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Uptake arsenic by plants: Effects on mineral nutrition, growth and antioxidant capacity

La absorción de arsénico por las plantas: efectos en la nutrición mineral, el crecimiento y la capacidad antioxidante

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ABSTRACT

Arsenic (As) is the one of the main environmental pollutant and phytoremediation is an effective tool for its removal of the environment. In this study, *Pistia stratiotes* were exposed to seven As concentrations (0, 3, 7, 10, 13, 16 and 20 µM) and then, the influence of this metalloid on growth, mineral nutrition and photosynthesis were analyzed. It was observed that this species have a high affinity for As and pollutant uptake occurs rapidly. The uptake of Cu, Mn, Fe and P increased until the concentration of 13 µM, decreasing in higher concentrations.The Mg content also decreased from this same concentration. No effects were observed in the uptake of K, Ca and Zn. Growth rate and photosynthetic pigments content were negatively affected by As. Despite this decrease, the growth was maintained up to the concentration of 13 µM of As. The maintenance of growth and the change in nutrients uptake are probably related with the increase in antioxidant capacity of the plant, indicating resistance to the pollutant. In this way, *P. stratiotes* is probably an efficient phytoremediator of As, even when in concentrations up to one hundred times greater than those permitted in water for human consumption.

Key words: Aquatic plant species; environmental pollution; phytoremediation.

RESUMEN

El Arsénico (As) es uno de los principales contaminantes ambientales y la fitorremediación se presenta como una herramienta efectiva para retirar este elemento del medio ambiente. En el presente estudio se analizó la influencia de este elemento en el crecimiento, nutrición mineral y fotosíntesis de Pistia stratiotes *bajo siete concentraciones de As (0, 3, 7, 10, 13, 16 y 20 µM). Encontrándose gran afinidad de esta especie por el As, siendo que la absorción de este elemento ocurre de forma rápida afectando la absorción de nutrientes esenciales. En este sentido, la absorción de Cu, Mn, Fe y P aumentó hasta una concentración de 13 µM de As, disminuyendo en el caso de las mayores concentraciones, entre tanto se observó una disminución en la absorción de Mg. No fueron observados efectos sobre la absorción de K, Ca y Zn. A pesar que tanto la fotosíntesis como el crecimiento fueron negativamente afectados por las diferentes concentraciones de As la planta consiguió mantener una tasa reducida de crecimiento hasta la concentración de 13 µM. Esta situación así como las alteraciones observadas en la absorción de nutrientes, probablemente esté relacionada con el aumento en la capacidad antioxidativa de la planta, indicando una posible respuesta de resistencia de la planta frente a este elemento contaminante. De esta forma es probable concluir que* P. stratiotes *actúa como una eficiente fitorremediadora de As, aun en concentraciones cien veces mayor que los niveles permitidos en el agua apta para consumo humano.*

Palabras clave: *plantas acuáticas, la contaminación ambiental, la fitorremediación.*

Introduction

Arsenic (As) is a metalloid which occurs naturally, although it can also be accumulated in the environment due to human actions. In Brazil, As contamination occurs mainly in regions with intensive mining activity, like in the Iron Quadrangle (McClintock *et al.,* 2012). Chronic arsenic exposure causes several cancerous and non-cancerous human diseases including urinary bladder, lung and liver

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cancer. Drinking water is the most common source for human exposure to As and the presence of this compound has therefore been declared as a major risk to human health in many regions in the world (Lièvremont *et al.,* 2009). A possible solution for this problem is the use of plants that are able to accumulate As by removing the metalloid from water and soil through a process called phytoremediation (Zhang *et al.*, 2009b).

Phytoremediation is a research field which has been developed in the recent years and numerous studies in this area are being carried out (Gonzaga *et al.,* 2012; Mandal *et al.,* 2012; Leão *et al.,* 2013). Aquatic macrophytes are an interesting tool for phytoremediation and they can effectively remove As from water (Sasmaz & Obek, 2009). In the present study, *Pistia stratiotes* L. (Araceae) was selected because of its fast growth, wide distribution and short life span, which are interesting features for phytoremediation (Rahman *et al.,* 2008). Furthermore, this plant was reported as being effective in removing various metals from water, including As. Although it has been shown that *P. stratiotes* is able to accumulate arsenic, the toxic effects of this pollutant in the plant remain unclear (Leão *et al.,* 2013).

Arsenic interferes in plant metabolism by inhibiting its growth and it may also influence nutrient uptake by competing directly with nutrients and/or altering metabolic processes. Phosphate is usually the nutrient whose uptake is the most affected by As. Arsenate (As^{5+}) and phosphate (P^{5+}) competes for uptaking since they are both taken up via phosphate transport systems. As^{5+} acts as a P^{5+} analog and it can disrupt phosphate metabolism (Farnese *et al.,* 2014). As also influences the uptake of other mineral nutrients in plants. In a hydroponic system, for example, it was reported that the addition of As influence significantly the concentration of potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn) and zinc (Zn) (Liu *et al.,* 2008). Other harmful effect of As is oxidative stress, as result of the increased production of reactive oxygen species (ROS). In this way, the tolerance of plants to As is related with the antioxidant capacity of the cells, which sometimes can be associated with the changes in nutrients uptake (Leão *et al.,* 2013).

The understanding of how these changes occurs and the behavior of the element in plants are important for the implementation of strategies to improve phytoremediation. So far, few researches have been carried out on the micro- and macronutrients responses of plants under high levels of stress due to As and the knowledge of the kinetics of As uptake for plants is in general poorly understood (Abedin *et al.,* 2002). Therefore, this study aimed to investigate the effects of As on mineral nutrition and growth of the plants, besides to evaluate the kinetics of As uptake.

Material and Methods

Pistia stratiotes plants were collected from the forest garden of the Federal University of Viçosa (Universidade Federal de Viçosa - UFV), separated according to their size (about 10 g fresh weigth) and grown in polyethylene pots containing 9 L of Clark nutrient solution (Clark, 1975) at half ionic strength and pH 6.5 in a growth chamber with controlled light and temperature (25 ± 2 ºC, 230 $\mu mol\ m^{-2}\ s^{-1}$) and a 16-hour photoperiod. Plants were maintained at these conditions for three days for acclimatizing to laboratory conditions.

Uptake kinetics measurements

The acclimatized plants were transferred for pots with different As concentrations and a depletion method (Zhang *et al.,* 2009a) was used to measure As uptake kinetics. The concentrations of As ($Na_2HAsO_4 \cdot 7H_2O$) were (µM) 0, 3, 7, 10, 13, 16 and 20. Each pot was weighed at the beginning and in the end of the experiment to calculate the water loss through evapotranspiration. Uptake rates were calculated by measuring the depletion from the solution based on the root dry weights and the concentration of As in roots and in the solution (Zhang *et al.,* 2009a). The As concentrations were measured by inductively coupled plasma spectrophotometer (Shimadzu, model AA-6701FG) (Marin *et al.*, 1993). Values for V_{max} and K_m were estimated for As by fitting Michaelis-Menten equation.

Determination of relative growth rate and chlorophyll contents

The acclimatized plants were transferred to pots containing As at the same concentrations described above. The plants were kept in these conditions for seven days. After harvesting, plants were rinsed in deionized water, weighed and dried in a conventional oven at 80 ºC until a constant dry mass. The relative growth rate of plants exposed to As was calculated using the equation proposed by Hunt (1978).

To determine the effect of As on photosynthetic pigments, approximately 0.3 g of fresh leaves were macerated in liquid nitrogen and added to 80% acetone and a small amount of silica. The homogenate was filtered and diluted to 25 mL with 80% acetone. The entire procedure was performed in the absence of light and the absorbance was measured using a spectrophotometer at 663, 645, 652 and 470 nm to determine the total chlorophyll a (chla), chlorophyll b (chlb), total chlorophyll and carotenoids, respectively, as proposed by Arnon (1949).

Mineral nutrition

The same plants utilized for assessing the uptake of As were utilized for assess mineral nutrition. The concentration of mineral nutrients (P, K, Ca, Mg, Fe, Mn, Cu and Zn) was measured by inductively coupled plasma spectrophotometer (Shimadzu, model AA-6701FG).

Total antioxidant capacity

The total antioxidant capacity of *P. stratiotes* was determined by the homogenization of 0.3 g of roots samples in 1.2 mL of 0.1 M sodium phosphate buffer, pH 7.6, containing 0.1 mM EDTA. The homogenate was centrifuged at 12,000 x g for 5 min. Then, 50 µL aliquots were removed from the supernatant and added to 1.5 mL of reaction medium consisting of 10 mM 2,4,6-tripyridyl triazine, 20 mM ferric chloride and 300 mM sodium acetate buffer, pH 3.6. After 5 min, the absorbance was read at 593 nm. The total antioxidant capacity was expressed as µM Fe g^{-1} FW (Szóllósi *et al.,* 2009).

Statistical analyses

The experiments were carried out in a randomized experimental design with five replicates. All statistical analysis were performed using SAEG 9.1 (System for Statistical Analyses). ANOVA was used to analyze the effect of As concentration on mineral nutrients uptake, and means were separated using Tukey's test at P≤0.05. The influence of As on plant growth and chlorophyll content were analyzed by linear regression.

Results and Discussion

Arsenate influx showed a linear increase with increment of As in solution (Figure 1). The kinects of As^{5+} influx was adequately described by the Michaelis-Menten equation, with K_m and V_{max}

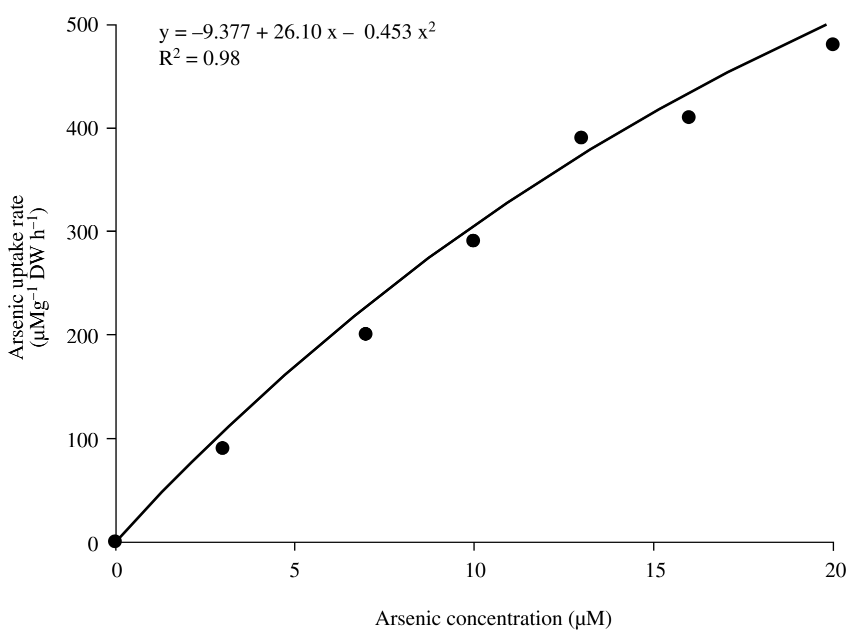

Figure 1. Arsenic concentration in the root of *Pistia stratiotes* L. (Araceae) under arsenic stress.

values for the saturable component of 2.9437 µM and 94.3396 µmol As g^{-1} FW h^{-1}, respectively. Characteristics of uptake kinetics can be considered as one important criterion for selecting a plant to be used in phytoremediation (Abedin *et al.*, 2002). The K_m for *Pistia stratiotes* appears to be lower than the values reported for others plant species (Zhang *et al.,* 2008), indicating an efficient As^{5+} uptake system, which may be the result of a higher density of transporters in the plasma membranes (Zhang *et al.,* 2008). In the vast majority of plants, this uptake occurs through high-affinity phosphate transporters, which apparently also occurs in *P. stratiotes*, since the values for V_{max} and K_m parameters are similar to the uptake kinetics of high affinity (Abedin *et al.,* 2002).

A higher growth rate associated with pollutants accumulation is one of the characteristics required for phytoremediation and changes in the relative growth rate have been used to differentiate the tolerance of plant species to toxic chemicals effects (Farnese *et al.,* 2014). The growth of *P. stratiotes* was significantly affected by the treatments with As. There was a linear reduction on growth with the increase of the metalloid concentration in solution, and at 20 µM of As, the relative growth rate was negative (Figure 2). Growth decrease is a typical plant response to As (Päivöke & Simola, 2001). Despite this decrease, the growth was maintained up to the concentration of 13 µM, indicating that the plant is tolerant to this As concentration.

The growth decrease in *P. stratiotes* may be related with the decrease in the photosynthetic pigments content and consequently in the photosynthetic process (Mascher *et al.,* 2002). Indeed, photosynthetic pigments were also negatively affected by As, and an increase in the concentration of the metalloid was accompanied by a decrease in the content of chla, chlb, total chlorophyll and carotenoids (Figure 3). The decrease in total chlorophyll content is one of the early symptoms of toxicity of several stress agents such as metals and metalloids (Leão *et al.,* 2013). This decrease is one of the parameter used as a bioindicator of oxidative stress caused by heavy metals (Macfarlane & Burchet, 2001). Some studies have reported As toxic effects on photosynthetic pigments (Farnese *et al.,* 2014; Leão *et al.,* 2013), which shows injuries in the photosynthetic apparatus and changes in the photosynthetic capacity, resulting in many negative consequences, such as reduction in carbon assimilation, growth, survival and reproduction (Rahman *et al.,* 2007).

Carotenoids are part of the photosynthetic pigments, playing an important role on the protection of chlorophyll pigment under stress conditions. Carotenoids are known to quench the photodynamics reactions leading to loss of chlorophylls, to replace peroxidation and to avoid the collapse of the

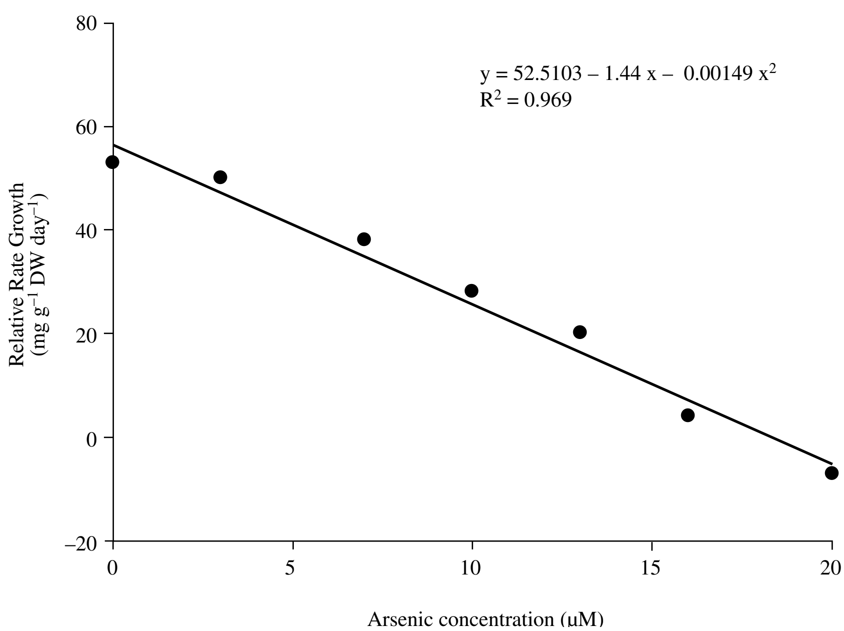

Figure 2. Relative growth rate of *Pistia stratiotes* L. (Araceae) specimens exposed to arsenate.

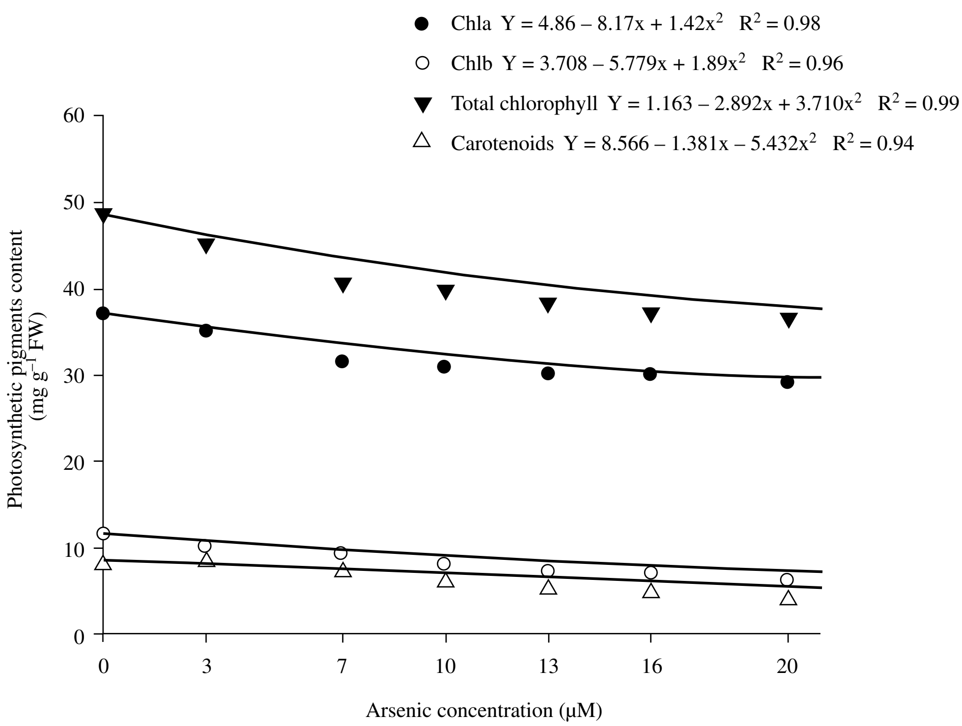

Figure 3. Concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in *Pistia stratiotes* L. (Araceae) under arsenic stress.

membranes in chloroplasts. Increase in carotenoids content in response to the pollutant was reported for *P. stratiotes* (Vajpayee *et al.,* 2001). However, in the present study, this response was not observed, possibly due to a severe damage resulted from toxic As concentrations associated with the long time of exposure to the pollutant.

No effects were observed in the uptake of K, Ca and Zn by *P. stratiotes* at any treatment, although changes in the uptake of these nutrients have been observed in other plants in the presence of the metalloid (Liu *et al.,* 2008). However, other nutrients were affected (Table 1). The presence of As caused an increase in the absorption of Cu, Mn and Fe up to the concentration of 13 µM, whereas in the highest concentrations decrease in uptake was observed. This result has also been found for other plants exposed to As (Päivöke & Simola, 2001; Liu *et al.,* 2008). Cu, Mn and Fe are micronutrients that have several functions in plants (Marschner, 1995),

Table 1. Concentrations (mg g^{-1} DW) of nutrients in the roots of *Pistia stratiotes* L. (Araceae) under arsenic stress. Copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), calcium (Ca), potassium (K), magnesium (Mg) and phosphate (P).

Treatment (µM As)	Cu*	Fe*	Mn*	Zn*	Ca*	K*	Mg*	P*
0	0.027a	3.888a	1.085a	0.254a	5.252a	22.350a	4.666a	1.869a
3	0.031a	4.685a	1.381b	0.235a	5.481a	22.396a	4.881a	1.875a
7	0.035ab	5.080a	1.384b	0.256a	5.722a	22.608a	4.773a	1.995b
10	0.038b	7.151b	1.602b	0.290a	6.017a	21.702a	4.729a	2.297b
13	0.044c	8.177b	1.725b	0.264a	6.579a	21.460a	3.521b	2.521b
16	0.035b	6.041a	1.231a	0.248a	5.871a	20.955a	3.519b	2.049a
20	0.027a	5.266a	1.046a	0.244a	5.554a	20.594a	3.272b	1.898a

Values in mg g^{-1} DW.
Values within one line not followed by the same letter are significantly different.

being structural components of the antioxidant enzymatic system of plant cells. One of the main damages of As in cells is the generation of reactive oxygen species (ROS). In this way, it is essential that an increase in the activity of enzymes of the antioxidant metabolism occur in order to scavenge the excess of free radicals, preventing the onset of serious injuries. Cu, Mn and Fe are constituents of the superoxide dismutase (SOD), which is considered to be the first line of defense against ROS. Studies have shown that deficiency in these elements cause a drastic reduction in SOD activity (Leão *et al.*, 2013). Fe is also a constituent of several peroxidases (POX) and catalases (CAT). Thus, the observed increase in the uptake of these nutrients can be directly related to the increase in the synthesis of antioxidant system enzymes. Indeed, the total antioxidant capacity of *P. stratiotes* roots significantly increased up to the concentration of 13 µM of As (Figure 4), indicating the activation of antioxidant mechanisms involved in the elimination of excess ROS generated by exposure to the metalloid. At concentrations higher than 13 µM, however, concentration of As is likely to become toxic, affecting the antioxidant capacity and consequently the uptake of nutrients. The phytotoxicity of As, including a major damage in the membrane caused by this metalloid, can also be the cause of this decrease in nutrients uptake (Päivöke & Simola, 2001).

The Mg uptake decreased when the metalloid concentration in solution increased. One major role of Mg is to be a cofactor in enzymes that activate phosphorylation processes, and it also serves as the central atom of the chlorophyll molecule. This decrease in Mg uptake probably is due to the fact that the As can uncouple oxidative phosphorylation and decrease the chlorophyll content, so the plant does not have high requirements of Mg; therefore its uptake is decreased. In addition, a reduction in Mg uptake may be a result from the toxic effect of As in roots (Marschner, 1995).

Phosphate in plants is important for energy transfer and protein metabolism (Marschner, 1995). Uptake of phosphorus, similarly to that observed for Cu, Mn and Fe, increased up to the concentration of 13 µM of As. At this concentration, phosphorus uptake was 42.45% higher than control. The increase in P uptake up to certain concentration of As was also reported in *Piteris vittata* (Singh & Ma, 2006). Because As can replace P but it is unable to carry out the role of P in energy transfer, the plant reacts as if there was a deficiency in P. Thus, the plant reacts by increasing P uptake. Accumulation of P in roots, like observed in this study, is an important parameter in plants that are tolerant to As. In fact, the P/As molar ratio in a plant tissue, defined as the P molar concentration divided by As molar concentration, may be the best indicator of their effects on plant

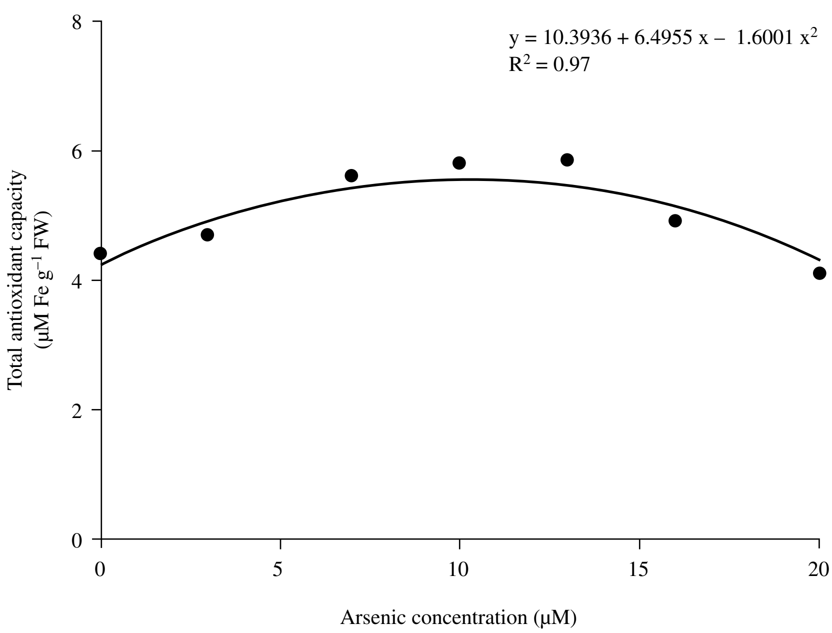

Figure 4. Effect of arsenic on the total antioxidant capacity of *Pistia stratiotes* L. roots (Araceae).

growth (Singh & Ma, 2006). At As concentrations higher than 13 µM, a decrease of phosphorus uptake was observed, probably by competing for the same transporter across the membrane.

Based on the data shown, can be concluded that *P. stratiotes* has high affinity to As, which is an interesting feature for phytoremediation programs. Increase in nutrients uptake, in the antioxidant capacity and maintenance of growth, even at a low rate, are indicators of the tolerance of *P. stratiotes* to the pollutant at the concentration of 13 µM. At concentrations higher than 13 µM of As, the plants showed various symptoms of toxicity, such as decrease in the uptake of most of the nutrients, in the growth and in the antioxidant capacity, indicating that these concentrations exceed the homeostatic capacity of *P. stratiotes*. Thus, *P. stratiotes* is a an efficient plant for phytoremediation of environments that are contaminated with As concentrations lower than 13 µM, which is 100 times higher than the allowed concentration in water for human consumption and four times higher than the concentration of As in contamined water bodies in all world, including several regions in Brazil, like Ouro Preto and Mariana (McClintock *et al.,* 2012). However, it is important that more studies on antioxidant metabolism be carried out in order to achieve a better understanding of As effects on this macrophyte, allowing the evaluation of its real potential for phytoremediation.

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