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Full Length Research Paper

Effect of cadmium on the morphology and anatomy of *Salvinia auriculata*

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This study aimed to evaluate the morphological and anatomical changes of *Salvinia auriculata* exposed to different concentrations 0, 2.5, 5, 7.5 and 10 μM of cadmium (Cd) and its effect on plant growth. The experiment was conducted in the laboratory of Plant Anatomy of the IF Goiano/Rio Verde Campus, Goiás. Cd free samples of *S. auriculata*, was obtained from the Aquários Plantados company, located in Belo Horizonte. The material was grown hydroponically for 20 days and after the experimental period, the leaf samples were fixed, including in historesin, cut to 5 μm thick thick, stained with toluidine blue and the images were obtained in an optical microscope. The toxic effects of Cd on *S. auriculata* was observed at lowest concentration with the appearance of chlorotic and necrotic spots. Microscopic analysis showed increased height and width of the aerenchyma gaps, mesophyll and a reduction in abaxial surface epidermal cells, due to increased doses of this metal. It was observed that *S. auriculata* is a plant sensitive to Cd, and thus indicated for environmental monitoring.

Key words: Ecological, aquatic species, pollution.

INTRODUCTION

The pollution of soil and water by heavy metals is due to industrial, agricultural activities and urbanization, and has become a serious problem with great environmental impacts (Carneiro et al., 2002). Contamination of water resources by heavy metals has been of concern to researchers and government agencies (Oliveira et al., 2001). Some of these metals, such as lead (Pb), zinc (Zn), arsenic (As) and cadmium (Cd), which in addition to being toxic in small quantities, are able to accumulate and interfere in the food web.

Cd is highly toxic, often disposed of improperly in the environment and may reach the soil, aquatic media or the air by the burning of municipal waste and fossil fuels, thus contaminating the environment and altering the ecosystem (Pino, 2005). The problems arising from this metal are not limited to the environmental area. Poisoning of living beings by this metal can bring specific problems according to the type of contamination, when

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poisoning occurs through the airways by inhalation of Cd dust, problems may occur in the respiratory tract and kidneys, which can lead to death; in the case of poisoning via oral ingestion of a significant amount of Cd, immediate poisoning and damage to the liver and kidneys may arise; poisoning through physical contact causes genetic changes (Brady and Humiston, 1986).

Worldwide, studies into the recovery of contaminated area by phytoremediation are being studied (Gardea-Torresdey et al., 2005; Vardanyan and Ingole, 2006). To evaluate the effectiveness of phytoremediation, it is necessary to expose the species in question to the contaminant, for a good phytoremediator, needs to accumulate relatively high concentrations of pollutants in its tissues without suffering toxic effects. However, if the plant is susceptible to the pollutant, presenting symptoms, it is considered an ecological pollution indicator.

According to Paiva et al. (2002), species, when exposed to heavy metal contaminated environments, respond in a very variable manner, it is necessary to test the behavior of each type of species, contamination period as well as evaluate the effect caused by the metal on morphology and growth, and resultant consequences to the macrophytes, confirming their use as bioindicadors. Due to lack of studies on the subject, this present study aims to evaluate the effects of Cd on the morphology and anatomy of *Salvinia* in order to contribute to information in environmental monitoring work.

MATERIALS AND METHODS

The experiment was conducted in plant anatomy laboratory at the Federal Institute of Education, Science and Technology Goiás (IFGoiano/Rio Verde Campus). Individuals of *S. auriculata* Aubl. (Salvinaceae) were purchased from the Aquários Plantados company, located in Belo Horizonte.

Plant growth

Adult *S. auriculata* individuals were disinfected using 1% sodium hypochlorite with immersion in this solution for 30 min. Plants were then washed with deionized water to remove excess hypochlorite. Subsequently, the plants were selected, to maintain homogeneity and placed in a 25 L capacity bowl containing Hoagland-Arnon nutrient solution modified with 1/5 ionic strength, pH 6.5 for 6 days for their adaptation.

After the adaptation period, plants were transferred to 1.2 L capacity pots with nutrient solution, maintaining the pH at 6.5 using 1 M HCl solution (hydrochloric acid) and 0.1 M NaOH (sodium hydroxide). To evaluate the effect of Cd, the following concentrations were adopted: 0 (T1), 2.5 (T2), 5 (T3), 7.5 (T4) and 10 µM (T5) in the form Cd $(NO_3)_2$. Morphological changes in the leaves were observed daily for 20 days. The solution was changed every three days and constantly aerated using a compressor.

Structural analysis

After 20 days of hydroponics 2 cm^2 leaf samples from leaf blade area were collected with the aid of forceps and cut with a disposable razor to one plant per pot, and fixed in Karnovsky solution (Karnovsky, 1965), for 24 h, dehydrated in increasing ethanol series, pre-infiltrated and infiltrated in historesin (Historesin, Leica) according to the manufacturer recommendations.

The material was sectioned to 5 µm thick, with a rotary microtome (RM 2155 model, Leica). The sections were stained with 0.05%, pH 4.0 toluidine blue (O'Brien et al., 1964) and mounted with Canada Balsam. The images were obtained in an Olympus BX61 microscope with a DP-72 camera.

The micro morphometric analyzes measurements were taken of the thicknesses of the epidermis (adaxial and abaxial faces), mesophyll and the aerenchyma gaps (height and width) of the leaves. All data were obtained with the aid of ImageJ - Image Processing and Analysis in Java software, Version 1.47, totaling 10 observations/ repetition for each structure evaluated.

The experimental design was completely randomized (CRD) with 4 replications and 5 treatments with each repetition consisting of 4 plants per pot. Data were subjected to analysis of variance (ANOVA) and regression using the statistical program Assistant.

RESULTS AND DISCUSSION

Plantlet growth

S. auriculata exposed to Cd show visible signs of toxicity, such as chlorosis and necrosis on the leaf surface with brown tint at the lowest dosage 2.5 µM (Figure 1B). With increasing doses of the metal added to the nutrient solution, the changes that the plant underwent were intensified (Figure 1C to F) compared to the control, free from the gradient (Figure 1A). This is a clear symptom observed in terrestrial plants, such as barley (Sridhar et al., 2007), as well as aquatic plants like *S. auriculata* (Siriwan et al., 2006) exposed to this metal.

According to Qian et al. (1999), the Cd^{2+} ions seem to be efficiently absorbed by the plant roots, their transport to other parts being very low, can be complexed and sequestered in cellular structures such as vacuoles, inhibiting its translocation to the shoot (Lasat, 2000). In addition, Cd can cause reduced growth, root development atrophy, leaf rolling and discoloration (Hutchinson and Czyrsk, 1975), necrotic tissue (Maine et al., 2000) and damage to metabolic systems or in protein synthesis (Oliveira et al., 2001).

At higher concentrations (10 and 20 µM of Cd) the toxicity symptoms most commonly observed by Oliveira and Mattiazzo (2001) working with water hyacinth was the presence of interveinal chlorosis in the leaves, which corroborates with the findings of the present study. Oliveira and Mattiazzo (2001) working with *Salvinia* observed reduction in plant survivability from Cd concentrations of 5 µM, and was not observed in this study.

Studies have shown that exposure of plants to high Cd concentrations causes growth inhibition and visible symptoms of chlorosis and necrosis in the leaves (Zhou et al., 2008; Clemens, 2006), caused by the photosynthetic decline and a decrease in the absorption and transport of nutrients (Larsson et al., 1998). When exposed to this gradient, plants are morphologically

Figure 1. *Salvinia auriculata* maintained for 20 days in nutrient solution with different concentrations of Cd. Control (A) and treated with 2.5, 5, 7.5 and 10 µM Cd (B, C, D, E, F) respectively. Dark spots were observed on the shoots (→).

damaged due to the substitution of Fe^{+2} by Cd^{+2} during physiological processes (Stohs and Bagchi, 1995). Yoshiara et al. (2006) working with *Nicotiana tabacum* observed that Cd causes Fe^{+2} deficiency.

Structural analyses

Compared to the control (Figure 2A), the leaf blade area showed alterations in the parenchymal architecture, with the increase in aerenchyma gaps of *S. auriculata* leaves (Figure 2B to F) evident. In aquatic plants, the aerenchyma developed mainly by the disintegration of cells, following several factors: oxygen deficiency promotes the production of ethylene by anaerobic stimulus, which causes an increase in the cellulase activity, which in turn leads to the cell disintegration and aerenchyma development (Fahn, 1982). In this case, the aerenchyma works as an alternative strategy for obtaining O_2 (Drew et al., 2000).

The same fact can be observed in the presence of Cd, as this metallic element stimulates ethylene production in plant species (Chen and Kao, 1995; Toppi et al., 1998). Although, the ethylene concentration in the different treatments was not evaluated in this present work, it is believed that the alterations observed in *S. auriculata* leaf aerenchyma may be related to changes in the concentrations of this hormone (Dantas et al., 2001).

In addition to alterations in epidermal cells, accumulation of content strongly stained was verified by toluidine blue (metachromasia) in the region beneath the adaxial epidermal (Figure 2B). Both at a Cd concentration of 7.5 µM as well as 10 µM, some points of the wall ruptured (Figure 2C, E and F).

The main changes caused by pollutants in plants are: increase or decrease in the production of some enzymes, genetic alterations and quantitative and qualitative alterations of metabolites (Pasqualini et al., 2003; Gerosa et al., 2003; Klumpp et al., 2006). As a result, symptoms arise such as chlorosis and necrosis in tissues and organs, leading to plant mortality, as are observed in this present study.

Increasing doses of Cd in the solution exerts adverse effect on plant growth. The plants were susceptible to this ion, since the lower dose of this element, besides causing visible symptoms on leaves, such as leaf tissue deterioration also caused a negative influence in plant growth during the twenty days of the experiment.

Sridhar et al. (2005) working with *Brassica juncea* (L) and *Palaquium ferrugineum* observed that the element Zn caused compression of the mesophyll and the reduction of intercellular spaces in the leaves. In *Elodea canadensis*, the mesophyll cells showed an increase in volume after Cd exposure, which led to a decline in the number of cells per area (Vecchia et al., 2005).

For the height of adaxial epidermis, it was observed

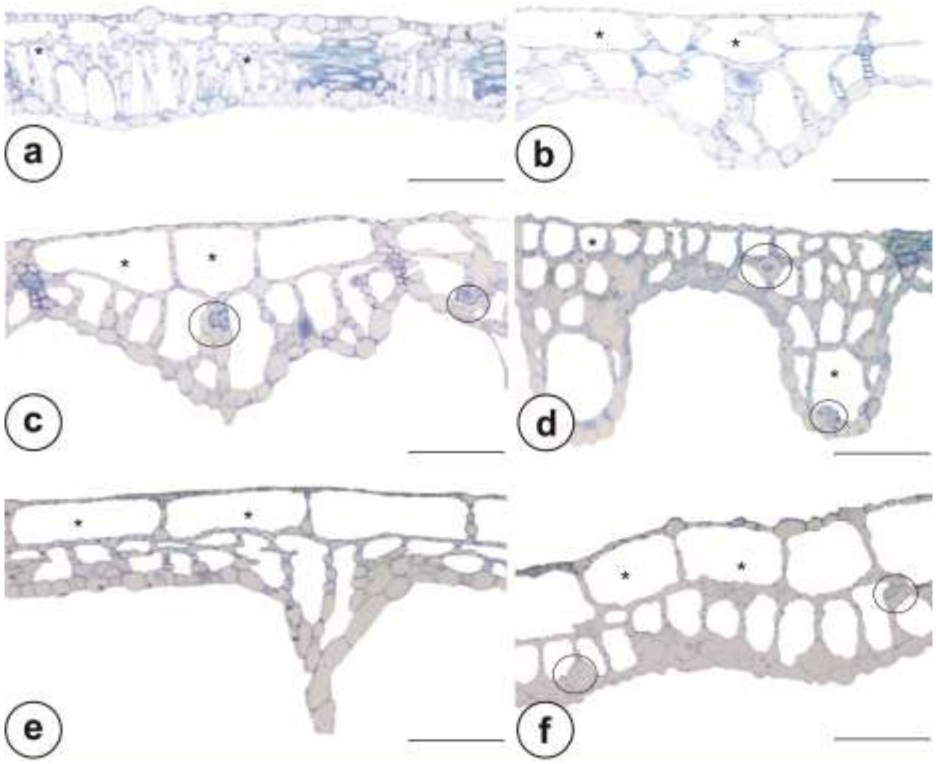

Figure 2. Leaf blade of *Salvinia auriculata* (cross sections stained with toluidine blue), after 20 days of experiment. A: control; B: 2.5 µM Cd; C: 5 µM Cd; D: 7.5 µM Cd; and E-F: 10 µM Cd. Disarrangement and increase of aerenchyma gaps (*). Accumulation of strongly stained contents (circle). Scale 200 µm.

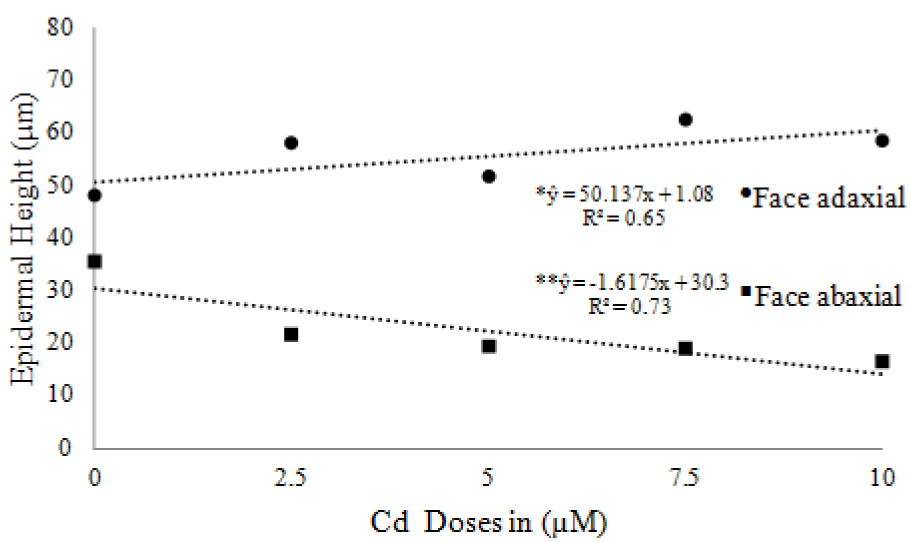

Figure 3. Regression analysis of the abaxial and adaxial face epidermis height of *Salvinia auriculata* leaves grown in Hoagland-Arnon solution, subjected to different concentrations of cadmium. *Significant at 5% probability. CV (%) = 13.56 (adaxial). **Significant at 1% level of probability. CV (%) = 26.29 (abaxial).

that from the 2.5 µM dose, the cells showed more elongation, resulting in increased thickness of this tissue with increased Cd concentrations (Figure 3). Plants grown without the presence of Cd showed larger abaxial epidermal cells, averaging 35.21 µm, while the lowest averages were observed in the treatments with 7.5 and

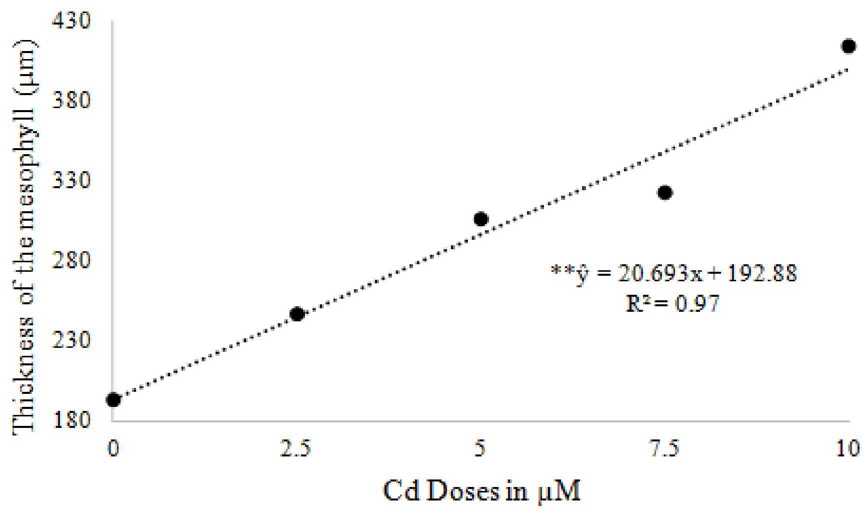

Figure 4. Regression analysis of the mesophyll thickness of the *Salvinia auriculata* leaves grown in Hoagland-Arnon solution, subjected to different concentrations of cadmium. **Significant at 1% level of probability. CV (%) = 7.43.

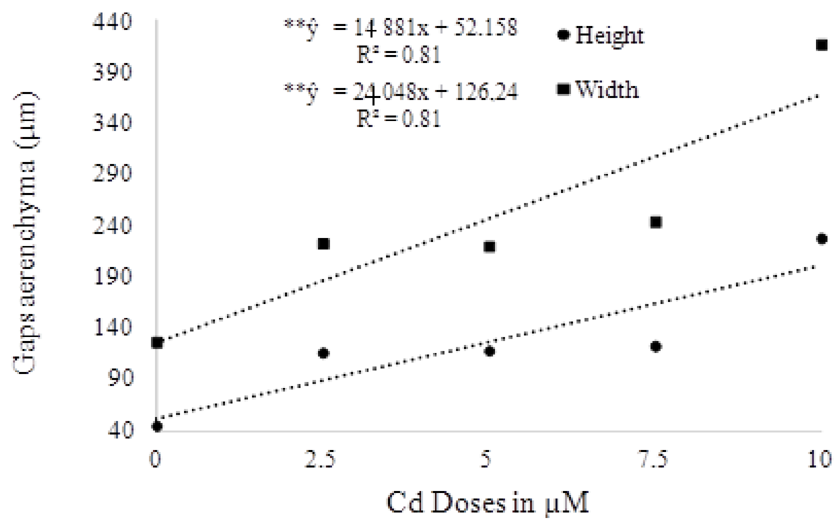

Figure 5. Regression analysis of *Salvinia auriculata* aerenchyma gap heights of leaves grown in Hoagland-Arnon solution, subjected to different concentrations of cadmium. **Significant at the 1% level of probability. CV (%) = 37.49 (gap height). **Significant at the 1% level of probability. CV (%) = 14:58 (gap width).

10 mM of Cd (Figure 3). In regression analysis, the model that best fit the data distribution was linear. Guglieri et al. (2007) working with *Platyhypnidium aquaticum* leaves observed reduction in the thickness of the leaf blade with the increase of Cd concentrations, not corroborating with results of the present work.

For the mesophyll thickness, the regression model that best fit the results was linear. The treatment with Cd at doses of 7.5 and 10 µM showed a mesophyll cell thickness increase (Figure 4). Alterations in the area and proportion of leaf tissue can be consequences of continuous exposure to heavy metals (Lux et al., 2011; Vaculik et al., 2012). Thus, the thickness increases in the mesophyll region at the higher Cd concentrations, which represents the larger diameter of the leaves.

Figure 5 presents the height of the aerenchyma gaps increased with higher doses of Cd compared to the control. The treatments influenced the aerenchyma elongation (Figure 5). The dose increases showed higher indices and it can be seen that treatment with 10 µM Cd presented a 426 µm thickness, differing from the other treatments (Figure 5). Pereira (2010) working with

Eichhornia crassipes submitted to Cd did not observe changes in the proportion of aerenchyma until it reaches the highest concentrations of this metal.

Conclusion

S. auriculata is a species sensitive to Cd, showing visible symptoms of toxicity, such as chlorosis and necrosis on the leaf surface from the lowest dose of that element, thus restricting its use in the decontamination of environments. On the other hand, it is indicated for environmental monitoring programs as an ecological indicator.

Conflict of interest

The author declares that there is no conflict of interest.

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