result, serious disorders on human population health have been observed due to the biomagnification processes and their toxic effects within the food chain (Tavares & Carvalho 1992; Cabana *et al.* 1994; Mergler *et al.* 1994; Doyle *et al.* 2003).

Manganese (Mn) is an essential trace element in physiological processes, both for plants and animals. However, high concentrations of this metal in the aquatic environment ($> 5,200 \mu\text{g/l}$) can be toxic (Mergler *et al.* 1994; Boucher & Watzin 1999; Doyle *et al.* 2003).

Besides its natural occurrence, the contamination of aquatic ecosystems by Mn is made worse by human activities, including the disposal of industrial waste. They involve manufacturing processes (metal, paper, and chemical agents), household waste and mining activities (Thornton 1995; Banks *et al.* 1997).

The National Council for the Environment (CONAMA - Resolution 357, 2005) establishes a total Mn limit of 0.1 mg/l for class 1 waters (waters that can be used for human consumption after simplified treatment), 0.5 mg/l for class 3 waters (waters that can be used for human consumption after conventional or advanced treatment) and 1 mg/l of dissolved Mn for discharging effluents. However, research has demonstrated that Mn concentration in waters of mining and mine drainage regions, as well as industrial waste waters, exceed the recommended concentrations (Banks *et al.* 1997; Sisinho 2003; Guimarães-Silva *et al.* 2007).

The recovery of areas contaminated by heavy metals can be done through conventional methods such as physical and chemical processes. They may include removing the source of contamination, isolating the contaminated area and/or flocculation (Richter & Azevedo Netto 1991), which are costly processes. For this reason, phytoremediation has been seen as an attractive, ecologically viable and low-cost technique. Moreover, as it is applied *in situ*, this technique causes minimal impact to the environment (Chaney *et al.* 2000; Raskin & Ensley 2000).

Studies show that water bodies contaminated by metals can benefit from phytoremediation using aquatic macrophytes such as *Lemna minor* L., *Pistia stratiotes* L., *Spirodela intermedia* W. Koch (Araceae) and *Ceratophyllum demersum* L. (Ceratophyllaceae) (Rai *et al.* 1995; Miretzky *et al.* 2004). This process has been very advantageous, as aquatic plants

usually show a high rate of vegetative reproduction, rapid growth and are easily handled (Dushenkov *et al.* 1995; Cunningham & Ow 1996).

Brazil is favorable for this technology, as this country has auspicious climatic and soil conditions, abundant aquatic environments and high diversity of plant species. However, few studies have reported the effects of the exposure of macrophytes to high concentrations of Mn, which makes it harder to indicate phytoremediation to minimize the impacts caused by this element on aquatic environments.

Research with such an approach must be encouraged, as toxicity by Mn has become a major environmental problem, especially in the state of Minas Gerais, where Mn occurs in abundance in the matrix rock.

This study aimed to evaluate Mn accumulation capacity and its effects on total chlorophyll content, carotenoids and growth rate of three tropical free-floating macrophytes: *Azolla caroliniana*, *Salvinia minima* and *Spirodela polyrhiza*. in order to investigate the potential of these species in phytoremediation processes.

Materials and Methods

The species *Azolla caroliniana*, *Salvinia minima* and *Spirodela polyrhiza* were obtained at the Botanical Garden of the “Universidade Federal de Viçosa (UFV)” and grown in a greenhouse, at “Unidade de Crescimento de Plantas (UCP)” / “Departamento de Biologia Vegetal (DBV/UFV)” / Minas Gerais, Brazil. To adapt to experimental conditions and to biomass obtaining, the macrophytes were grown in Hoagland nutrient solution (Hoagland & Arnon 1950), with 1/5 ionic strength and pH 6.5 ± 0.5 . The plants were placed on plastic trays in a growth room at $25 \pm 1^\circ\text{C}$ temperature, $230 \mu\text{mol}$ of photons $\text{m}^{-2}\text{s}^{-1}$ illumination and photoperiod of 12 hours of light: dark, during 15 days, to allow daughter plant/propagule uniformity and to assure homogeneity in the plant material used in the treatments, as suggested by Outridge & Hutchinson (1991). The nutrient solution was replaced every three days.

After the acclimatization period, the experiment began with 1.5 g *A. caroliniana* and *S. polyrhiza* fresh mass (FM), and 2.0 g *S. minima* fresh mass, since this species has individuals with greater plant mass in relation to the other species. All plants were placed in polyethylene vessels containing 350 ml of Hoagland solution and increasing Mn doses (0.05; 0.1; 0.2; 0.3; 0.4 mM) in

the form of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. Control plants were grown in Hoagland solution only. On a daily basis, the pH of the solutions was measured and, when necessary, corrected to 6.5 ± 0.5 with HCl (1 M) or NaOH (1 M). The three species were simultaneously tested, in a single experiment, conducted during seven days, in a completely randomized design; five repetitions were made for each treatment, and the plants were kept in the same microclimatic conditions set during the acclimatization period.

On the seventh day after the beginning of the experiment, the plants were removed from the vessels, washed in 1% nitric solution and rinsed with deionized water. The final weight of sample fresh matter (FM) was recorded, and then the plants were placed in a ventilated greenhouse at 80°C to arrive at the final dry weight (DM). The dry matter initial weight for each species was estimated from 10 samples of plants randomly chosen from the same batch as those used for the experiment. The DM data was used in relative growth rate analysis, according to the equation proposed by Hunt (1978).

Total chlorophyll content and carotenoid content for each species were determined from 0.5 g of fresh plant matter, and 80% acetone v/v was used as extractant, with CaCO_3 (Arnon 1949). The absorbance reading was made at 470 nm (carotenoids), 646.8 and 663.2 nm wavelengths (total chlorophyll) in UV Visible spectrophotometer (UVmini-1240, Shimadzu, Japan). From the data obtained, total chlorophyll content and carotenoid content were calculated using the equations proposed by Lichtenthaler (1987).

In order to determine Mn content, dry matter samples underwent wet mineralization with 3 ml of nitro-perchloric mixture (2:1, v/v) to 120-140°C, until organic matter digestion was completed (Tedesco *et al.* 1995). The mineralized samples were diluted in 25 ml of deionized water, and Mn content determined by atomic absorption spectrometry (AAS 220 FS, Varian Australia) at 279.8 nm wavelength.

The data was analyzed using ANOVA (significance level $p < 0,05$) and the averages were compared by Tukey testing. The software used for the statistical analysis was SAS (SAS Institute, Cary, NC).

Results

The macrophytes *Azolla caroliniana* and *Salvinia minima* presented visual symptoms of toxicity from the fifth day of Mn exposure. Chlorotic spots were identified on fronds of both species,

but only on plants subjected to 0.4 mM of Mn (Fig. 1a-d). In *A. caroliniana*, these visual symptoms were less intense.

In *Spirodela polyrhiza* plants, the symptoms were visible from 0.2 mM concentration of Mn, being more pronounced in 0.3 and 0.4 mM concentrations. After the fourth day of exposure to Mn, the leaves showed chlorosis at the basal region, gradually extending to the apical region (Fig. 1e-f).

At the end of the experiment, the relative growth rate of *A. caroliniana* and *S. minima* did not decrease significantly with excess Mn in the nutrient solution (Fig. 2a-b). However, there was a reduction (16.22%) of *S. polyrhiza* growth rate beginning at 0.2 mM Mn concentration. A more pronounced decrease (21.34%) was observed in at 0.3 and 0.4 mM Mn concentrations (Fig. 2c).

Excess Mn in the solution modified total chlorophyll content in the three macrophytes studied. The changes happened in different ways among the species, as well as among the different treatments (Fig. 3a-c). Total chlorophyll content for *A. caroliniana* showed no statistical difference between control plants and plants exposed to 0.05 and 0.1 mM concentrations of Mn. Starting at 0.2 mM concentration, total chlorophyll content was significantly reduced (26.67%), but did not change at 0.3 and 0.4 mM concentrations of Mn (Fig. 3a). In *S. minima* plants, there was a 39.69% reduction of total chlorophyll content, but only in plants exposed to 0.4 mM concentration (Fig. 3b). The decrease in chlorophyll content in *S. polyrhiza* plants (16.49%) was statistically different from the control plants starting at 0.2 mM concentration of Mn. A more pronounced decrease (32.98%) was recorded for 0.3 and 0.4 mM Mn concentrations (Fig. 3c).

Carotenoid content was significantly reduced in *A. caroliniana* (27.02%) from 0.2 mM concentration of Mn, but was not different at 0.3 and 0.4 mM concentrations (Fig. 4a). Statistically, in *S. minima*, there was no change in carotenoid content between plants exposed to Mn and control plants (Fig. 4b). In *S. polyrhiza*, carotenoid content displayed a reduction of 25.34% in plants exposed to 0.3 and 0.4 mM concentrations of Mn, which were not different among themselves (Fig. 4c).

The Mn content in the DM of plants exposed to different concentrations of that element rose significantly in relation to control plants for the three macrophytes studied. The increase observed was proportional to the concentrations of Mn in the solution (Fig. 5a-c).

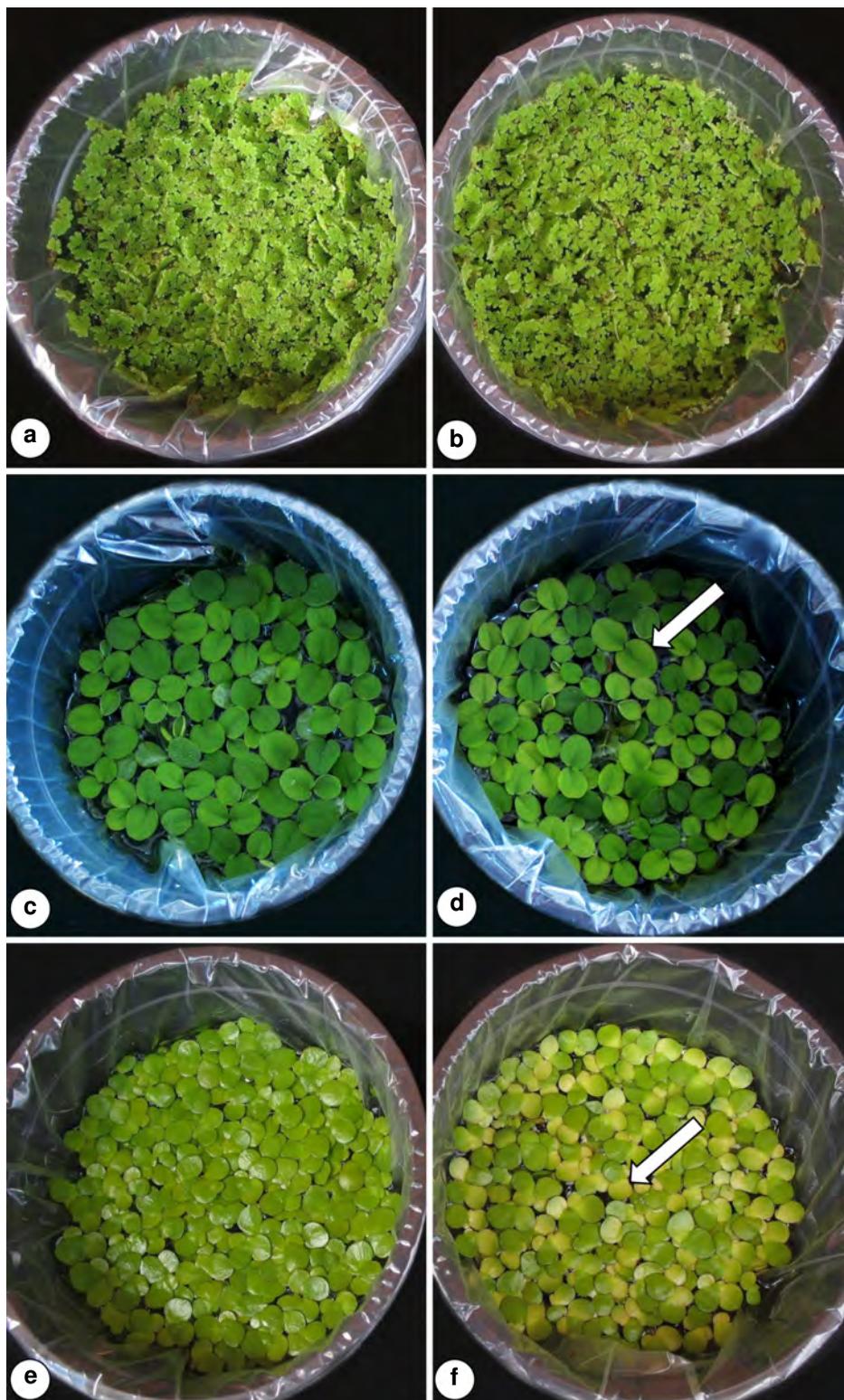


Figure 1 – Characterization of the visual symptoms of aquatic macrophytes – a, c, e. control plants; b, d, f. plants exposed to 0.4 mM Mn; a-b. *Azolla caroliniana*; c-d. *Salvinia minima*, d. overview of plants exposed to Mn, showing fronds chlorosis (arrow); e-f. *Spirodela polyrrhiza*; f. overview of plant exposed to Mn showing leaves chlorosis (arrow).

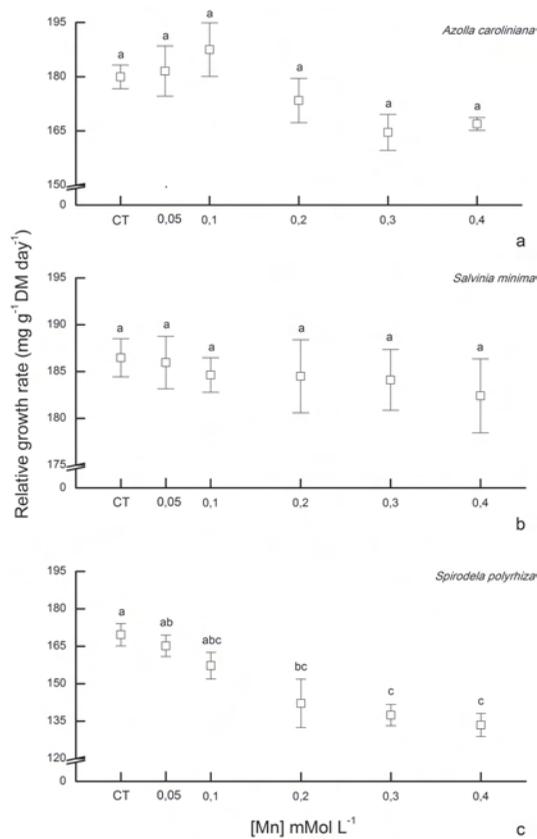


Figure 2 – Relative Growth Rate for *Azolla caroliniana* (a), *Salvinia minima* (b) and *Spirodela polyrhiza* (c), for different concentrations of Mn. Significant differences are indicated by different letters (a-c) above the bars ($P < 0,05$). CT = Control.

However, Mn accumulation for *A. caroliniana* did not show statistical differences among treatments 0.05; 0.1 and 0.2 mM, but rose in 0.3 and 0.4 mM concentrations (Fig. 5a). In *S. minima*, accumulated Mn was similar between treatments 0.05 and 0.1 mM, increasing in treatments 0.2; 0.3 and 0.4 mM, yet, the latter concentrations were not statistically different between themselves (Fig. 5b). The content of accumulated Mn in *S. polyrhiza* increased proportionally in relation to its concentration in solution, and showed significant differences among all Mn concentrations (Fig. 5c).

Discussion

Knowledge of plant response to Mn accumulation is limited. Literature provides some descriptions for woody or shrubby species, and mostly for vascular plants with economic value (Memon & Yatazawa 1980; Caldwell 1998; Fecht-Christoffers *et al.*

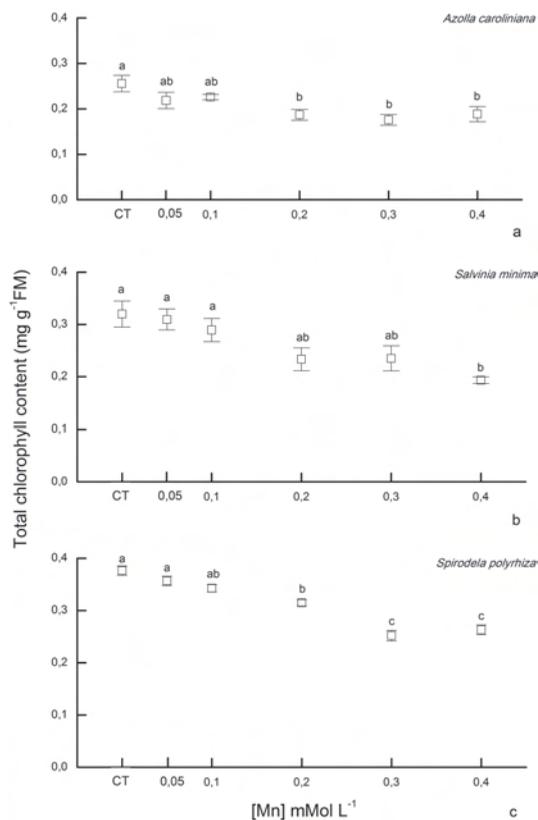


Figure 3 – Total chlorophyll content in *Azolla caroliniana* (a), *Salvinia minima* (b) and *Spirodela polyrhiza* (c), for different concentrations of Mn. Significant differences are indicated by different letters (a-c) above the bars ($P < 0,05$). CT = Control.

2003; Fernando *et al.* 2006). However, information on aquatic macrophytes is scarce.

In this study, the aquatic macrophytes *Azolla caroliniana*, *Salvinia minima* and *Spirodela polyrhiza* accumulate Mn in their tissues. Yet, these species can be distinguished for their capacity to accumulate the element.

Azolla caroliniana showed low values (0.080 – 1.341 mg g⁻¹ DM) of accumulated Mn in relation to the other two species. Studies by Santos (2006) and Guimarães (2006) assessed the potential of *A. caroliniana* to accumulate Arsenic (As) and found that this species also presented low capacity to accumulate the metalloid in relation to *Lemna gibba* L. and *S. minima* plants. Presumably, *A. caroliniana* has an exclusion mechanism in response to metal excess in solution, including Mn, as well as As. Thus, by reducing metal accumulation in the tissues, it reduces the chance of plant toxicity,

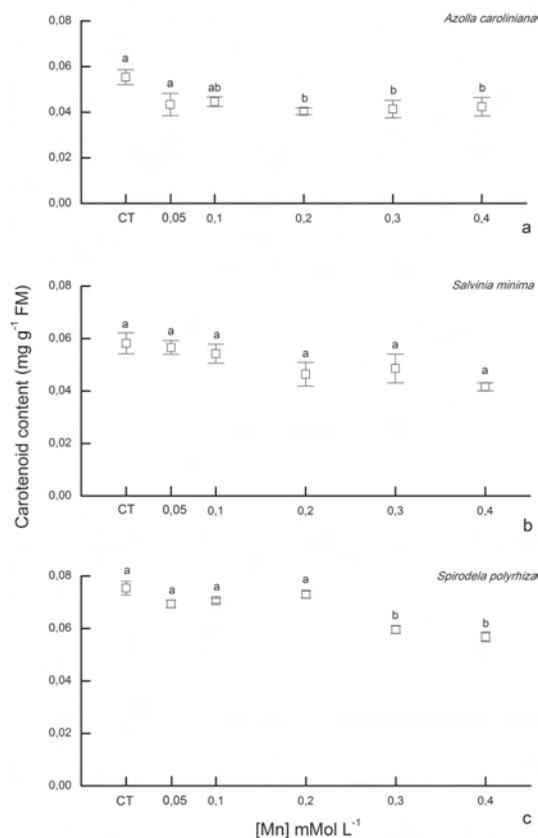


Figure 4 – Content of carotenoids in *Azolla caroliniana* (a), *Salvinia minima* (b) and *Spirodela polyrhiza* (c), for different concentrations of Mn. Significant differences are indicated by different letters (a-b) above the bars ($P < 0,05$). CT = Control.

granting more tolerance to the plant (Reichman 2002). This fact was verified in the present study when *A. caroliniana* plants exposed to the highest concentration of Mn showed less visible symptomatology than the other tested species.

Salvinia minima showed intermediate values (0.097 – 4.283 mg g⁻¹ DM) for Mn accumulation in relation to *A. caroliniana* and *S. polyrhiza*. This data provides positive indications for the use of this species to mitigate water bodies contaminated with Mn. The accumulated Mn values and the fact that these plants did not have their growth affected even when exposed to 0.4 mM of Mn are promising results, since aquatic macrophytes used in any treatment system must primarily comply with two important criteria: pollutant absorption capacity and high growth rate (Visoottiviseth *et al.* 2002).

The implementation of this genus (*Salvinia*) in metal removal programs has been recommended

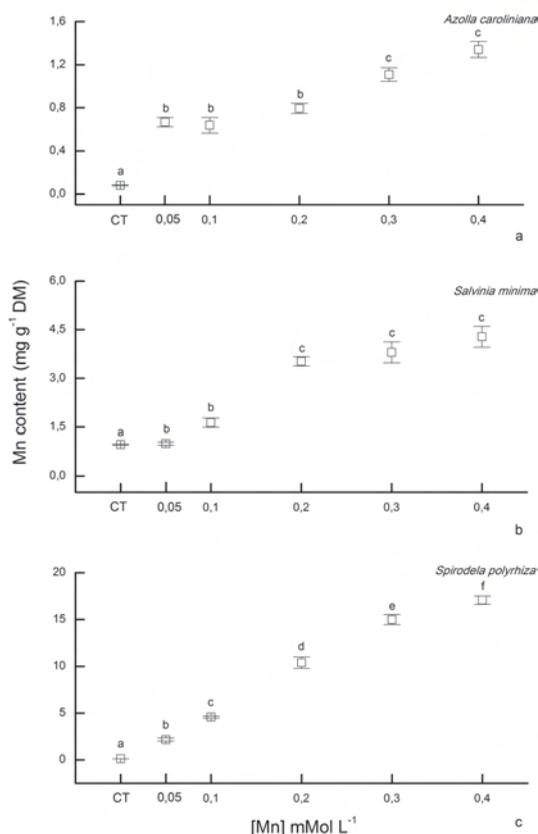


Figure 5 – Accumulation of Mn in plants of *Azolla caroliniana* (a), *Salvinia minima* (b) and *Spirodela polyrhiza* (c) for different metal concentrations. Significant differences are indicated by different letters (a-f) above the bars ($P < 0,05$). CT = Control.

by some researchers (Olguín *et al.* 2002; Hoffmann *et al.* 2004; Soares *et al.* 2008). Accumulation and toxic-effect responses were reported for that species in stress situations with Cadmium (Cd) (Olguín *et al.* 2002), Lead (Pb) (Hoffmann *et al.* 2004), Chrome (Cr) (Nichols *et al.* 2000), Arsenic (As) and atrazine (Guimarães 2006). However, information in the literature on *S. minima* potential for absorbing and accumulating Mn is scarce, and the results of the present study are pioneer.

Spirodela polyrhiza species accumulated high concentrations of Mn (17.062 mg g⁻¹ DM) in plant tissues. Sinha *et al.* (1994) also observed the capacity of that species to accumulate Mn. *S. polyrhiza* potential to accumulate different contaminants has been reported by many authors (Tripathi & Chandra 1991; Sinha *et al.* 1994; Rai *et al.* 1995; Noraho & Gaur 1996), and the use of that species for phytoremediation has been considered

very promising. Rai *et al.* (1995) observed that *S. polyrhiza* was able to reduce the copper level in 90% in a solution. Sinha *et al.* (1994) reported that *S. polyrhiza* accumulated significant amounts of iron (71 μmol), this capacity being similar to the data obtained in this study for Mn.

The main characteristic required for the use of plant species in phytoremediation technology is pollutant bioaccumulation, which is related to high productivity of plant biomass (Vissottiviset *et al.* 2002). However, excessive accumulation of metals in plants usually leads to changes in physiological and biochemical processes, which can be damaging to plant metabolism. This affects the growth and development of the plant (Barceló & Poschenrieder 1990; Oliveira *et al.* 2001), and therefore can make the use of plants in phytoremediation technology impossible.

In this study, we found that Mn accumulation in plants exposed to 0.2–0.4 mM concentrations is associated with toxicity symptoms in all three species studied; yet, such events displayed different characteristics. A reduction of total chlorophyll content was observed in *A. caroliniana*, *S. minima* and *S. polyrhiza*. The reduction of chlorophyll content in plants exposed to Mn has been associated, at least partially, to the fact that Mn leads to iron deficiency in plant tissues, an element necessary for chlorophyll synthesis (Csatorday *et al.* 1984; Beale 1999). Another possibility would be the fact that Mn can replace the Mg ion in the chlorophyll molecule, and so impede the metabolic function of the molecule (Mukaopadhyay & Sharma 1991; Hauck *et al.* 2002).

The decrease in carotenoid content for *S. polyrhiza* and *A. caroliniana* reinforce the results found by Caldwell (1998) and Asrar *et al.* (2005) for different terrestrial plants exposed to Mn excess. Carotenoid reduction was proven to be related to an increase in Mn content in thylakoids, presumably interfering with stacking and pigment content in the chloroplasts (Lidon *et al.* 2004). This hypothesis could explain the carotenoid-content reduction observed in *A. caroliniana* and *S. polyrhiza* after the increase in Mn concentrations (0.2 to 0.4 mM) in solution, which was followed by a Mn content increase in macrophyte dry matter.

The fact that vegetative growth was not significantly affected in *A. caroliniana* and *S. minima*, even in the presence of high concentrations of Mn, may be due to lower Mn content accumulated in macrophyte tissues. On the other hand, the reduction in *S. polyrhiza* growth

rate after exposure to Mn excess may have been a consequence of the high concentration of the element in plant tissues, leading to nutritional imbalance, and also to a decrease in total chlorophyll content observed in the plants, since that pigment is substantial to the process of carbon conversion to biomass.

Responses to Mn accumulation among the three species studied are probably related to differences in absorption and Mn content accumulated in the plant tissues, which grants different tolerance patterns to the element. Results show that *A. caroliniana* and *S. minima* tolerate Mn excess in solution. However, the plants were not effective in accumulating high amounts of the metal in their tissues.

Further studies must be conducted, including more refined analysis, with the purpose of enhancing knowledge about aquatic plants response to Mn excess. The combined and/or alternate use of the species studied could be a promising practice capable of aiding Mn sequestration in contaminated aquatic environments, especially those where metal concentrations are not too high.

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