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Biomass reduction of *Salvinia molesta* exposed to copper sulfate pentahydrate (CuSO$_4$.5H$_2$O)

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ABSTRACT

Copper in the aquatic ecosystem may remain adsorbed or be incorporated into the biomass and undergo biomagnification causing unwanted effects to aquatic macrophyte communities. This study evaluated the biomass reduction of *Salvinia molesta* (Mitchell) exposed to copper sulphate pentahydrate (CuSO$_4$.5H$_2$O) under laboratory conditions. Approximately 20.5 g of fresh mass (FM) of *S. molesta* (0.74 g dry matter, DM) were placed in glass tanks with different concentrations (n = 3) of CuSO$_4$.5H$_2$O as follows: 0.0; 2.0; 4.0; 6.0; and 8.0 mg L^{-1} for 28 days. The dry mass was determined after each seven-day interval over 28 days and submitted to repeated ANOVA measures, followed by a Tukey test ($P<0,05$). The results show that macrophyte increased until the seventh day of exposure in all treatments. After this period, the biomass of *S. molesta* decreased; but there was no significant difference between treatments with copper, except for the 8.0 mg L^{-1} treatment. The copper treatments decreased the *S. molesta* biomass an average of 43.2% (0.50 g DM) after 28 days. At the end of the experiment, copper absorption in the treatments with 6.0 and 8.0 mg L^{-1} was on average 77.9% higher than in the treatments with 2 and 4 mg L^{-1}. The treatments with 6.0 and 8.0 mg L^{-1} reached their maximum bioaccumulation capacity after 14 days. The results show that contamination of the aquatic environment at concentrations above 2 mg L^{-1} Cu^{2+} can reduce the *S. molesta* biomass by approximately 43%.

Keywords: aquatic macrophytes, heavy metal, phytoremediation.

Redução da biomassa de *Salvinia molesta* exposta ao sulfato de cobre pentahidratado (CuSO$_4$.5H$_2$O)

RESUMO

No ecossistema aquático, o cobre pode permanecer adsorvido ou ser incorporado à biomassa e sofrer biomagnificação ocasionando efeitos indesejados às macrófitas aquáticas. Este trabalho avaliou a redução da biomassa da *Salvinia molesta* (Mitchell) exposta ao sulfato de cobre pentahidratado (CuSO$_4$.5H$_2$O), em condições de laboratório. Aproximadamente 20,5 g de massa fresca (MF) de *S. molesta* (0,74 g de massa seca, MS) foram dispostas em aquários de vidro e submetidas (n=3) às concentrações de 0,0; 2,0; 4,0; 6,0; e 8,0 mg L^{-1} por

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28 dias. A massa seca foi determinada a cada sete dias durante 28 dias e os dados submetidos à ANOVA de medidas repetidas, seguida do teste de Tukey ($P<0,05$). Em todos os tratamentos *S. molesta* cresceu até o sétimo dia de exposição. Após esse período houve redução da biomassa de *S. molesta*, porém não ocorreu diferença significativa entre os tratamentos com cobre, exceto para o tratamento com 8.0 mg L^{-1}. A redução média da biomassa de *S. molesta* nos tratamentos com cobre foi 43,2% (0,50 g MS) aos 28 dias. Ao final do experimento a absorção do cobre nos tratamentos com 6,0 e 8,0 mg L^{-1} foi em média 77,9% maior em comparação à absorção nos tratamentos com 2 e 4 mg L^{-1}. Os tratamentos com 6,0 e 8,0 mg L^{-1} atingiram sua capacidade máxima de bioacumulação aos 14 dias. Os resultados mostram que contaminação de ambientes aquáticos com concentrações acima de 2 mg de Cu^{2+} L^{-1} pode ocasionar redução de aproximadamente 43% da biomassa de *S. molesta*.

Palavras-chave: fitorremediação, macrófitas aquáticas, metal pesado.

1. INTRODUCTION

Human activities cause multiple environmental impacts on aquatic environments. Among these activities, the incorrect use of fertilizers and increasing industrial activities have led to increasing contamination of soil and water bodies by metal ions (Moyo and Phiri, 2002; Espinoza-Quiñones et al., 2005; Penning et al., 2008).

Copper sulfate ($CuSO_4$) can contaminate aquatic ecosystems indirectly during the spraying of crops due to entrainment caused by rain and, naturally, by soil erosion. The copper ion also reaches aquatic environments directly when industrial effluents are released into water bodies and when copper sulfate is used to control algae in reservoirs and irrigation equipment (Who, 1998; Boyd, 2015). In Brazil, the use of copper sulphate pentahydrate ($CuSO_4.5H_2O$) is not allowed; but it is widely used in other countries to control parasitoids in aquaculture (e.g. *Ichthyophtirius mutifiliis)*, diseases of bacterial and fungal origin (Cardeilhac and Whitaker, 1988) and algae blooms, as well (Boyd and Massaut, 1999). In Brazil, the maximum value of copper concentration released in the receiving water bodies is 1.0 mg L^{-1} (Conama, 2011); however, continuous use on crops and in industrial activities may result in the accumulation of ions in aquatic ecosystems.

Copper is essential for certain cellular activities at low concentrations, but it can be toxic, causing mortality or sub-lethal stress in high concentrations (Mazon et al., 2000; Pourahmad and O' Brien, 2000). The presence of copper in the environment poses a toxicity threat to animals and plants (McBride et al., 1997). In aquatic ecosystems copper can be adsorbed to the sediment by ion complexation reactions and influence the bioavailability, transport and migration of metallic cations (Bezerra et al., 2009). As a result of its conservative characteristics, copper may result in bioconcentration and biomagnification in the food chain (Amarante et al., 2010; Marengoni et al., 2013).

Aquatic macrophytes play a key role in nutrient cycling and primary production of aquatic ecosystems, because they promote the exchange of chemical elements of the sediment to the water column and are an important food source for aquatic and terrestrial animals (food web), organic matter and debris (detritivores web), in addition to providing substrate, shelter and refuge for fish, zooplankton and macro-invertebrates (Camargo et al., 2003; Thomaz and Cunha, 2010; Mormul et al., 2010; Batista-Silva et al., 2012; Súarez et al., 2013).

Salvinia molesta (Mitchell) is a small, floating aquatic macrophyte of the Salvinaceae family, widely distributed in Brazil. The *Salvinia* genus is native to the Neotropics, extending from Mexico and the Galapagos Islands through Central America and Caribbean and most of South America (Sculthorpe, 1967). This macrophyte presents high growth rates, high rates of reproduction and the ability to accumulate nutrients (McFarland et al., 2004; Cary and

Weerts, 1984). The sensitivity to different substances allows the use of *S. molesta* as a biological indicator of contaminated environments (Suñe et al., 2007) while its high growth rate favors its use as phytoremediation agent. In addition to the ability to absorb nutrients, Kumar et al. (2008) and Ladislas et al. (2012) reported that some species of macrophytes have great potential for removing heavy metals from water and sediments. Although the metal removal capacity is a desirable feature in macrophytes used in phytoremediation of contaminated aquatic environments, it is important to know the morphological and physiological damage caused by heavy metal accumulation on macrophyte tissues (Milan et al., 2006).

The accumulation of metals can reduce plant biomass and, consequently, habitat heterogeneity and primary production in aquatic environments. Proença et al. (2012) demonstrated that the aquatic macrophytes *Lemna minor* were able to grow in low copper concentrations (0.01, 0.1 and 1.0 mg L^{-1}) (i.e., the stimulatory effect), but the biomass was reduced when exposed to a concentration of 10 mg L^{-1} (inhibitory effect). Cruz et al. (2009) used *Pistia stratiotes* L. (Araceae) to treat mining effluents containing Mn and Zn and reported that the accumulation of heavy metals in plant tissue may cause phytotoxicity and reduce plant biomass.

Floating aquatic macrophytes with large biomass, such as *Eichhornia crassipes* and *Pistia stratiotes* are often used for phytoremediation because they are more resistant to the adverse conditions of the contaminated water (Mondardo et al., 2006; Cruz et al., 2009). In an experiment using *E. crassipes* for 21 days, Mokhtar et al. (2011) reported the removal of 97.3, 95.6 and 61.6% of the copper present in solution with 1.5, 2.5 and 5.5 mg L^{-1}, respectively.

The effects of copper on floating macrophyte with small biomass such as *Salvinia molesta* (Mitchell) are poorly understood. The wide geographic distribution of *S. molesta* in Brazil, even in environments with low nutrient concentrations (Rubim and Camargo, 2001), and the fact that *S. molesta* may be subjected to possible biomass reduction resulting from contamination by copper ions (Cu^{2+}) in aquatic environments, led us to evaluate the effects of copper sulfate pentahydrate on *S. molesta* biomass.

2. MATERIALS AND METHODS

An experiment was conducted to evaluate how copper affects *Salvinia molesta* biomass. The plants were exposed (in triplicate) to five copper sulphate pentahydrate concentrations (0.0, 2.0, 4.0, 6.0 and 8.0 mg L^{-1}) in 5.0 liter capacity tanks in laboratory conditions for 28 days.

The plants were selected by size and appearance, in good health and nutritional status. The tanks were filled with two liters of water and an average 20.5 g of fresh mass (FM) of *S. molesta,* corresponding to approximately 0.74 g dry matter (DM). The fresh weight was determined after removing excess water by keeping the plants in the shadow, inside sieves for 5 minutes and, after that, they were laid on paper to drain for 2 minutes. The initial dry matter was estimated by simple linear regression between fresh (FM) and dry matter. The regression between FM and DM yielded a correlation value of R²=0.997 (n=15) and the obtained equation was DM = 0.0313*(FM) + 0.1019. The dry weight was determined after the plants were dried at 105°C for 24 hours, as recommended by Wetzel and Likens (1991).

The copper sulfate pentahydrate ($CuSO_4.5H_2O$, *Pro analisis*®) was 99.99% pure. The water added to the laboratory tanks came from the *S. molesta* maintenance tanks to reduce the impact of the transfer on the plants. Table 1 presents the water physical and chemical variables.

Table 1. Mean (n=3) and standard deviation values of temperature (temp.), pH, dissolved oxygen (DO), electrical conductivity, total Kjeldahl nitrogen (TKN) and total phosphorus (TP) of water in the tanks.

Physical and chemical variables	Temp. (ºC)	pH	DO (mg L^{-1})	Electrical Cond. (mS cm^{-1})	TKN (mg L^{-1})	TP (µg L^{-1})
Macrophytes tanks collection	27.1± 0.6	7.9 ± 0.4	6.0 ± 0.6	0.094±0.023	0.50±0.12	205.0 ± 29.1

The experiment was conducted in 60 tanks under controlled conditions (26.5 ± 2.0ºC and photoperiod of 8 hours). Every seven days, 15 tanks were removed and all macrophyte biomass was used to determine fresh mass (FM, g), dry matter (DM, g) and copper content. Copper content in the biomass of macrophytes (mg g^{-1}) was determined following the B-3120 method described in APHA (2005). The 15 tanks corresponded to five treatments (0.0; 2.0; 4.0; 6.0 and 8.0 mg L^{-1}) and three repetitions.

The data regarding dry matter and copper content in the macrophytes biomass at 7, 14, 21 and 28 days were subjected to descriptive statistics. Subsequently, the dry weight data were submitted to the Kolmogorov-Smirnov' and Bartlett's test to verify the normality and homoscedasticity, respectively. If the assumptions were confirmed, the dry weight data were submitted to repeated ANOVA measures to verify the effects of copper (0.0; 2.0; 4.0; 6.0 and 8.0 mg L^{-1}) on the *S. molesta* biomass. Significantly different means *(P<0.05)* were then compared by the Tukey test.

3. RESULTS AND DISCUSSION

The repeated ANOVA measures pointed to significant effects of treatments (i.e. copper concentrations) and time (samplings days). Interactions among the effects of time and copper concentrations were also observed (Table 2). In the control treatment, *S. molesta* biomass was significantly higher than in the others. *Salvinia molesta* exposed to 8.0 mg L^{-1} had lower biomass; and no significant difference was observed among 2.0, 4.0 and 6.0 mg L^{-1} treatments (Table 2).

Table 2. Mean (n=12) of *Salvinia molesta* biomass (g DM) in different copper concentrations with results from the repeated measures ANOVA upon the effects of treatment (Treat.), time and interaction (Treat. x time).

Copper concentration (mg L^{-1})	Treatments (Treat.)					Anova *P* value		
	0.0	2.0	4.0	6.0	8.0	Treat.	Time	Treat. x Time
	0.98a	0.63b	0.64b	0.64b	0.58c	<0.001	<0.001	<0.001

The biomass of macrophyte increased until the seventh day of exposure in all treatments. After this period *S. molesta* biomass reduced, although not significantly different *(P<0.05)* between treatments, except compared to the control (Figure 1). In the control treatment, biomass increased an average 29.7% throughout the experiment. At the end of the experiment, *S. molesta* biomass (mean 0.50 g DM) reduced on average 43.2% in the treatments with copper, compared to 7th day (mean 0.88 g DM). After 28 days, biomass (0.50 g DM) reduced 45.1% for 2.0 mg L^{-1} copper concentration and, 44.4% (0.50 g DM), 40.2% (0.52 g DM) and 41.7% (0.49 g DM) for 4.0, 6.0 and 8.0 mg L^{-1}, respectively (Figure 1). This result suggests that the contamination of the aquatic environment with concentrations greater than 2.0 mg L^{-1} can cause heavy losses to the *S. molesta* biomass. Exposure of *Lemna minor* to the

concentration of 10 mg L^{-1} Cu^{2+} reduced the biomass macrophyte by 62.1% (Proença et al., 2012). *Salvinia auriculata* exposed to zinc (ZnSO$_4$.7H$_2$O) concentrations of 7.5 and 10.0 mg L^{-1} decreased by 25 and 38%, respectively (Wolff et al., 2009).
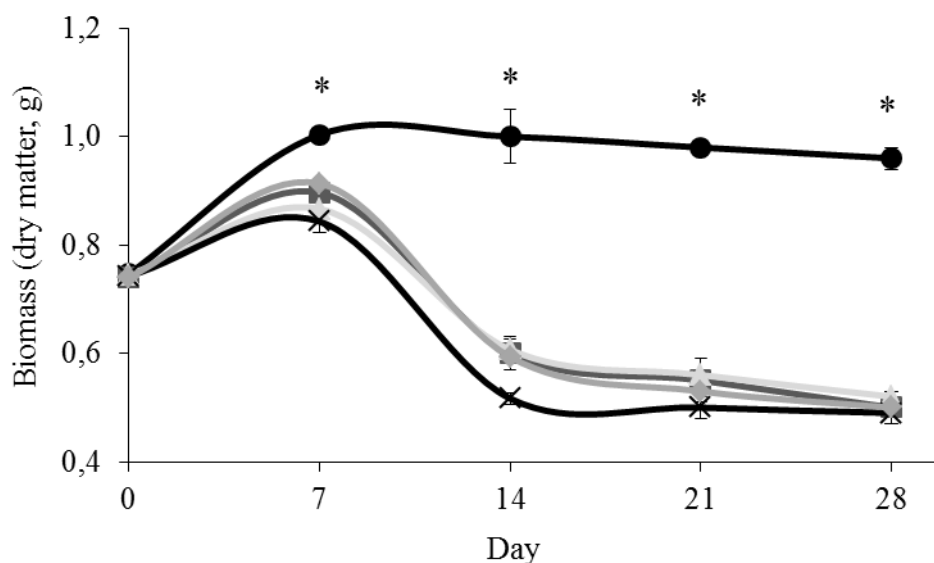

Figure 1. Means (n=3) and standard deviation of *Salvinia molesta* biomass in the 0.0 (●), 2.0 (♦), 4.0 (■), 6.0 (▲) and 8.0 mg L^{-1} (x); * denotes significantly different (*P*<0.05) between treatments.

The biomass decreased with the copper treatment due to leaf necrosis after 14 days of trial and, especially, to early root senescence from the 7th day. After this period, senescence and detachment from the plant roots could be observed. Several authors reported that heavy metals are not accumulated in the roots (Kumar et al., 2008; Mansouri et al., 2012; Rascio and Navari-Izzo, 2011); however, the floating aquatic macrophytes absorb most of the nutrients by the roots (Tundisi and Tundisi, 2008). This fact suggests that *S. molesta* biomass reduction begins in the root system caused by ion copper absorption. For example, in *P. stratiotes* mercury accumulation was about 4 times higher in roots than in the upper parts (Snow and Ghaly, 2008; Ayyasamy et al., 2009). So, although floating aquatic macrophyte with high biomass are more resistant to adverse environmental conditions, copper contamination of aquatic environments can reduce the biomass of species such as *Eichhornia crassipes* and *Pistia stratiotes* due to higher ion absorption caused by greater root area. The length of *S. molesta* roots (i.e., root-like structures that are actually modified leaves) ranges from about 2.4 to 10.5 cm (Room, 1983), whereas *E. crassipes* root length can reach 30 cm (Meerhoff et al., 2003).

At the end of the experiment, copper absorption in the treatments with 6.0 (4.3 mg g^{-1} DM) and 8.0 mg L^{-1} (6.2 mg g^{-1} DM) was on average 77.9% higher than in the treatments with 2.0 (2.0 mg g^{-1} DM) and 4.0 mg L^{-1} (3.9 mg g^{-1} DM). *Salvinia molesta* exposed to copper concentrations of 6.0 and 8.0 mg L^{-1} absorbed more rapidly and reached maximum bioaccumulation capacity at 14 days, when the copper content in the macrophyte biomass reached 4.8 and 6.9 mg Cu^{2+} g^{-1} DM, respectively (Figure 2). Probably this result is due to the higher initial availability of copper. Wolff et al. (2009) reported that the zinc content in *S. auriculata* biomass showed a positive relationship with the highest concentrations in the solution. In this study, a relationship between the initial concentration and the copper content in the macrophyte biomass was also observed. However, no positive correlation was observed between decreasing biomass and increasing concentration in the solution, because the

reduction was similar in all concentrations, except for 8.0 mg L^{-1}. In addition to the absorption process, the ion adsorption by macrophyte structures (e.g., root structures) favor the reduction of copper. In fact, Módenes et al. (2013) related that *E. crassipes* dry matter reduced about 50% of Zn, Cu and Cd ions from aqueous solutions.

The copper content in the *S. molesta* dry matter also suggests that after 14 days copper starts to be transferred to the medium (i.e. solution), especially in the 6.0 and 8.0 mg L^{-1} treatments (Figure 2). This fact may be related to loss of copper absorption capacity resulting from ion saturation in the plant biomass. In the treatments with 2.0 and 4.0 mg L^{-1} copper content increased steadily in the macrophyte biomass, indicating that at these concentrations *S. molesta* did not reach maximum bioaccumulation capacity. Heavy metals such as cadmium, copper, lead, zinc and nickel can trigger toxic effects on organisms due to bioaccumulation and biomagnification in the food chain (Dembitsky and Rezanka, 2003; Ugya et al., 2015).
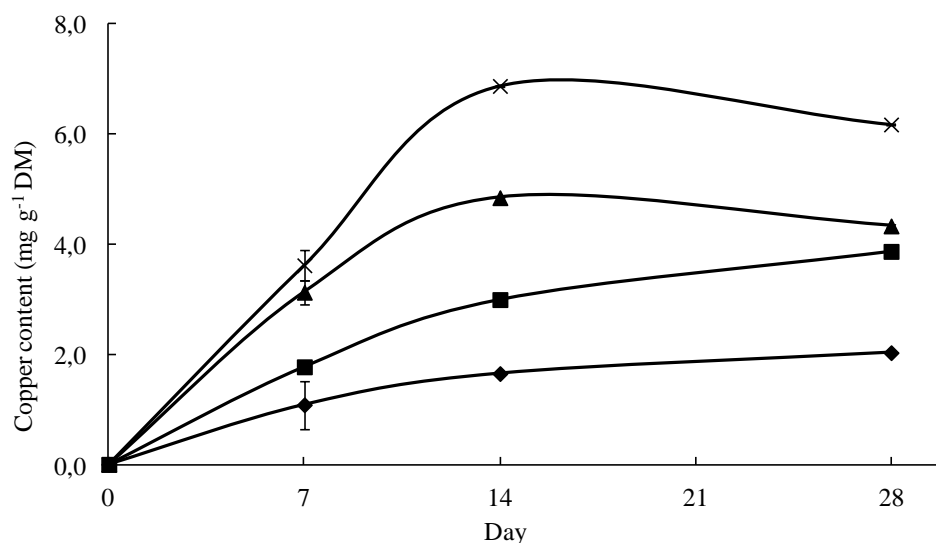

Figure 2. Copper content in *Salvinia molesta* biomass for the treatments with 2.0 (♦), 4.0 (■), 6.0 (▲) and 8.0 mg L^{-1} (x).

4. CONCLUSION

The exposure of *Salvinia molesta* to copper sulphate pentahydrate causes the macrophyte biomass to decrease; however, this decrease was similar in the 2.0; 4.0 and 6.0 mg L^{-1} treatments, and slightly higher in the 8.0 mg L^{-1} treatment. Thus, no positive correlation was observed between copper concentration and biomass reduction. Nevertheless, at higher concentrations (6.0 and 8.0 mg L^{-1}) accumulation was also higher and possibly reached copper saturation due to rapid absorption of the ion. These results also suggest that contamination of the aquatic environment at concentrations of 2 mg L^{-1} Cu^{2+} can reduce *S. molesta* biomass by approximately 43%.

5. ACKNOWLEDGEMENTS

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