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

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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 Hoalgand 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 co-factor 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 interferes 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.,

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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 (Beeby, 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 Aksoy, 2004; Fritioff and Greger, 2006; Yabanli et al., 2014). However, Guilizzoni (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 D:12 L and 23 °C, respectively. Light was provided by daylight lamps with irradiance of 114.33 ± 4.70 μmol/m^2/s, measured by a light sensor (Li-Cor Radiation sensor LI-1400 Data Logger, Lincoln, Nebraska USA).

2.2. Copper accumulation test

Acclimated *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 $CuCl_2{\cdot}2H_2O$, 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% HNO_3) 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 HNO_3 (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 acclimated *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 $CuCl_2{\cdot}2H_2O$, 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 cooper; 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% HNO_3) 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/m^2/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.