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Evaluation of the potential of *Pontederia parviflora* Alexander in the absorption of copper (Cu) and its effects on tissues

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ABSTRACT. The study sought to evaluate the potential of the aquatic macrophyte *Pontederia parviflora* Alexander in the absorption of copper (Cu) and possible variations in its tissues after 21 days of exposure to this metal. The concentration of Cu was analyzed in the solution and in the vegetal tissues (stem, root and leaves). The experiment was set up in triplicates with weekly measurements of pH, temperature and DO. The results indicated that *P. parviflora* reduced 96% of Cu in the solution and the root was the vegetal tissue, which accumulated more Cu. Values of pH, DO and temperature were in accordance with the metabolic activities of the plants. In the anatomic analyses, dark spots were identified in the vascular bundles and in the epidermis of the aerial parts, highlighting the variation resulting from the presence of this substance. However, these variations were not sufficient to damage the development of the individuals. *P. parviflora* showed high capacity of extraction and storage of the metal, being a good alternative to aquatic environments with high concentrations of Cu.

Key words: aquatic macrophyte, phytoremediation, copper.

RESUMO. Avaliação do potencial de *pontederia parviflora* Alexander na absorção de cobre (Cu) e efeitos nos tecidos. O estudo avaliou o potencial da macrófita aquática *Pontederia parviflora* Alexander na absorção de cobre (Cu) e possíveis alterações nos tecidos, após 21 dias de exposição. O teor do metal foi analisado na solução e nos tecidos vegetais (caule, raiz e folhas). O experimento foi montado em triplicatas com medidas semanais do pH, temperatura e OD. Os resultados mostraram que *P. parviflora* reduziu 96% do Cu na solução e a porção vegetal que acumulou mais o metal foi a radicular. O pH, OD e temperatura apresentaram valores condizentes com as atividades metabólicas das plantas. Nas análises anatômicas, foram identificadas manchas escuras nos feixes vasculares e na epiderme das partes aéreas, evidenciando a alteração provocada pela presença da substância. No entanto essas alterações não foram suficientes para prejudicar o desenvolvimento dos indivíduos. *P. parviflora* mostrou-se com alta capacidade de extração e armazenamento do metal, sendo uma boa alternativa para ambientes aquáticos com altas concentrações de Cu.

Palavras-chave: macrófitas aquáticas, fitorremediação, cobre.

Introduction

The increase in environmental problems in Brazil is related to the industrialization process initiated in the 1950s, an agricultural model of monoculture and exportation instituted from the seventy years, accelerated urbanization, and to socioeconomic inequality (LAMEGO; VIDAL, 2007). In this context, the lack of adequate treatment and disposal of wastes, nutrients and pollutants from industrial and domestic activities in water bodies contribute directly to the enhancement of these problems, causing degradation and making impossible the utilization of these resources, which are fundamental to the maintenance of life.

The discharges of liquid effluents can cause variations in the physical-chemical characteristics of a water body such as pH, temperature, composition and concentration of each chemical component (for instance nutrients, heavy metals) and bring drastic consequences to the living beings that depend directly or indirectly on that water body (BRAGA et al., 2002). Contamination by heavy metals represents a great problem among many pollutants that are discharged in the aquatic environment, as they are not biodegradable and are accumulated into the food chains.

Although copper (Cu) is an essential microelement to many metabolic activities, it becomes extremely toxic in higher concentrations.

This metal is present in industrial and domestic effluents, and the most common sources of its introduction in the aquatic environment are: corrosion of copper and alloy pipes by acid waters, algaecides, fungicides used in the preservation of wood and in the industry of mining, casting, electro planting process and refining (BLAYLOCK; HUANG, 2000; RASKIN et al., 1994).

Phytoremediation becomes a great potential tool for environmental decontamination. According to Salt et al. (1998), phytoremediation is a technique of decontamination that applies plants to remove pollutants from the environment or to transform them in less dangerous forms to living beings. The application of this technique has minor environmental impact and lower installation and maintenance costs when compared with physical-chemical methods. It can be used in soils and waters contaminated with metals, organic compounds, radioactive chemical elements, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents and industrial toxic sub-products (CUNNINGHAM et al., 1996).

The application of aquatic macrophytes in effluent treatment systems is being used with success in polluted rivers and lakes. In environmental sanitation, their use is associated with domestic sewage treatment in humid land systems, known as constructed wetlands. Their usage is justified because these plants present fast growth, intense absorption of nutrients, ease of withdrawal from lakes and the possibility of reusing the biomass (COSTA et al., 2003).

Many research studies have been carried out to evaluate the phytoremediation potential of native aquatic macrophytes of Campo Mourão region, Paraná State. Thus, this work was developed in order to accomplish the efficiency of the aquatic macrophyte *Pontederia parviflora* Alexander in the removal of copper (Cu) and its effect in the alterations of the tissues of root, stem and leaves of these plants.

Material and methods

The experimental apparatus was set up in six aquariums with eight individuals of the mentioned specie. Three aquariums received Cu solution in the water and the others were kept with no addition of this metal solution, as control. For the experimental set up, the plants were randomly collected in the Papagaios river in the rural area of Campo Mourão in January 2007. The analyses were carried out weekly in the Laboratory of Ecology of the Federal Technology University of Paraná – UTFPR, Paraná State, during 21 days.

The aquariums were set up in triplicate with eight seedlings of the specie in each aquarium and control. The collected plants were washed in tap water to remove soil adhered to the roots. Basaltic crushed stone was used as substrate, washed several times with distilled water, hydrochroloric acid 10% and distilled water, until reaching neutral pH. Then, the aquariums received 6 L of distilled water and were kept seven days for acclimatization of the specie to the conditions of luminosity and temperature of the laboratory.

After the acclimatizing period, the water of the aquariums was replaced by the nutritional solution of Clark (1975), distilled water and copper sulfate (until reaching a concentration of 10 ppm), totaling a volume of 6 L. The control aquarium contained only the nutritional solution. The level of the aquariums was kept weekly by adding distilled water. Dissolved oxygen (DO), temperature and pH were measured weekly in the aquariums by using potentiometers.

Metal concentration in the beginning of the experiment was measured in a sample of the solution and in the vegetative portions of the plant. After 21 days of the treatment, copper concentration was analyzed in new samples of the solution and in the aquarium plants. The sediment in the bottom of the control aquarium was also evaluated, but the same procedure was not carried out with the plants because this material is composed by the plants roots. The results were analyzed by *t* test to paired samples.

Transversal and paradermal sections were made on the roots, stem and leaves of the plants by free hand of each aquarium in order to carry out the anatomical and histological analyses. To build the slides, the cuts were clarified with sodium hypochlorite 1%, washed with distilled water, stained with astra blue and fuchsine and fixed with glycerin in semi permanent slides for viewing in an optical microscope. Photomicrographs were done by using a Motic photomicroscope and the Motic 2000 program.

Results and discussion

Variations in pH, DO and temperature were observed during the weekly accomplishment in the control aquarium and in the treatment. The zero day indicates the addition of Cu in the aquariums after the acclimatizing period of the plants. The control aquarium presented slightly acid values during the entire period and the aquariums containing Cu showed an increase of this parameter that remained similar to 7.3 (Table 1).

According to Kadlec and Knight (1996) and Metcalf and Eddy Inc. (1991), the indirect effect of pH on aquatic ecosystems is very important because it can contribute to the precipitation of toxic chemical elements such as heavy metals in some conditions and have an effect on the solubility of the nutrients. H^+ ions are essential to guarantee the availability of some nutrients to life processes and also can help the dissolution of heavy metals that are highly toxic (RICKLEFS, 2003).

Table 1. Mean concentrations of Cu present in the control solution and in the treatment with *P. parviflora* of the samples analyzed after 21 days of treatment.

Analysed parts	1st Day	21st Day
Control solution	9.67 ± 0.05 mg L^{-1}	0.06 ± 0.04 mg L^{-1} (8.23 ± 0.35 mg L^{-1}, precipitate)
Solution	9.67 ± 0.05 mg L^{-1}	0.05 ± 0.03 mg L^{-1}
Aerial	14.94 ± 0.04 mg kg^{-1}	33.53 ± 6.03 mg kg^{-1}
Root	56.88 ± 0.05 mg kg^{-1}	377.40 ± 127.21 mg kg^{-1}
pH	5.6	7.3
DO	4.4 mg L^{-1}	2.4 mg L^{-1}

Results expressed as mean ± standard deviation (N = 3), in which standard deviation of the first day and control are related with the measure and of the plant is related with the treatment.

Concentrations of dissolved oxygen were unstable during the process. Initially, a mean concentration of dissolved oxygen of 4.4 mg L^{-1} was obtained. However, this value was reduced in the treatment aquariums in the measurement carried out after seven days, while it remained equal to 3.0 mg L^{-1} in the aquarium with no metal solution. This decrease can be explained by the biodegrading activity of the bacteria that were adhered to the plants after their collection from the Papagaios river.

The mean temperature was 28ºC in the four aquariums during the 21 analyzed days. Room temperature varied from 25ºC (minimum) to 33ºC (maximum). As the experiment was carried out during the summer, high values were recorded for room temperature, typical for that period, with a minimum of 25ºC and maximum of 33ºC. Although high temperatures favored the development of aquatic macrophytes from different ecological groups, each specie presents a optimum value of temperature (CAMARGO et al., 2003).

Analyses referring to Cu concentration in the samples (Table 1) indicated that this specie accumulated higher quantity of this metal in its roots than in its aerial parts. It is important to highlight that even though the concentration of Cu was reduced in the control solution, it was accumulated in the basaltic crushed at in the bottom of the aquariums. The statistical test, in a level of 0.05, indicated the difference among the treatments as significant ($t = 3.99$; $p = 0.0005$). In accordance with Moreira and Siqueira (2006), plants can accelerate the removal processes of metals, acting under the contaminants and contributing indirectly by the rhizospheric effect under the biodegradable microbiota or accumulating the metal in their roots.

The treatment efficiency of this plant was corroborated by the concentration of copper lower than 1.0 mg L^{-1} observed after 21 days of the experiment. This concentration was in accordance with the discharge patterns described in Resolution no. 357/2005 of Conama.

The principle of phytoremediation technique is based on the mechanisms of tolerance to the accumulation of metals existent in the plants in order to keep their cellular functions even in the presence of great quantity of metals. These mechanisms include the compartmentalization of metals in cellular structures, exclusion or reduction of the transport by the membrane and the formation of chelating peptides, rich in cysteine, as phytochelatins and metallothioneins that assist, directly and indirectly, the accumulation of metals by the plants (COBBETT; GOLDSBROUGH, 2002).

Histological cuts were done in the control individuals and in the individuals in contact with the Cu solution to verify the alterations in the structure of the tissues of *P. parviflora*. Anatomical analyses of the tissues of the leaves showed that the epidermis has cylindrical and slightly flattened cells (Figure 1a). The leaf mesophyll is composed by the sustentation tissues of palisade parenchyma (below the epidermis), sponge parenchyma and by silica spicules that also assist in the sustentation (Figure 1b). In accordance with Scremin-Dias et al. (1999), the mesophyll is also composed by many intercellular air spaces that transport gases to the entire plant, offer mechanical resistance to the submersed parts and assist in fluctuation.

Anatomical analyses of the leaves tissues indicated that the plants in contact with Cu presented dark substances stored in the mesophyll and in the epidermis (Figures 2a and b). This dark substance was apparently produced by the glandules (Figure 2a).

Sections done in the stem did not present any alteration in the conduction tissues of xylem, phloem and aerenchyma. The plants in contact with Cu also presented many dark spots in the glandules of the vascular bundles (Figures 3a and b).

Roots of *P. parviflora* presented a typical primary growth with cortical zone filled by aerenchyma with big and fragile intercellular spaces that are disintegrated with the cut. Sections were done in the mid regions of the roots, but it was not possible to make good observations and records of the slides in the microscope due to their sensitivity. Due to this, it was difficult to evaluate their anatomy. Although it was verified that the roots absorbed more Cu by the chemical analysis, no relevant anatomic alteration was noted.

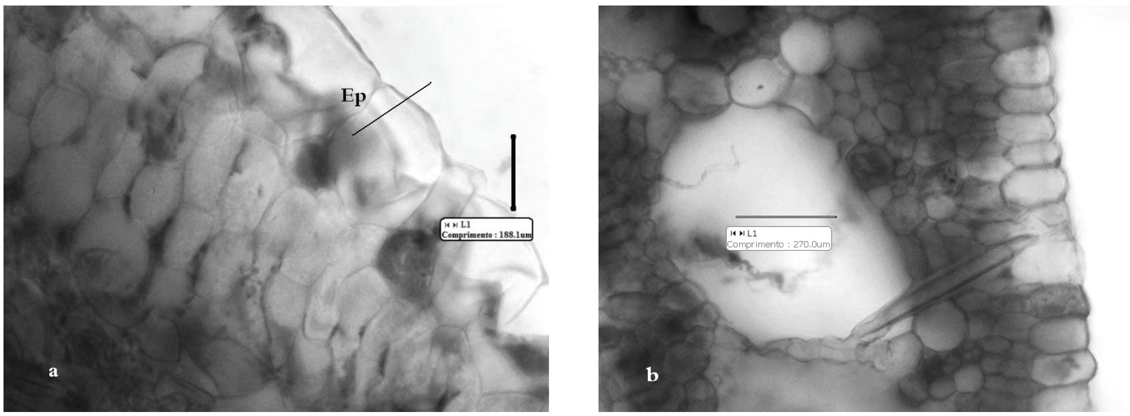

Figure 1. Photomicrographs of the transversal sections of the leaves of *P. parviflora*, with no contact with the solution containing Cu. a) detail of the epidermis (Ep) 100 x and b) detail of the foliar mesophyll 40x.

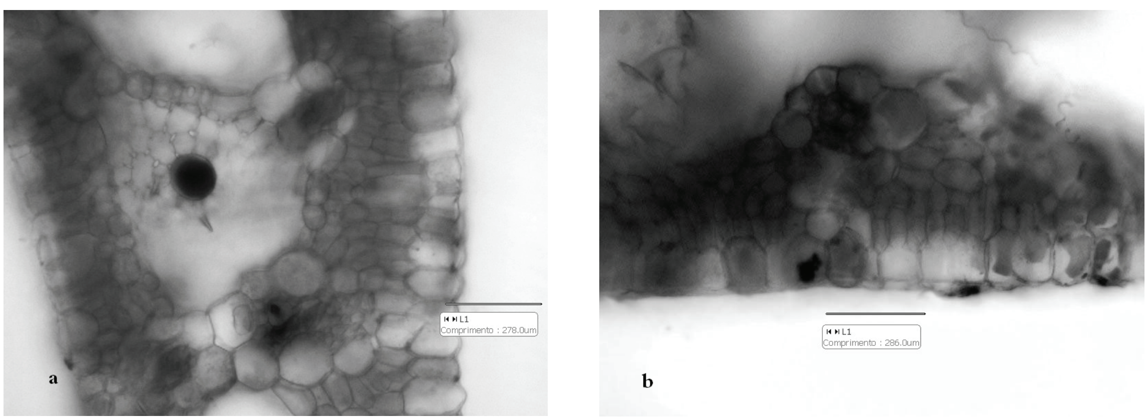

Figure 2. Photomicrographs of the transversal sections of the leaves of *P. parviflora*, increase of 40 x a), detail of the presence of dark spots in the aerial spaces and in the vascular bundle, b) detail of the dark spots in the epidermis. Scale_____ 0.28 mm

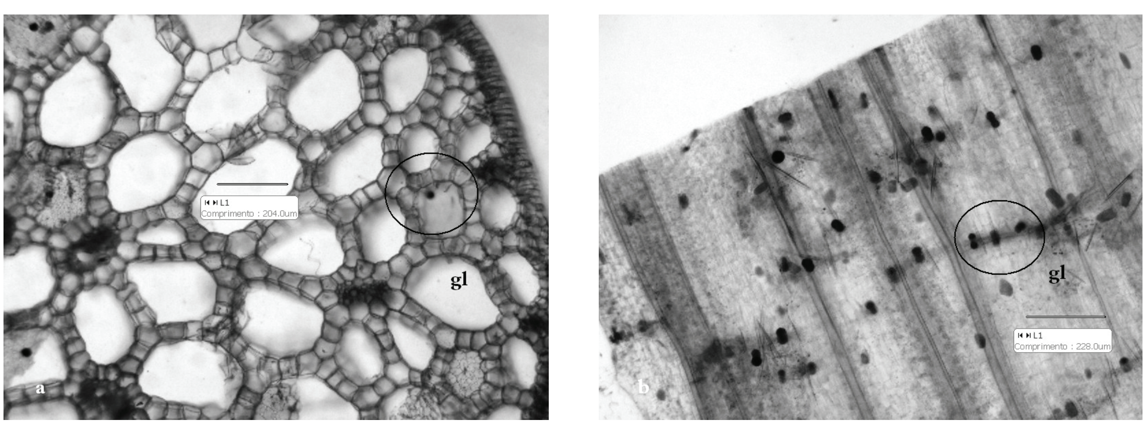

Figure 3. Photomicrographs of the transversal sections of the stems of *P. parviflora* in a) (40 x) and longitudinal in b) (10 x). Detail of the presence of alteration of the glandule (gl) in the vascular bundle. Scale _____ 0.2 mm.

The high concentrations of heavy metals in the environment does not always indicate high bioaccumulation by the vegetal species, as the interpretation of the results can be complicated and the withdrawal system, via root, is not completely discovered (MULGREW; WILLIAMS, 2000). Moreover, the species did not react similarly to different metals. It is common that the plant is an excellent accumulator of a type of metal and less efficient to others, indicating its utilization as bioindicator (MKANDAWIRE; DUDEL, 2005; ODJEGBA; FASIDI, 2004).

Comparing the cuts in the non contaminated plants with those in the plants contaminated with Cu, it was verified that the specie absorbed the metal by its roots, transporting and accumulating it in its aerial parts. Thus, it can be considered as a hyperaccumulator plant with high capacity of phytoextraction and storage of Cu. The application in civil construction, production of bricks, cement pastes and mortars are alternatives for its discharge and later utilization of its biomass.

Conclusion

Based on the results obtained in the experiment, it was possible to conclude that *Pontederia parviflora* was efficient in the phytoremediation of Cu, as the concentration of this metal in the solution of the aquariums was reduced and remained in the discharge patterns established in Resolution no. 375/2005 of Conama, which varies from 0.009 mg L^{-1} to 0.0013 mg L^{-1} for freshwater of classes I to IV of rivers. However, more research studies must be carried out with equal or higher concentrations of Cu with this specie during longer periods of time.

As the principles of the phytoremediation technique are based on the tolerance to the contaminants, values observed for the parameters pH, DO and temperature were acceptable to the metabolism of the plants that kept their cellular functions even in the presence of the added metal.

This specie can be classified as hyperaccumulator with phytoremediation mechanism of phytoextraction as it absorbed, transported and accumulated the metal by its roots and aerial parts.

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