Accumulation and effects of copper on aquatic macrophytes *Potamogeton pectinatus* L.: Potential application to environmental monitoring and phytoremediation

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**ARTICLE INFO**

**Keywords:**
- Plant
- Metal
- Bioconcentration
- Photosynthesis
- Biomonitor

**ABSTRACT**

This study investigated the ability of *Potamogeton pectinatus* L. to accumulate copper and its effects on plants. In accumulation tests, macrophytes were exposed (96 h) to different copper concentrations (0–1000 µM) and the metal was measured in media and plant tissues (roots, stems and leaves) to determine the bioconcentration factor (BCF). Plants accumulated high concentrations of copper in a dose-dependent manner and roots was the main organ for copper accumulation. However, the more copper increased in water, the more BCF values decreased. It may be due to either saturation of copper uptake or down-regulation of metal uptake by plants. In the physiological and morphological analyses, plants were kept (96 h) in Hoagland nutrient solution without copper, in full Hoagland solution (0.5 µM Cu) and in Hoagland medium with copper from 1 to 100 µM. The absence and the presence of copper above to 1 µM inhibited photosynthesis. Chlorophylls and carotenoid levels also decreased with the excess of copper, a fact that may have affected the photosystem II-dependent of chlorophyll and caused photosynthesis suppression. Only macrophytes at 10 µM Cu showed decrease in length and number of leaves on the 10th day of the test, when they died. Chlorosis and necrosis were observed in control groups and groups with extra copper, but not in Hoagland group. Overall, the macrophyte *P. pectinatus* can be considered a suitable plant for monitoring environments contaminated by copper, based on results of copper accumulation in the plant, decrease in pigment concentration and presence of chlorosis and necrosis. However, values of BCF based on fresh water tissues was not proper to indicate the use of *P. pectinatus* for cleaning environments contaminated by copper.

1. Introduction

Copper is an essential micronutrient required by plants as a cofactor for enzymes involved in respiration and photosynthesis. However, copper can be toxic when it is found at high concentrations in the environment. Chloroplasts are the most vulnerable sites of copper toxicity. Among its effects, copper not only binds different sites of photosystem II (PS II), affecting the electron transport chain (Maksymiec, 1997) but also interferences in the synthesis and degradation of pigments involved in photosynthesis (Yan and Pan, 2002; Mysliwa et al., 2004; Upadhyay and Panda, 2009). Due to its redox property, copper can also induce oxidative stress by increasing the production of reactive oxygen species (ROS) that react with biomolecules and cause damage, such as membrane lipid peroxidation (Teisseire and Guy, 2000; Upadhyay and Panda, 2009; Monferrán et al., 2009). All these effects of copper exposure lead to failure in photosynthesis, thus, affecting plant growth and survival.

Although copper can cause harm, many plants are highly resistant to this metal and can absorb and accumulate huge amounts of it. Because of their ability to accumulate metals, aquatic macrophytes have been suggested for environmental monitoring to monitor levels of metals in the environment (Demirizen and Askoy, 2004; Zhou et al.,...
2008; Peng et al., 2008). For any organism to be classified as either a biomonitor or a sentinel, it needs to reflect the amount of pollutants in the environment (Rebeby, 2001). Likewise, hyperaccumulating macrophytes have also been used for cleaning sites contaminated by metals. This technique, called phytoremediation, uses hyperaccumulating plants to remove the maximum volume of an element or substance in the shortest possible time from contaminated areas (water and soil), thus, decreasing pollutant contents in the environment. Phytoremediation has been considered an environmentally friendly option to restore polluted aquatic resources and is cost-effective alternative by comparison with most treatments that have already been established in areas contaminated by metal (USEPA, 2000). Application of this technology has been suggested for metal clearance (Odjegba and Fasidi, 2004; Tangahu et al., 2011; Melignani et al., 2015) and for removal of toxins from water (Pflugmacher et al., 2015). For example, Pflugmacher et al. (2015) use the phytoremediation potential of aquatic macrophytes for water purification in the concept of Green Liver System.

Several studies show that aquatic macrophytes, such as plants of the Potamogeton genus, are able to accumulate metals in their tissues at concentrations above the ones found in the environment (Jain et al., 1989; Zayed et al., 1998; Miretzky et al., 2004; Demirizen and Askoy, 2004). Accumulation of metals in plants is organ-dependent and metal concentration is usually higher in a root than in a leaf, since the former is the main absorptive organ in plants (Cardwell et al., 2002; Demirizen and Askoy, 2004; Fritiof and Greger, 2006; Yabanli et al., 2014). However, Guillizzi et al. (1991) observed that absorption of metals by leaves of submerged macrophytes becomes especially high in heavily contaminated environments.

The species Potamogeton pectinatus has been proposed as a hyperaccumulator, which is useful for monitoring of metal levels (Demirizen and Askoy, 2004; Peng et al., 2008). It is a submersed macrophyte with cosmopolitan distribution in systems of rivers, lakes and coastal areas. Among the Potamogetons, only P. pectinatus tolerates high salinities, alkalinity and eutrophication. This species grows nearly all bottom substrates and, like most submersed vascular plants, has adapted to grow its roots in sediments with low oxygen levels (Kantrud et al., 1990; Ganie et al., 2016).

The objective of this study was to assess not only the ability of the aquatic macrophyte P. pectinatus to accumulate copper but also the effects of this metal on photosynthetic and respirations rates, pigment content and plant growth in order to access the use of P. pectinatus as a monitor and/or remediator in copper contaminated water bodies. Because P. pectinatus was previously described as a hyperaccumulator plant and due to its tolerance to variations in abiotic factors, such as salinity and alkalinity, this study hypothesized that P. pectinatus has potential for both monitoring and remediation of copper as a tolerant species.

2. Material and methods

2.1. Plant material and growth conditions

Macrophytes P. pectinatus, whose stems were around 10–15 cm in length and had approximately 6 leaves, were manually collected in lakes of an Environmental Protection Area (EPA) (32’07.923’S and 52’10.858’W), located close to the road RS-734 in southern Brazil (Rio Grande, RS). Due to variations in root and leaf lengths, the stem length was considered the size of the plants. After sampling, plants were gently washed in dechlorinated tap water and then transferred to a BOD (Biochemical Oxygen Demand) germination chamber, where they were acclimated for 15 days in glass aquariums (50 cm × 30 cm × 20 cm) filled with 5 L of water enriched with Hoagland nutritive solution, full strength (Hoagland and Arnon, 1950). Plants were acclimated in a ratio of 1 plant per 1 L water. In the acclimation period, the medium was renewed every three days for nutrient replacement. Photoperiod and temperature were fixed at 12 L:12D and 23 °C, respectively. Light was provided by daylight lamps with irradiance of 114.33 ± 4.70 µmol/m²/s, measured by a light sensor (Li-Cor Radiation sensor LI-1400 Data Logger, Lincoln, Nebraska USA).

2.2. Copper accumulation test

Accumulated P. pectinatus were gently washed in dechlorinated tap water and measured in length (15.84 ± 0.72 cm was the length of the stem). Then, they were individually placed in 1 L glass beakers filled with 500 mL dechlorinated tap water with copper at 1, 10, 100 and 1000 µM CuCl₂·2H₂O, where they were kept for 96 h. A control group, without copper, was also maintained throughout the experiment. Five plants were used in each experimental condition. The experiment was conducted in a BOD germination chamber under the same conditions of photoperiod, temperature and irradiance of the acclimation period. Copper was added to the water 24 h before the experiment to pre-equilibrate the media that were renewed every 24 h over the 96 h test. Both non-filtered and filtered (0.45 µm mesh filter, Millipore, São Paulo, SP, Brazil) water samples (10 mL) were collected from both control and copper-contaminated media prior to the introduction of the plants in the beaker (0 h) and after 24 h (before renewing the water). Water samples were placed in clean plastic Falcon® tubes and acidified (0.5% HNO₃) for later determination of total (non-filtered samples) and dissolved (filtered samples) copper concentrations.

After 96 h exposure, plants were washed with EDTA solution (12 mM) – to have the adsorbed metal removed –, dried with filter paper and separated into leaves, stems and roots. Organs were individually weighed (fresh weight - FW), dried (4 days at 60 °C), weighed again (dry weight - DW) and completely digested in HNO₃ (65% - ultra pure, Merck) at 60 °C in the following 4 days (at 60 °C). Digested samples were used for copper determination. The Bioconcentration Factor (BCF) was calculated for each treatment (see Section 2.7).

2.3. Physiological assays

Specimens of accumulated P. pectinatus (10.83 ± 0.040 cm, the length of the stem, and 0.71 ± 0.003 g FW) were exposed to copper at 1, 10 and 100 µM, as CuCl₂·2H₂O, or kept in control conditions. In this experiment, copper was added to the Hoagland solution without copper, instead of dechlorinated tap water used in the accumulation test. Moreover, there were used 2 different control groups: the first was made by modified Hoagland nutrient solution without the micronutrient copper; whereas, in the second group, plants were maintained in a full Hoagland nutrient solution with nominal copper at 0.5 µM (Epstein and Bloom, 2006). The first group was named the control group while the second one was the Hoagland group. For physiological assays, plants were individually exposed in 1 L glass beakers filled with 500 mL of the different media. Copper was added to media 24 h before the experiment to pre-equilibrate the medium. Media was renewed every 24 h over the 96 h test. Both non-filtered and filtered (0.45 µm mesh filter, Millipore, São Paulo, SP, Brazil) water samples (10 mL) were also collected at 0 and 24 h and acidified (0.5% HNO₃) for copper measurements. The following endpoints were analyzed: photosynthesis, respiration and pigment concentration.

Photosynthesis was measured after 24 and 96 h exposure to copper in different lighting conditions, i.e., dark, 17, 100, 300 and 500 µmol/m²/s. In the absence of light (dark condition), respiration was measured. For this experiment, 3 plants were submitted to each condition of light and different treatments with copper: control, Hoagland and 1, 10 and 100 µM of Cu groups. At 24 and 96 of exposure, plants (total of 75) were moved from the beakers and individually placed in 300 mL BOD transparent glass bottles filled with the corresponding experimental media. The bottles were covered with different filters (varied meshes) to get light intensities mentioned above, and then submerged in an incubation chamber with circulating water at 23°C for two hours.
Readings of dissolved oxygen concentration in the media were carried out before and after incubation, by an oximeter (Dissolved Oxygen Meters DO5519, Lutron, Twain, São Paulo). Plants used for photobiological analyses were removed from the BOD bottle, dried with a filter paper and weighed. Determination of photosynthesis and respiration rates was based on oxygen evolution values (described in Colares et al., 2007) and calculated by the equations of Strickland and Parsons (1972), using FW for standardization. Results were expressed as mg of O₂/L/h/g of plant FW.

Chlorophyll a and b and carotenoid contents were measured in agreement with Lichtenthaler (1987). In this analysis, 10 plants were used per treatment (control, Hoagland and 1, 10 and 100 µM of Cu). As mentioned before, plants were individually exposed in 1 L glass beakers filled with 500 mL of the different media. Abiotic parameters were kept in the same conditions of the acclimation period (12 D:12L, 23°C and irradiance of 114.33 ± 4.70 µmol/m²/s). About 0.5 g leaf of each plant was homogenized in 7 mL 80% acetone and filtered by a funnel lined with filter paper wetted in 2 mL 80% acetone. Residue which remained on the filter paper was washed with 4 mL 80% acetone. The filtrate volume was filled up to 20 mL with 80% acetone and analyzed by spectrophotometry at three different wavelengths: 470 nm (carotenoid), 663 nm (chlorophyll a) and 647 nm (chlorophyll b). Concentrations of pigments were standardized by g of FW.

2.4. Morphological assays

Copper effects on the macrophytes morphology were evaluated in the course of 30 days by measurements of the longitudinal length of the stems, number of leaves and occurrence of chlorosis and necrosis. Plants with approximately same size and weight (10.83 ± 0.53 cm was the length of the stem and 0.71 ± 0.05 g FW) were exposed to copper. Experimental groups were control group (Hoagland nutrient solution without copper), Hoagland group (full Hoagland nutrient solution), 1, 10 and 100 µM of Cu, as CuCl₂·2H₂O added to Hoagland solution with no copper. Ten plants were exposed to each experimental condition in a ratio of 1 plant per 1 L of water. In this case, the ten plants were divided in 2 replicates placed in aquariums (50 cm × 30 cm × 20 cm) filled up to 5 L of media. Conditions of plant accommodation, photoperiod, temperature and luminosity were the same as the acclimation period (12 D:12L, 23°C and 114.33 ± 4.70 µmol/m²/s, respectively). Initially, plants were properly identified with colored strings tied to their stems so that there would be no exchange of specimens during the analysis. On the 10th, 20th and 30th day, growth was estimated, based on stem length and number of leaves. Besides, after the 30th day of exposure, chlorosis and leaf necrosis were qualitatively (presence or absence) identified in the macrophytes. The chlorosis condition was recognized when yellow spots were identified on the leaves whereas the necrosis condition meant brown stains.

Copper was also added to the water 24 h before the experiment to pre-equilibrate the media that were renewed every 24 h over the 30-day test. Both non-filtered and filtered (0.45 µm mesh filter, Millipore, São Paulo, SP, Brazil) water samples (10 mL) were collected at 0 and 24 h water renewal on the 7th, 14th and 28th day of the experiment for total and dissolved copper measurements.

2.5. Analytical techniques

Copper concentration in water samples (non-filtered and filtered ones) from control media, Hoagland solution and 1 µM of nominal copper exposure, were determined by graphite furnace atomic absorption spectrophotometry (AAS, ANALYST 700, Perkin Elmer, limit for copper detection 0.0014 ppm). Copper concentration in the other water samples (non-filtered and filtered samples above 1 µM of CuCl₂·2H₂O), as well as in the biological samples (leaves, stems and roots), was analyzed by atomic absorption spectrometry with flame atomization (AAS, 932 Avanta Plus - GBC, Hampshire, IL, USA, detection limit of copper: 0.01 – 4 ppm). Mean recovery values of standards used as quality criteria for quantification of copper ranged from 90% to 110%, which is considered acceptable in a chemical individual analysis (Ribani, 2004; SANCO, 2013). Results in biological samples were expressed as µg of copper / g FW tissue, and in water sample as µM.

2.6. Quality control

All reagents were of analytical grade. Ultra-pure water (Millipore, Milli-Q system) was used for preparing both standard and nutrient solutions, dilutions and blanks. Standard solutions (Merk, Brazil) were used in the experiments and for copper measurement by atomic absorption spectrometry. All glassware was kept in nitric acid solution overnight and then washed with ultra-pure water before use. Chemical residues produced by this study were sent to a company (Saniplan, Rio de Janeiro, Brazil) that works with treatment and storage of residues of chemical products, in agreement with the Brazilian legislation.

2.7. Calculation and statistics

The bioconcentration factor (BCF) was calculated as:

\[
\text{BCF} = \frac{\text{µM in tissue}}{\text{µM dissolved in the media}} \times \left( \frac{\text{g fresh weight}}{\text{µM in tissue}} \right)
\]

where copper dissolved in the media is given by the average of all samples collected at 0 and 24 h in each experimental condition (Xue et al., 2010). See item 2.2.

Data are expressed as mean(s) ± standard error. The ANOVA (one way analysis of variance), followed by the Tukey’s test, was used for determining differences among pigment concentration between treatments and differences in size and number of leaves between days of exposure. The ANOVA (two way analysis of variance), followed by the Tukey’s test was used for detecting: (1) differences between treatments and tissues regarding the copper load; (2) differences between treatments and time of experiment for respiration (24 and 96 h); and (3) differences between treatments and light intensity in the photosynthesis experiment. The significance level adopted for all tests was 95% (α = 0.05). Pearson regression equation was used for determining the relationship between copper burden in plant tissues and copper dissolved in water, and also for the relationship between pigments concentration in leaves and copper dissolved in water. Assumptions of the analysis of variance (Shapiro-wilk test for normality and Levene test for homogeneity of variances) were previously checked. Sigmamaplot 11.1 was used for all statistical calculations.

3. Results

Concentrations corresponding to the nominal control, Hoagland, 1, 10, 100 and 1000 µM of copper were: 0.06 ± 0.01, 0.18 ± 0.06, 1.10 ± 0.11, 10.12 ± 1.32, 113.15 ± 10.53 and 1057.63 ± 82.42 µM of total copper; and 0.08 ± 0.01, 0.18 ± 0.02, 1.42 ± 0.30, 15.85 ± 2.29, 121.47 ± 10.01 and 955.39 ± 52.06 µM of dissolved copper in water. Total copper and dissolved copper are presented as the average of all samples collected at 0 and 24 h for each treatment, regardless of the experiment under investigation. There were differences in copper concentrations neither among experiments nor between periods of 0 and 24 h.

3.1. Copper accumulation test

Copper was measured in all organs of plants exposed to each experimental condition. Its concentration in tissues increased significantly as a function of copper concentration dissolved in water in a dose-dependent manner (correlations were: r = 0.90, r = 0.86 and r = 0.96 for leaves, stem and roots, respectively) (Fig. 1). However, differences
among copper contents in the tissues were observed only in the plants exposed to 10 µM nominal copper. In this case, roots concentrated more copper than leaves and stems. Table 1 shows that, in copper treatments, roots had higher BCF in comparison with leaves and stems, but this difference among organs dropped with the increase in copper concentration in the water. Besides, BCFs calculated for leaves, stems and roots showed that decrease follows an increase in copper concentration in the media.

### 3.2. Physiological assays

Plant respiration is shown in Fig. 2. In general, the highest respiration rates were found in macrophytes submitted to a 96 h test by comparison with the ones submitted to a 24 h test. Regarding the copper effect, after 24 h of experiment, respiration rate was higher in plants exposed to 100 µM Cu than in macrophytes from control, Hoagland and 1 µM Cu groups. On the other hand, at 96 h, respiration was also higher in plants exposed to 100 µM copper but it was significant only in comparison with plants in the 10 µM Cu treatment. Photosynthesis was affected by copper, as well as by light intensity at 24 h of experiment (Fig. 3). Fig. 3A shows that plants immersed in full Hoagland solution presented better photosynthetic performance, followed by plants from the control group and by plants exposed to 1 µM Cu. The best light intensity for *P. pectinatus* photosynthesis was 100 µmol/m²/s. At 24 h, photosynthesis could not be observed in plants exposed to 10 and 100 µM Cu. There were significant differences among groups when light was 300 µmol/m²/s, i.e., plants exposed to 100 µM Cu had their photosynthesis reduced by comparison with others. However, it increased again at 500 µmol/m²/s. Moreover, photosynthetic performance of plants immersed in Hoagland nutrient solution throughout 24 h was two-fold higher in comparison with their performance at 96 h (Fig. 3A and B, respectively).

Concentrations of pigments (chlorophyll a, chlorophyll b and
carotenoids) measured in P. pectinatus at 96 h were inversely correlated to copper concentrations in the water with values of 0.43 for chlorophyll a, 0.53 for chlorophyll b and 0.66 for carotenoids (Fig. 4). The highest pigment concentrations were detected in P. pectinatus immersed in full Hoagland nutrient solution, followed by plants from the control group (without copper in the medium) and by plants exposed to copper at concentrations ≥ 1 µM. However, significance was only observed in plants which underwent copper treatments.

3.3. Morphological assays

Regarding growth based on the size, only plants exposed to 10 µM Cu were reduced both in stem length and in the number of leaves (Tables 2, 3, respectively), while significant increase in the number of leaves was just observed in plants immersed in full Hoagland nutrient solution (Table 3). All macrophytes exposed to 10 µM Cu died after 10 days of test; therefore, the status of growth was registered for those plants on the 10th day. Plants from the Hoagland group did not show any sign of chlorosis and necrosis; however, in control condition, 50% of plants had chlorosis and 20% had necrosis. At 1 µM, 100% plants showed chlorosis and 20% showed necrosis whereas at 10 and 100 µM Cu, all plants had both symptoms.

4. Discussion

Concentrations of waterborne copper were similar in both total and dissolved forms. When in aqueous medium, copper ionizes and complexes with organic and inorganic agents found in water reaching different molecules (metal speciation) that influence in metal bioavailability and toxicity (Mersch et al., 1993; Paquin et al., 2002). The most reactive species of copper is the free ion and other molecules measuring less than 0.45 µM and, thus, present in the dissolved fraction (Paquin et al., 2002). This was the reason why dissolved metal concentrations in water were used to calculate BCF and carry out other relations shown by this study.

According to our results, P. pectinatus can be considered suitable to monitor sites contaminated by copper as it accumulates this metal in a dose-dependent manner with a positive correlation of approximately 0.90 for all tissues (Fig. 1). A study from Demirizen and Aksoy (2004) showed a correlation of 0.79 between copper concentrations in the same aquatic macrophyte and sediment and a correlation of 0.74 between copper in the plants and water. Another study with P. pectinatus carried out by Peng et al. (2008), presented a significant positive relationship (.73) of copper concentrations in water and in leaves of macrophytes. Thus, the ability of P. pectinatus to accumulate copper and the proposal of its use in environmental monitoring is reinforced by this study. It is worth saying that Demirizen and Aksoy, (2004) and Peng et al. (2008) correlated the amount of copper present in plants and in the water from wetlands or wastewater, where parameters influencing copper bioavailability are not controlled, as it is in Hoagland media under laboratory conditions. This may be the reason for the high correlation values found by this study (≈ 0.90) in comparison with these other (≈ 0.70). Another important point to address is that because the dry weight (DW) of roots was extremely low, results of copper accumulation presented here were expressed as µg Cu per gram of fresh weight (FW) to ensure consistent measurements. This makes it difficult to compare our data of copper accumulation with those available in the literature, since most studies express accumulation standardized by dry weight (DW).

Despite data shown in Fig. 1, Table 1 reports an inverse relationship between BCF values and the concentration of copper in water (Table 1). The BCF takes into account the concentration of an element not only in the organism but also in the surrounding environment. As much as higher is the BCF the better is the efficiency of metal extraction from the environment. BCF value is typically above 1 in metal hyperaccumulator species (Zayed et al., 1998). In this study, BCFs were lower than 1 for isolated tissues (leaves, stem and roots) of P. pectinatus exposed to copper. However, it is important to consider that BCF were calculated based on the FW tissues, therefore, data from present study are possibly underestimating the ability of P. pectinatus to bioconcentrate copper in comparison to other results that express higher values of BCF based on DW of Potamogeton sp. (Bielmyer-Fraser et al., 2017).

Taking into account the potential of Potamogeton sp. in bioconcentrate metals as copper, a study of meta-analysis of metal absorption capacity of plants conducted by Li et al. (2015) showed that BCF from the Potamogetonaceae family had a mean value around 3-fold lower than Gramineae, Pontedericeae and Ceratophyllaceae families. Another study showed that Potamogeton sp can bioconcentrate more copper than the macrophytes Utricularia sp. and Nymphae aodorata, but less than Microlythrum sp, Eleocharis sp. and Myriophyllum sp. (Bielmyer-Fraser

### Table 2

Longitudinal stem length (cm) of P. pectinatus (n = 10) exposed (30 days) to Hoagland nutrient solution without copper; full Hoagland solution (0.5 µM Cu); and Hoagland solution with copper from 1 to 100 µM. Data are expressed as mean ± standard error. (*) indicates differences among the same treatments in the different periods (ANOVA – one way, p < .05).

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Time of exposure (Day)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>Hoagland</td>
<td>12.1 ± 0.8</td>
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<tr>
<td>1 µM Cu</td>
<td>10.7 ± 0.3</td>
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<tr>
<td>10 µM Cu</td>
<td>10.3 ± 0.4</td>
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<tr>
<td>100 µM Cu</td>
<td>11.9 ± 1.0</td>
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### Table 3

Number of leaves of P. pectinatus (n = 10) exposed (30 days) to Hoagland nutrient solution without copper; full Hoagland solution (0.5 µM Cu); and Hoagland solution with copper from 1 to 100 µM. Data are expressed as mean ± standard error. (*) indicates differences among the same treatments in the different periods (ANOVA – one way, p < .05).

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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>12.4 ± 1.3</td>
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<tr>
<td>Hoagland</td>
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<tr>
<td>1 µM Cu</td>
<td>13.3 ± 1.1</td>
</tr>
<tr>
<td>10 µM Cu</td>
<td>10.0 ± 0.8</td>
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<tr>
<td>100 µM Cu</td>
<td>11.9 ± 1.6</td>
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et al., 2017). Results from these studies indicate a better performance of the other macrophytes in hyperaccumulate metals than Potamogeton sp. Nevertheless, this work defines important patterns of copper accumulation by P. pectinatus. In addition to the dose-dependent accumulation (Fig. 1), results also showed that roots are the main site of copper accumulation (Fig. 1 and Table 1) and that the efficiency of P. pectinatus to hold copper diminishing when it increases in the medium (Table 1). In the same context, McGeer et al. (2003) showed that higher BCF values are indicative of lower metal exposure levels for a range of aquatic biota, including algae and macrophytes. The inverse relationship between BCF and copper in the media may be due to either a saturation of copper uptake or a down-regulation of metal uptake. The process of copper accumulation has been shown to occur initially by bioadsorption, followed by transport through the membrane and bioaccumulation in the cytoplasm, in a dose-dependent and time-dependent course (Monferrán et al., 2009). Previous studies about metal hyperaccumulating aquatic plants, including those of the genus Potamogeton, show that the accumulation of most metals is higher in roots than in other parts of plants (Cardwell et al., 2002; Demirizen and Aksoy, 2004; Fritioff and Greger, 2006; Yabanli et al., 2014), which agrees with our results. Containment, immobilization and accumulation of metals in the root may be due to the process of rhizofiltration, which is commonly observed in aquatic plants (Dhir and Srivastava, 2011). However, Guizzioni (1991) and Fritioff and Greger (2006) observed that the absorption of metals by leaves and stems is also significant in submerged macrophytes. In this context, Table 1 shows that at 1000 µM Cu, BCFS were basically the same in the 3 tissues. These data suggest that roots are potentially the primary site of metal absorption, but once its capacity exhausts due to high concentration of copper, metal uptake is managed by other tissues, such as leaves and stems (Hedges and Curtis, 1999). Copper absorbed by the plant may also be transported around its tissues (Xue et al., 2010). Since copper is an essential nutrient, plants can control cellular levels of this metal by regulating copper absorption, besides either augmenting or reducing free copper concentrations. Copper circulating is mediated by metalloproteins, which are soluble metal-binding proteins able to transport copper through plant tissues and deliver the metal to the sites where it is required (O’Halloran and Calotta, 2000). These proteins can also prevent toxicity by avoiding high levels of free copper as well as its translocation to sensitive parts of the plant (Chen et al., 2000).

Accumulation of metals in various parts of aquatic macrophytes is often accompanied by induction of cellular and physiological changes; some of them directly contribute either to metal tolerance or to toxicity in plants. In this study, copper bioaccumulation in P. pectinatus resulted in considerable changes, in the plant physiology and morphology, affecting its health and survival. This indicates a relatively high sensitivity of P. pectinatus to copper. Copper (100 µM), for example, increased respiratory rates at 24 h of experiment that reflected in photosynthesis depression; and it also caused significant inhibition of photosynthesis at 1 µM. On the other hand, the absence of copper in the medium also seems to be negative for photosynthesis, indicating how essential this micronutrient is.

The redox property of copper makes it a very reactive molecule. Thus, it has high potential for interacting with proteins, affecting their morphology and function. In addition, over Fenton-type reactions copper raises free radicals (Sutton et al, 1989). The antioxidants play an important role in cellular defense against ROS, but copper can also affect them (Monferrán et al., 2009). Copper directly interacting with proteins from PSII and/or inducing ROS production and decreasing antioxidant capacity may have led to degradation of chlorophyll pigments, contributing to the inhibition of photosynthesis observed by this study (Figs. 4 and 3A, respectively). Likewise, Prassard et al. (2001) reported inhibition of photosynthesis and decreasing in pigment concentration in Lemma sp. exposed to copper at concentrations ranging from 2 to 50 µM Cu – 48 h.

Although inhibited, photosynthesis was detected in plants exposed to 1 µM of copper at 24 h but not after a 96 h test. According to Monferrán et al. (2009), variations in antioxidant defenses occur as a function of time and copper concentration, a fact that may affect copper toxicity. The authors showed that the defense system of P. psilus reacts at low copper concentrations (≤ 20 µg/L) and short exposure (1 day), but is strongly affected at high concentrations (≥ 20–100 µg/L) and prolonged exposure (3 and 7 days) to the metal. Exposure to copper at 1 µM (63 µg/L) for a short period of 24 h may induce the antioxidant defense to protect plants and contribute to photosynthesis in this group, but not at 96 h. In fact, at 96 h, only plants from control, Hoagland and exposed to 100 µM performed photosynthesis. This result for plants submitted to 100 µM Cu was unexpected and the collected data in present study do not allow indication of which mechanisms could be involved in that. Perhaps, induction of certain enzymes only triggered by high copper accumulation for metal detoxification (Assche and Clusters, 1990), or a decrease in susceptibility of enzymes to metal inhibition, as observed in copper tolerant soil plants (Cox and Hutchinson, 1980), could have happened allowing photosynthesis in plants from 100 µM Cu group at 96 h.

Luminosity also affected photosynthesis (Fig. 3). P. pectinatus had better photosynthetic performance in light intensity of 100 µmol/m2/s, in agreement with results found by Colares et al. (2007). Decrease in the photosynthesis rate to its total inhibition at 500 µmol/m2/s may have been caused by the excess of radiation (photoinhibition) and the presence of copper. These conditions together could intensify potential injuries to the reaction center of photosystem II. It is worth considering that fluctuations in physiological performance, e.g., photosynthesis and respiration rates, happen as consequences of either a contaminant or variations in natural parameters in a process known as phenotypic plasticity, which supports the survival of plants under stressful conditions (Bennett, 1997).

As mentioned before, another symptom of copper toxicity was the decrease in the levels of pigments chlorophyll a and b and carotenoids (Fig. 4). Several studies reported decrease in concentrations of photosynthetic pigments in aquatic plants exposed to heavy metals (Pietrini et al., 2005; Hou et al., 2007; Sivacic et al., 2008; Upadhyay and Panda, 2009, Upadhyay, 2014). According to Prasad et al. (2001), drop in chlorophyll is attributed to the copper-induced modification of chlorophyll molecule, leading to structural and functional damage. For example, copper can replace the magnesium atom of the chlorophyll molecule and damage it (Kupper et al., 1998). On the other hand, carotenoids can serve as antioxidants, diminishing cell injuries (Czerpak et al., 2006). However, it was observed that copper can lead to decrease in carotenoids in P. pectinatus exposed to Cu. Previous studies showed that decrease in carotenoid content is a common response to metal toxicity (Rai et al., 2004), but increase can be associated to its important role in detoxification of ROS (Chandra et al., 2009). Moreover, the lack of difference between control and Hoagland groups on Fig. 4 may be due to the fact that copper is the second micronutrient less required by Potamogeton (Epstein and Bloom, 2006) and it is not part of chlorophyll or carotenoid molecules, thus, over 96 h the absence of copper did not affect pigment concentration.

Reduction in photosynthesis caused by copper may affect production of energy required by plants for biomass synthesis and growth. In our study, only plants from Hoagland group had their number of leaves significantly increased from the beginning to the end of exposure time (30 days of test), indicating plant growth on this treatment. Inhibition of growth based on stem weight was detected for Elodea canadensis exposed to copper for 25 days (Mal et al, 2002) and for H. verticillata exposed to copper for 4 days, both in a dose-dependent manner. While, Odjegba and Fasidi (2004) observed reduction in the leaf area in Pistia stratiotes exposed to 1000 µM Cu (10 fold superior than the highest concentration tested here which was 100 µM). Unlike studies mentioned above, in this work cooper did inhibit growth only at 10 µM. The concentration of 10 µM Cu appeared to be very critical for P. pectinatus. Plants submitted to this concentration died on the 10th day of the test.
However, before plant death, there was reduction in size as well as leaf fall. The lack of inhibitory effect of copper on stem size and number of leaves of plants exposed to 1 and 100 µM Cu, showed that even under copper stress, these plants are still alive, at least for 30 days.

Chlorosis and necrosis imply early senescence of plants and such effects were clear after exposure to copper. Plants submitted to Hoagland solution did show the best functioning with the best photosynthetic performance and no signs of chlorosis and necrosis. In fact, Hoagland culture medium contains all elements at desired concentrations required by these Potamogeton sp. to grow, including copper (Hoagland and Arnold, 1950; Spencer, 1986; Tripathi et al., 2003; Colares et al., 2007; Upadhyay et al., 2014). Finally, taking into account that this study used young specimens of P. pectinatus, the negative effects of copper could also influence plants colonization and establishment in aquatic environments.

5. Conclusions

Results shown by this study reflect the intensity and diversity of disorders generated by the absence of copper, as well as of high concentrations of metal exposure. The macrophyte P. pectinatus can be considered a suitable plant for monitoring environments contaminated by copper. This conclusion is drawn from the dose-dependent curve of accumulation of copper in the plant and decrease in pigment concentration. Chlorosis and necrosis can also be used as parameters for copper monitoring. However, values of BCF based on FW is not proper to indicate the use of P. pectinatus for phytoremediation in copper contaminated sites. Furthermore, negative effects of copper investigated by this study should call attention to the future maintenance of P. pectinatus in its natural environment.

Acknowledgements

This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) in the scope of the Instituto Nacional de Ciência e Tecnologia - Toxicologia Aquática (INCT-TA / Proc. 573949/2008-5). The authors thank the coordinator of INCT-TA, Dr. Adalto Bianchini. MBC is supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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