Short-term physiological responses to copper stress in *Salvinia auriculata* Aubl.

Respostas fisiológicas de curta exposição ao estresse por cobre em *Salvinia auriculata* Aubl.

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Abstract: Aim: To evaluate, in a short-time exposure, the physiological responses of *Salvinia auriculata* Aubl. under different concentrations of Cu. **Methods:** The plants were exposed to treatments with 0.01, 0.1, 1 and 10 mM of Cu in a period of 2 days. Then development variables of *S. auriculata* (weight, photosynthetic pigments, and soluble carbohydrate), lipid peroxidation (malondialdehyde, aldehydes, and electrolyte leakage) and production of antioxidants (anthocyanins, carotenoids, flavonoids, and proline) were evaluated. **Results:** It was observed fresh weight reductions in concentrations above 1 mM of Cu. Chlorophyll *a* decreased with the increase of Cu concentrations unlike chlorophyll *b*. The ratio chlorophyll *a* / chlorophyll *b* was changed due to the degradation of photosynthetic pigments. The reductions of carotenoids were more pronounced than that of total chlorophyll. The values of electrolyte leakage ranged from 14 to 82 % and lipid peroxidation from 7 to 46 nmol.g^{-1}. Flavonoids and soluble carbohydrates showed reductions with the increase of Cu concentration. Anthocyanins, phenolic compounds, and proline when subjected to 0.1 mM of Cu had increased, suggesting adaptability of plant stress caused directly by metal and reactive oxygen species. In higher concentrations, degradation and/or direct modifications of these molecules possibly occurred. **Conclusions:** The data suggest that *S. auriculata* is provided with an efficient mechanism against stress caused by Cu in the concentration of 0.1 mM. As for higher concentrations (1 and 10 mM), despite its role as micronutrients, Cu was toxic to the plant due to the redox behavior of this metal, which leads to the exacerbated formation of reactive oxygen species, inducing to severe damage such as biological membrane degradation and protein denaturation.

Keywords: lipid peroxidation, antioxidant, proline, metal stress.

Resumo: Objetivo: avaliar em curto tempo de exposição às respostas fisiológicas de *Salvinia auriculata* Aubl. sob diferentes concentrações de Cu. **Métodos:** as plantas foram expostas a tratamentos com 0,0; 0,01; 0,1; 1 e 10 mM de Cu em um período de 2 dias. Em seguida foram avaliadas variáveis de desenvolvimento de *S. auriculata* (peso, pigmentos fotossintéticos e carboidratos solúveis), peroxidação lipídica (malonaldeído, aldeídos e extravasamento de eletrólitos) e produção de antioxidantes (antocianinas, carotenóides, flavonóides e prolina). **Resultados:** Foram verificadas reduções das massas frescas nas concentrações acima de 1 mM de Cu. A clorofila *a* reduziu com o aumento da concentração de Cu, diferentemente da clorofila *b*. A razão clorofila *a*/clorofila *b* foi alterada, devido à degradação dos pigmentos fotossintéticos. As reduções dos carotenóides foram mais acentuada do que a de clorofila total. Os valores de extravasamento de eletrólitos variaram de 14 a 82 % e peroxidação lipídica de 7 a 46 nmol.g^{-1}. Flavonóides e carboidratos solúveis mostraram reduções com o aumento da concentração de Cu. Antocianinas, compostos fenólicos e prolina quando submetidas a 0.1 mM de Cu apresentaram aumento, sugerindo adaptabilidade da planta ao estresse causado diretamente pelo metal e espécies reativas

de oxigênio. Nas concentrações superiores, possivelmente, ocorreram degradação e/ou modificações diretas destas moléculas. **Conclusões:** Os dados sugerem que *S. auriculata* é provida de um mecanismo eficiente contra estresse causado por Cu na concentração de 0.1 mM. Já nas concentrações mais elevadas (1 e 10 mM), apesar de seu papel como micronutriente, Cu foi tóxico para a planta devido ao comportamento redox deste metal, que leva à formação exacerbada de espécies reativas de oxigênio, induzindo danos severos como degradação de membranas biológicas e desnaturação protéica.

Palavras-chave: peroxidação lipídica, antioxidante, prolina, estresse metal.

1. Introduction

Cu is an essential micronutrient that is toxic to the plants at high concentration (Brown and Rattigan, 1979). This element can be found naturally in water bodies (Delmail et al., 2011) up to a maximum value of 0.009 mg L^{-1} ($\cong 0.141$ µM) for freshwater according to Resolution 357/05 of the National Council for the Environment in the Brazil - CONAMA (Brasil, 2005). Though, this metal is between one of the most frequently discharged elements into the environment (Moore, 2011).

Among the factors that contribute most to the increase of Cu in the aquatic environment is urban manipulation (algae control in swimming pools and aquariums), agricultural (pesticides) and industrial (batteries and electroplating) (Guida et al., 2008; Song et al., 2011). Excessive intake of this metal in water can threaten human health through the consumption of aquatic organisms and the water itself (Hu et al., 2007). Because of this, the United States Environmental Protection Agency (USEPA) lists the Cu as a priority pollutant (Salomons et al., 1995).

Plants that inhabit locations with high concentrations of metals in water have mechanisms to tolerate and/or resist toxicity of these elements (Hanikenne and Nouet, 2011). The excess metal can induce many changes in plant metabolism, such as ionic imbalance, changes in photosynthetic and respiratory enzymes as well as formation of complex with substances such as amino acids and phenolic compounds (Sharma et al., 1998; Yruela, 2005).

Commonly, the heavy metals attack the biological membranes, causing changes in the permeability and selectivity of these structures (Solanki and Dhankhar, 2011). These changes may relate to the direct action of the metal or reactive oxygen species (ROS). ROS production occurs continuously in varied organelles, and when plants are exposed to high concentrations of this metal, there is an increase in this production (Gratão et al., 2005). Cu is a metal redox, which can catalyze the formation of ROS in Haber-Weiss and Fenton reaction types (Banci et al., 2011). These molecules can induce degradation of biological membranes, chlorophyll breakdown, protein denaturation and DNA mutation (Apel and Hirt, 2004). Thus, ROS reaction with fatty acids of biological membranes produces aldehydes (lipid peroxidation), especially malondialdehyde (MDA). This compound is an indicator of oxidative damage, and it can be related to increased permeability of cell membranes under stress conditions.

Some macrophytes have great ability to remove and accumulate various elements and compounds present in water and sediment (Núñez et al., 2011), and they are indicated for maintenance of environmental quality. *Salvinia auriculata* Aubl. (Salvinaceae) is a floating aquatic macrophyte that has potential for wastewater treatment, due to its high rate of growth in lentic systems that are rich in nitrogen and phosphorus (Nahlik and Mitsch, 2006). This plant has floating leaves with some modified submerged leaves, similar to roots that act by absorbing water and ions (Sculthorpe, 1967).

The genus *Salvinia* demonstrates ability to accumulate large quantities of metals (Dhir and Srivastava, 2011). However, very little is known about the *Salvinia auriculata* response to stress by Cu. In studies with *Salvinia minima*, it was observed that increases in Cu concentration from 1.0 to 3.0 mg L^{-1} ($\cong 15.7$ to 47.2 µM) negatively impacted plant growth. However, the reduction in plant growth was not severe enough to inhibit plant growth. The plants demonstrated the ability to accumulate significant concentrations of Cu in its tissues (Al-Hamdani and Blair, 2004). Thus, the aim of this study was to examine the effect of increasing concentrations of Cu (0.01, 0.1, 1, and 10 mM) in a short time exposure on (i) the development of *S. auriculata* (weight, photosynthetic pigments, and soluble carbohydrate) (ii) lipid peroxidation (MDA, aldehydes, and electrolyte leakage), and (iii) production of antioxidants (anthocyanins, carotenoids, flavonoids, and proline). The data acquired indicate the high tolerance of *S. auriculata* to Cu, suggesting its use in the remediation of polluted water systems.

2. Material and Methods

2.1. Plant material, growth conditions and metal treatment

S. auriculata were obtained from uncontaminated aquatic ecosystems around Campos dos Goytacazes, Rio de Janeiro, Brazil between 21°43'10.49"S and 41°19'49.68"W. The plants were transported to the laboratory within water tanks (20 L). They were thoroughly washed with tap water to remove attached inorganic and organic matter.

S. auriculata were cultured in half-strength Hoagland's solution at pH 5.8 ± 0.1 without aeration for about 2 months before to start the experiments. The plants were grown under greenhouse conditions in natural sunlight with mean temperature compensated of 27 ± 2 °C and relative humidity of 74 ± 7 %. The National Institute of Meteorology (INMET) provided daily data from Meteorological Station of Campos dos Goytacazes in the period of October 2009 to March 2010.

The plants were acclimatized for 7 days before the metal treatment in half-strength Hoagland's solution under laboratory conditions (16:8 h light/dark cycles at 30 ± 2 °C, 50 μmol photons $m^{-2}s^{-1}$). Subsequently, 5 g of plants were placed in 1,000 mL flasks containing half-strength Hoagland's solution with different concentration of Cu for 48 h. The solutions were prepared with $CuCl_2$ (Merck) using 0.01, 0.1, 1, and 10 mM of Cu. These high concentrations were used to test the ability of the *S. auriculata* to resist stress caused by Cu. In total, there were nine samples per treatment.

2.2. Development of Salvinia auriculata Aubl.

The fresh weight (FW) of plants was measured from each treatment. For determination of dry weight (DW), the samples were oven dried at 70 °C for 96 h. The plant water content (WC, mL g^{-1} DW) was estimated using the equation: WC = (FW – DW)/DW.

The growth rate (GR) was calculated based on the increase in FW over 48 h and it was expressed in percent. For this analysis, we used the formula: GR (%) = (FW_{final} – $FW_{initial}$) x 100.

The photosynthetic pigments (chlorophyll *a*, *b*, total and carotenoids) were determined according to the method of Wellburn (1994). *S. auriculata* plants (50 mg of FW) were placed in plastic test tubes containing 5 mL dimethylsulfoxide reagent (DMSO) as organic solvent and kept in the dark. After 4 days, the extract was analyzed in a spectrophotometer at 480 nm, 649 nm and 665 nm. The total chlorophyll, chlorophyll *a/b* and total chlorophyll/carotenoid ratios were calculated. All the laboratory procedures were carried out in a low light environment.

Soluble carbohydrate content was determined according to the method of Dubois et al. (1956) and Robyt and White (1987). A total of 100 mg of fresh tissue was mixed with 1 mL of methanol (80 %) at 70 °C for 30 min. After the mixture had cooled, 1 mL of the extract was mixed with 1 mL of phenol (5 %) and 5 mL of sulfuric acid (95 %). Thus, the plastic test tubes were incubated for 1 h at room temperature and the absorption was measured at 640 nm. Glucose was used as a standard.

2.3. Lipid peroxidation

The lipid peroxidation was measured by determination of malondialdehyde (MDA) (Heath and Packer, 1968) and other five aldehydes (propanal, butanal, hexanal, heptanal and propanal dimethylacetal) (Meirs et al., 1992), which are breakdown products of lipid peroxidation. A total of 100 mg of fresh plant samples was homogenized in 1 mL of 0.1 % tri-chloroacetic acid (TCA) and centrifuged at 15,700 g for 15 min at room temperature. Thus, 1 mL of the supernatant was vortexed with 3 mL of 20 % TCA containing 0.5 % 2-thiobarbituric acid (TBA). This solution was heated for 30 min at 96 °C. The non-specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement, and for other aldehydes at 455 nm. For the calculation of MDA and other aldehydes, it was used the extinction coefficients (e) 1.55×10^5 M^{-1} cm^{-1} and 0.457×10^5 M^{-1} cm^{-1}, respectively.

The electrolyte leakage (EL) was determined as described by Ben Hamed et al. (2007). The plant samples (300 mg of FW) were placed in plastic test tubes containing 15 mL of ultra-pure water. These tubes were incubated in a water bath at 25 °C for 3 h and the initial electrical conductivity (EC) of the medium was measured by an EC meter (Metrhom, Switzerland). Then, the samples were heated for 20 min at 96 °C to release all the electrolytes. After the samples were cooled at 25 °C, and the final EC was measured. The EL was calculated by using the formula: EL = ($EC_{initial}$/EC_{final}) x 100.

2.4. Antioxidants

Phenolic compounds were determined according to the method of Soland and Laima (1999). The plant samples (100 mg of FW) were incubated

with 5 mL of absolute ethanol at room temperature for 24 h. The extract was mixed with 0.5 mL of Folin–Ciocalteu's reagent (diluted 1:1 with water) and 1 mL of 5 % sodium carbonate. The absorbance was measured at 725 nm after 1 h. Tannic acid was used as a standard.

For determination of anthocyanin content, the plant samples (50 mg of FW) were placed in 5 mL of methanol:HCl, 99:1 (v/v). The tissues were crushed and kept in the dark at 25 °C for 24 h. Then, the extract was centrifuged at 15,700 g for 10 min at room temperature and the absorbance of the supernatant was measured at 550 nm. To calculate the concentration of anthocyanins was used the extinction coefficient 33,000 mol^{-1} cm^{-1} (Wanger, 1979).

The flavonoids content was determined as described by Krizek et al. (1998). The plant samples (50 mg of FW) were mixed with 5 mL of ethanol:acetic acid, 99:1 (v/v). Then, the samples were gently boiled for 10 min in a water bath at 80 °C. The absorbance was measured at 270, 300 and 330 nm using spectrophotometer.

Free proline was determined according to Bates et al. (1973). Plant samples (100 mg of FW) were mixed in 5 mL of 3 % (w/v) sulfosalicylic acid and the homogenate was centrifuged at 15,700 g for 10 min at room temperature. Then, 1 mL of mixture was heated at 96 °C for 1 h in a water bath together with 1 mL of acid ninhydrin (2.5 % ninhydrin and 50 % phosphoric acid) and 1 mL of glacial acetic acid. After cooling, the reaction was measured at 518 nm. Proline was used as a standard.

2.5. Sensitivity index

The sensitivity index (SI) was calculated from the difference between dry matter production of copper-treated plants and the control. It was expressed in percent according to the expression: $SI_{Cu} = [100 \times (DW_{Cu} - DW_{control})]/DW_{control}$. This parameter was more negative when the plant was sensitive to copper (Saadallah et al., 2001).

2.6. Statistical analysis

All values shown in the figures are expressed as mean ± standard error. Normal distribution for data was analyzed by D'Agostino & Pearson omnibus normality test. Data were subjected to a one-way analysis of variance (ANOVA) to confirm the variability of data and validity of results. The Tukey test was performed to determine significant differences between treatments. In the figures, the values are marked with a different letters for the significance level ($p < 0.05$) as compared to the control. Discriminant analysis was used to group treatments based on increasing concentrations of Cu.

3. Results

It was found that 0.01 mM of the metal did not affect plant development negatively. The damage began to be observed visually from 0.1 mM (Figure 1). The effects of increasing concentrations of Cu on physiological parameters in *S. auriculata* are shown in Table 1. It is found that the increase in concentration of Cu in the environment led to a reduction in weight, significant ($p < 0.05$) loss in water content, chlorophyll, carotenoids, phenolic compounds, proline, anthocyanins, flavanoids, and soluble sugars. In contrast, there was an increase ($p < 0.05$) in MDA levels, aldehydes and electrolyte leakage.

S. auriculata did not survive at high availability of Cu after 48 h of experimentation. Thus, the plants were separated by using SI as shown in Figure 2. *S. auriculata* exposed to 0.01 mM of Cu showed higher tolerance, and from 0.1 mM on SI decreased with the concentration.

The Figure 3 identifies the physiological parameters simultaneously to group the Cu treatments with similar responses. Control and 0.01 mM were pooled. The treatments with 1 and 10 mM Cu also were pooled. The treatment with 0.1 mM Cu kept isolated.
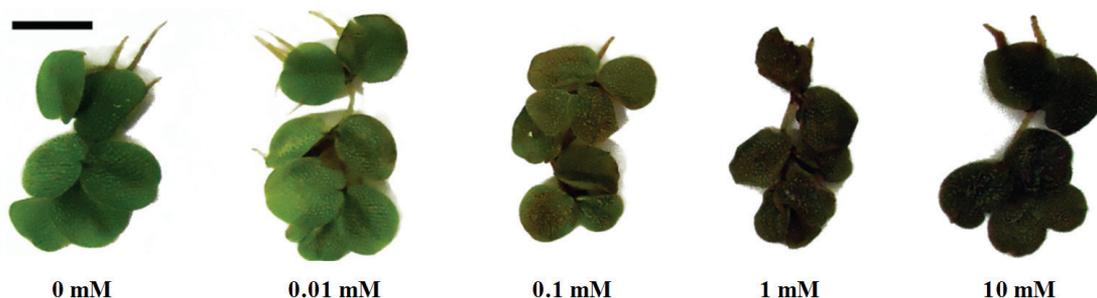

| 0 mM | 0.01 mM | 0.1 mM | 1 mM | 10 mM |

Figure 1. Plants of *Salvinia auriculata* Aubl. exposed to Cu. The horizontal bar equals 1 cm.

Table 1. Effects of the increasing concentrations of Cu on physiological variables of the *Salvinia auriculata* Aubl.

	Control	0.01 mM	0.1 mM	1 mM	10 mM
Growth rate (%)	100.5 ± 0.8 a (100 %)	102.1 ± 4.3 a (102 %)	71.4 ± 4.3 b (71 %)	57.4 ± 2.1 c (57 %)	54.4 ± 2.0 c (54 %)
Water content (ml g⁻¹ dw)	38.5 ± 1.2 a (100 %)	34.1 ± 0.7 b (89 %)	31.2 ± 0.4 b (81 %)	27.3 ± 0.8 c (71 %)	27.5 ± 0.7 c (71 %)
Chlorophyll *a* (µg g⁻¹ fw)	544.7 ± 21.3 a (100 %)	430.2 ± 47.5 bc (79 %)	495.4 ± 30.4 ab (91 %)	329.5 ± 12.4 cd (60 %)	270.3 ± 14.6 d (50 %)
Chlorophyll *b* (µg g⁻¹ fw)	205.4 ± 10.1 b (100 %)	193.2 ± 14.9 b (94 %)	216.0 ± 8.5 b (105 %)	302.6 ± 12.3 a (147 %)	339.7 ± 17.8 a (165 %)
Total chlorophyll (µg g⁻¹ fw)	750.2 ± 30.7 a (100 %)	632.4 ± 21.3 ab (84 %)	711.3 ± 32.0 ab (95 %)	623.4 ± 50.9 ab (83 %)	610.0 ± 16.0 b (81 %)
Carotenoids (µg g⁻¹ fw)	86.5 ± 4.8 ab (100 %)	79.6 ± 9.3 b (92 %)	101.6 ± 5.6 a (117 %)	9.6 ± 2.0 c (11 %)	ND ND
Malondialdehyde (nmol g⁻¹ fw)	6.9 ± 0.7 b (100 %)	6.2 ± 0.8 b (90 %)	32.0 ± 2.3 a (463 %)	35.1 ± 3.1 a (506 %)	45.8 ± 3.7 a (661 %)
Other aldehydes (nmol g⁻¹ fw)	41.2 ± 1.7 c (100 %)	57.3 ± 4.3 c (139 %)	215.1 ± 14.2 b (522 %)	195.3 ± 25.3 b (474 %)	297.9 ± 34.6 a (723 %)
Electrolyte leakage (%)	14.1 ± 0.6 c (100 %)	11.7 ± 1.4 c (83 %)	17.9 ± 0.9 c (127 %)	59.8 ± 5.3 b (425 %)	81.8 ± 1.8 a (582 %)
Proline (µg g⁻¹ fw)	53.8 ± 2.9 b (100 %)	50.3 ± 4.4 b (93 %)	82.1 ± 5.7 a (153 %)	6.3 ± 1.0 c (12 %)	4.0 ± 0.5 c (8 %)
Phenolic compounds (µg g⁻¹ fw)	517.8 ± 42.9 ab (100 %)	440.0 ± 52.0 b (85 %)	627.8 ± 36.1 a (121 %)	238.9 ± 10.9 c (46 %)	252.2 ± 6.6 c (49 %)
Anthocyanins (nmol g⁻¹ fw)	255.6 ± 17.2 ab (100 %)	171.1 ± 40.8 b (67 %)	315.6 ± 21.4 a (123 %)	195.6 ± 15.8 b (77 %)	175.6 ± 11.8 b (69 %)
Flavonoids (% A 270 nm)	11.7 ± 1.6 ab (100 %)	14.2 ± 2.2 a (122 %)	4.5 ± 1.4 c (39 %)	6.2 ± 1.6 bc (53 %)	6.2 ± 1.6 bc (53 %)
Flavonoids (% A 300 nm)	15.7 ± 1.2 a (100 %)	12.4 ± 0.9 b (79 %)	9.2 ± 0.6 c (58 %)	6.8 ± 0.6 d (43 %)	6.0 ± 0.6 d (39 %)
Flavonoids (% A 330 nm)	25.3 ± 1.3 a (100 %)	16.0 ± 1.0 b (63 %)	17.6 ± 0.8 b (70 %)	10.5 ± 0.4 c (41 %)	8.4 ± 0.4 c (33 %)
Soluble sugar (mg g⁻¹ fw)	119.2 ± 13.6 a (100 %)	102.4 ± 17.0 a (86 %)	99.4 ± 7.5 a (83 %)	57.7 ± 2.6 b (48 %)	48.1 ± 3.3 b (40 %)

Mean (n = 9) ± standard error. The different letters indicate significant differences (p <0.05) according to Tukey's test. In parentheses is relative resistance. ND: not determined; dw: dry weigh; fw: fresh weigh.
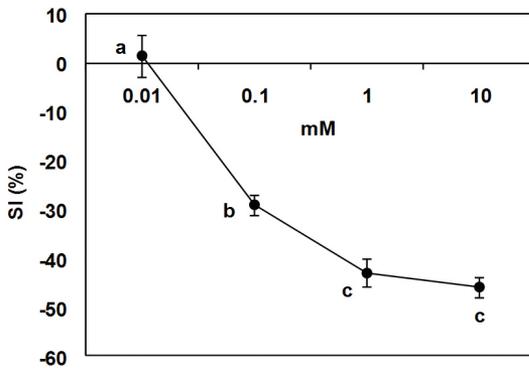
Figure 2. Plant sensitivity index (SI) in *Salvinia auriculata* Aubl. exposed to increasing concentrations of Cu. The different letters indicate significant differences ($p < 0.05$) according to Tukey's test.
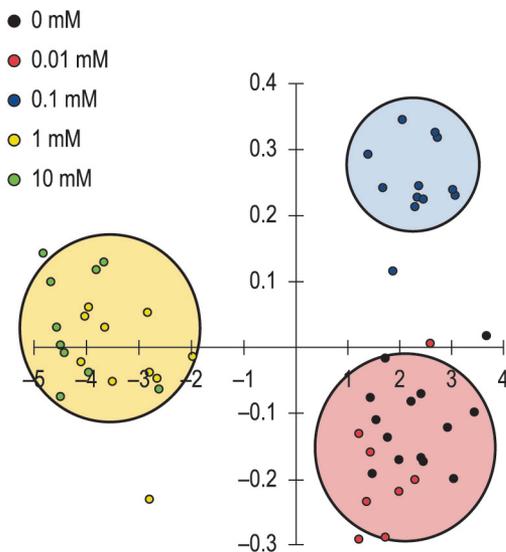

Figure 3. Discriminant analysis of the treatments of Cu in *Salvinia auriculata* Aubl.

4. Discussion

The availability of heavy metals in the environment showed an increase in the last century due to the increased of anthropogenic activities such as: mining, industrial sewages, and solid waste disposal (Fu and Wang, 2011). Large portion of these contaminants is carried to aquatic ecosystems and strongly affect the organisms that live there. *S. auriculata* is admittedly an aquatic macrophyte widespread in Neotropical aquatic environments and has a high growth rate. Therefore, it has high potential for uptake of compounds available in the environment (Nahlik and Mitsch, 2006). When evaluating its physiological response to increasing concentrations of Cu, differences were found in the symptoms of toxicity (weight loss and chlorosis) for

inducing a variety of metabolic changes that directly contributed to the tolerance to this metal.

During the study, it was observed that responses to the stress in the concentration at 0.01 mM of Cu in *S. auriculata* have values similar to the control situation. Even occurring damage to the photosynthetic apparatus, it is possible that the synthesis of defensive compounds such as carotenoids, proline and phenolic compounds is energetically more costly, which makes up for living in a stress situation, since there was no increase in antioxidants. This behavior changed when the plants were exposed to 0.1 mM of Cu concentration. There was energy displacement for the synthesis of defensive compounds to be kept alive, tolerating the stressful condition for 48 h. As for those plants exposed to 1 and 10 mM, they presented metabolic breakdown. These data are corroborated by the discriminant analysis.

The weight loss and decrease in the concentration of soluble carbohydrates seem to be directly associated with inhibition of Cu on cell growth by preventing the plant survival under high concentrations (Alaoui-Sossé et al., 2004). This can be due to enzymatic reactions in carbon fixation, especially in the synthesis and Rubisco activity mediated by the metal (Lidon and Henriques, 1991). In addition, it is noteworthy that the excess Cu can interact on plastocyanins (transporter of electron) and superoxide dismutase (antioxidant enzyme), affecting the process of photosynthesis and detoxification in chloroplasts (Nouet et al., 2011).

The high concentration of Cu significantly accelerates the degradation of pigments in *S. auriculata*. The reduction of these pigments probably relates to the increase in reactive oxygen species acquired from the damage of the photosystems ($r = -0.60$, $p<0.05$). The ionic imbalance caused by excessive Cu may affect aminolevulinic acid synthesis (precursor of chlorophyll) and replace Fe^{2+} and Mg^{2+} in chlorophyll molecules, increasing degradation of the pigments (Stobart et al., 1985). As for the moderate concentrations of Cu (up to 0.1 mM), they caused an increase in carotenoids, which protect against stress, since these pigments are responsible for the suppression of singlet oxygen ($^1O_2$), reducing lipid peroxidation and consequent oxidative damage (Juan et al., 2005).

The excess Cu in the cellular environment causes changes in photosynthetic and respiratory system, intensifying the formation of ROS (Møller et al., 2007). It is known that lipid peroxidation by free radicals induced in the lipid membrane results in a greater oxidative damage mediated by a chain

reaction. In this study, the accumulation of MDA and other aldehydes was increasing with the highest concentration of Cu in the environment along with electrolyte leakage (r = 0.76; p<0.05), which represents the extent of membrane damage (Bandeoglu et al., 2004). This biological membrane rupture has led to reduction of turgor and, concomitantly, to likely changes in the elasticity of the cell walls (Maksymiec et al., 1995), causing cell death at higher concentrations.

Cu adverse effects on the metabolism of nitrogen were also observed. The content of proline increased under exposure of Cu (up to 0.1 mM), indicating its role in the response to the metal as antioxidant agent (Singh et al., 2010). Moreover, the proline acted as an osmotic regulator, taking part in the stabilization of membranes (Shah and Dubey, 1998) and the formation of non-toxic complex (chelators) with this metal (Rauser, 1999). At 1 and 10 mM concentrations, there was a drastic reduction in the content of proline associated with excess ROS and Cu, which shows that plant defenses were no longer sufficient to respond to the excess metal; and the cell collapsed.

Phenolic compounds responded to stress with decrease in their concentration. However, at 0.1 mM the values presented a significant increase when compared to the control. Thus, these compounds may be acting as antioxidants (Solecka, 1997; Sakihama et al., 2002), as well as playing the role of chelating agents (Michalak, 2006). The increase observed in the concentration of phenolic compounds is confirmed by the data shown for anthocyanins and carotenoids, which are also compounds formed by aromatic rings. All these molecules along with proline (neutralization of OH^- and $^1O_2$), which presented the same behavior, probably have protective role in plant physiology in response to Cu stress in different cellular compartments, since anthocyanins are found in the vacuole, carotenoids in the chloroplast as well as proline in the cytosol. The same analysis for flavonoids shows that they were not as effective in acting as antioxidants, since there was not increase in their concentration. In this sense, in the Figure 4, it is proposed a model that it summarizes the physiological changes in *S. auriculata* caused by high levels of Cu.
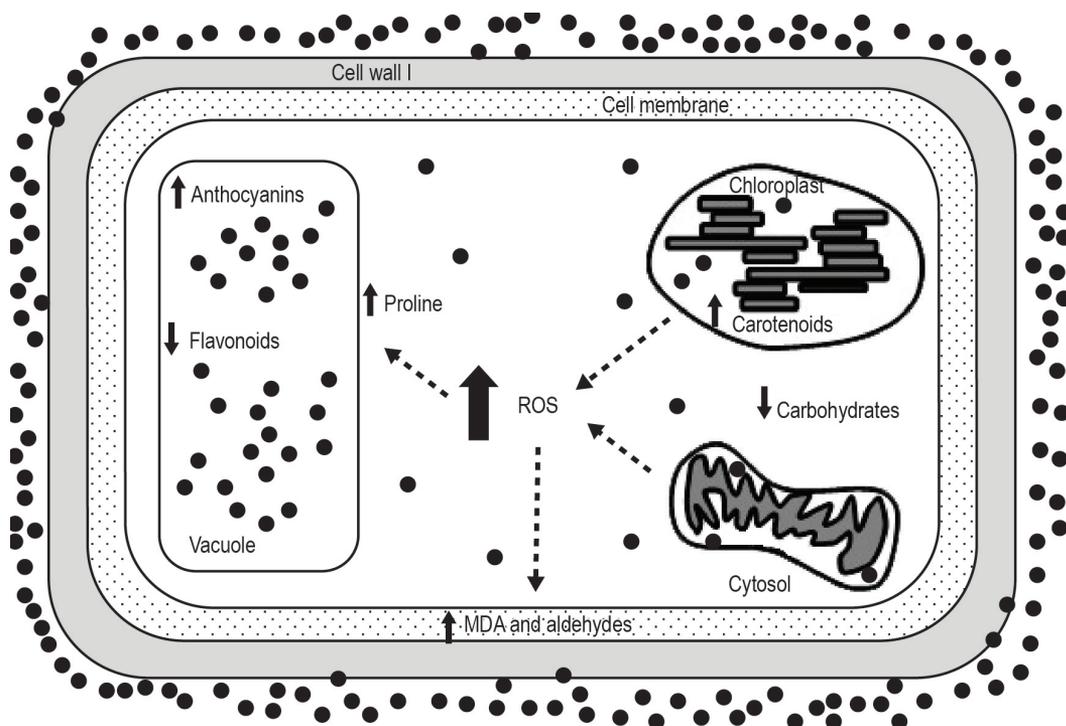

Figure 4. Physiological responses of *Salvinia auriculata* Aubl. to the addition of Cu (●). Chloroplast and mitochondria are compartments that produce reactive oxygen species (ROS) continuously. With the increase in the concentration of Cu in the intracellular environment, disorders occur in these organelles, which increase the production of ROS and affect the synthesis of carbohydrates and flavonoids. This makes the plant cell import Cu for the vacuole, which is rich in ROS, activating non-enzymatic systems in this organelle (production of anthocyanins and flavonoids) as well as chelators in the cytosol (proline). Carotenoids reduce ROS in the chloroplasts and the anthocyanins act similarly in the vacuole. Added to this, the excess Cu and ROS affect biological membranes through the degradation of fatty acids causing lipid peroxidation, and consequently the production of the malondialdehyde (MDA) and others aldehydes.

The concentrations of metal tested in this study were high because our interest was to verify if *S. auriculata* would be a possible candidate for decontamination of environments polluted with Cu in extreme situations. The results obtained show the high resistance of the aquatic plant to the stress caused by the metal. This, in addition to its high growth rate and ease in harvesting, make it a potential candidate for phytoremediation.

Currently, the use of aquatic macrophytes is under investigation as a viable alternative for remediation of a wide range of contaminants including heavy metals (Hughes et al., 1996; Lee et al., 1998; Rodgers and Bunce, 2001; Miglioranza et al., 2004; Miretzky et al., 2004; Polomski et al., 2009; Augustynowicz et al., 2010; Rahman and Hasegawa, 2011). These researches emphasize that the plant-based approach to remediation of the environments highly contaminated has an interesting cost-effective, because it is cheaper than other methods and safe for human.

5. Conclusion

In this study, the results suggest that *S. auriculata* has an efficient mechanism against the stress caused by Cu at concentrations up to 0.1 mM, which is promising for phytoremediation of contaminated aquatic systems. As for higher concentrations (1 and 10 mM), severe damage was observed along with the exacerbated formation of ROS. Thus, both the metal and ROS induced damage detected in biological membranes and primary sources of energy, leading to cell death of the plant.

Acknowledgments

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