Evaluation of the potential of *Pistia stratiotes* L. (water lettuce) for bioindication and phytoremediation of aquatic environments contaminated with arsenic

Farnese, FS.[a], Oliveira, JA.[b], Lima, FS.[c], Leão, GA.[a], Gusman, GS.[a] and Silva, LC.[a]*

[a]Departamento de Biologia Vegetal, Universidade Federal de Viçosa – UFV, Av. Peter Henry Rolfs, s/n, Campus Universitário, CEP 36570-000, Viçosa, MG, Brazil.

[b]Departamento de Biologia Geral, Universidade Federal de Viçosa – UFV, Av. Peter Henry Rolfs, s/n, Campus Universitário, CEP 36570-000, Viçosa, MG, Brazil.

[c]Departamento de Ciências Biológicas, Universidade Federal do Triângulo Mineiro – UFTM, Av. Getúlio Guaritá, 159, Abadia, CP 136, CEP 38025-440, Uberaba, MG, Brazil.

*e-mail: jalves@ufv.br

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Abstract

Specimens of *Pistia stratiotes* were subjected to five concentrations of arsenic (As) for seven days. Growth, As absorption, malondialdehyde (MDA) content, photosynthetic pigments, enzymatic activities, amino acids content and anatomical changes were assessed. Plant arsenic accumulation increased with increasing metalloid in the solution, while growth rate and photosynthetic pigment content decreased. The MDA content increased, indicating oxidative stress. Enzymatic activity and amino acids content increased at the lower doses of As, subsequently declining in the higher concentrations. Chlorosis and necrosis were observed in the leaves. Leaves showed starch accumulation and increased thickness of the mesophyll. In the root system, there was a loss and darkening of roots. Cell layers formed at the insertion points on the root stems may have been responsible for the loss of roots. These results indicate that water lettuce shows potential for bioindication and phytoremediation of As-contaminated aquatic environments.

Keywords: antioxidant enzymes, water contamination, structural analyses, photosynthetic pigments.

Avaliação do potencial de *Pistia stratiotes* L. (alface d'água) para a bioindicação e fitorremediação de ambientes aquáticos contaminados com arsênio

Resumo

Espécimes de *Pistia stratiotes* foram submetidos a cinco concentrações de arsênio (As), durante sete dias. Crescimento, absorção de As, concentração de malondialdeído (MDA), pigmentos fotossintéticos, atividades enzimáticas, concentração de aminoácidos e alterações anatômicas foram avaliadas. O acúmulo de As pelas plantas aumentou com o incremento do metaloide na solução, enquanto que a taxa de crescimento e o teor de pigmentos fotossintéticos diminuiu. O conteúdo MDA aumentou, indicando estresse oxidativo. A atividade de enzimas antioxidantes e os teores de aminoácidos aumentaram nas doses mais baixas de As, declinando nas concentrações mais elevadas. Nas folhas foram observados clorose e necrose. As folhas apresentaram acumulação de amido e aumento da espessura do mesofilo. No sistema radicular houve perda e escurecimento das raízes. Camadas de células formadas nos pontos de inserção da raiz podem ter sido responsáveis pela queda das raízes. Estes resultados indicam que a alface da água apresenta potencial para bioindicação e fitorremediação de ambientes aquáticos contaminados com As.

Palavras-chave: enzimas antioxidantes, contaminação da água, análise estrutural, pigmentos fotossintéticos.

1. Introduction

Arsenic (As) is a toxic metalloid and carcinogen found naturally in the environment, though toxic concentrations of this element are caused by anthropical actions. As contamination normally occurs from the ingestion of contaminated water (Kazi et al., 2009), and in Brazil it arises mainly in regions with intensive mining activity (McClintock et al., 2012). Several technologies have been proposed for the remediation of As-contaminated areas,

including phytoremediation. This technique uses plants to remove, transform or contain toxic compounds in the soil, water or atmosphere.

Plants used in phytoremediation, besides being able to remove the pollutant from the environment, should also be tolerant to damage caused by it. The toxic effects of this absorption primarily result from the oxidative stress caused by the increased production of reactive oxygen intermediates (ROIs) (Sharma et al., 2012). The increased production of ROIs alters the normal metabolism of plants and might cause damage to cell membranes, inhibition of photosynthesis and growth (Farnese et al., 2014) and cell death (Sharma et al., 2012). However, plants have developed mechanisms to mitigate these effects using enzymatic antioxidants, such as superoxide dismutase (SOD: EC 1.15.1.1), peroxidases (POX: E.C.1.11.1.7) and catalases (CAT: EC.1.11.1.6). Besides the enzymes, another stress tolerance mechanism in plants is associated with the accumulation of amino acids, which have several roles in plants (Tripathi et al., 2012).

The heavy metals absorption has also a harmful effect in morphology and anatomy of plants. The structural changes, in turn, directly influence the plant physiology (Barcelo et al., 1988; Han et al., 2004). Thus, knowledge of the anatomical harm resulting from heavy metals is essential to understanding the phytoremediation process (Sinha et al., 2005). In some plants, structural changes may occur rapidly, even when the metal is found at low concentrations. These plants can be useful as biomarkers for toxicity (Barcelo et al., 1988). The characterisation of biomarkers of toxicity is essential, since bioindicators are, most of the time, more efficient than the physical and chemical parameters normally measured in the field. However, there is still little information regarding structural and ultrastructural changes in plants that are induced by heavy metals (Favas et al., 2012; Wolff et al., 2012).

Aquatic plants have particularly attractive characteristics for use in phytoremediation and bioindication programmes (Favas et al., 2012). *Pistia stratiotes* is a perennial aquatic macrophyte widely distributed throughout the world and is capable of removing several heavy metals from water, including As (Farnese et al., 2014). Considering these facts, this paper aims to verify the potencial of *P. stratiotes* for the phytoremediation and bioindication of aquatic environments contaminated with As, emphasising molecular mechanisms of tolerance to metalloid.

2. Material and Methods

Specimens of *P. stratiotes* collected in non-polluted dams at the Federal University of Viçosa, Brazil, were used in all experiments. Plants of similar size were collected and transferred to polyethylene pots with 10 L of Clark's nutrient solution, pH 6.5 (Clark, 1975), and maintained in a growth room with controlled temperature and irradiance (25 ± 2 °C; 230 mmol m^{-2} s^{-1}), under a photoperiod of 16 hours, for an adaptation period of 3 days.

After the adaptation period, plants were transferred to 1.0 L polyethylene pots containing 0.5 L Clark's nutrient solution, pH 6.5 and exposed to arsenic (As) (in the form of heptahydrated sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$)) at concentrations of 0, 5, 10, 15 and 20 μM. The plants remained in this solution for seven days in the growth room.

2.1. Determination of relative growth rate and arsenic determination

After the exposure period, plants were divided into roots and leaves, rinsed in deionised water, weighed and placed in a conventional oven at 80 °C, where they remained until a constant dry mass was obtained. The relative growth rate was calculated using the equation proposed by Hunt (1978). Oven-dried (80 °C) plant materials were digested in a nitric-perchloric acid mixture (2:1) (Marin et al., 1993), and the As concentration was determined by coupled plasma mass spectrophotometry (Optima 3300 DV, Perkin-Elmer, Norwalk, CT).

2.2. Determination of photosynthetic pigments

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 absorbance was measured using a spectrophotometer set at 663, 645, 652 and 470 nm to determine the chlorophyll a (chla), chlorophyll b (chlb), total chlorophyll and carotenoids, respectively, as proposed by Arnon (1949).

2.3. Assessment of damage to cell membranes

The level of lipid peroxidation, expressed as MDA (malondialdehyde) content, was determined as 2-thiobarbituric acid (TBA) reactive metabolites. Plant fresh tissues (0.2 g) were homogenised and extracted in 10 ml of 0.25% TBA made in 10 ml trichloroacetic acid (TCA). Then extract was heated at 95 °C for 30 min and then rapidly cooled in ice. After centrifugation at 10,000g for 10 min, the absorbance of the supernatant was measured at 532 nm. Non-specific turbidity was corrected by subtracting the absorbance value taken at 600 nm. The concentration of MDA was calculated using extinction coefficient of 155 m M^{-1} cm^{-1} (Zhang, 1992).

2.4. Enzyme extraction and assays

To assess enzyme activities of POX, CAT and SOD, samples were ground in liquid nitrogen and homogenised in the following buffer media: 0.1 M potassium phosphate buffer, pH 6.8, 0.1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM phenymethanesulfonyl fluoride (PMSF) and 1% polyvinylpolypyrrolidone (PVPP) (Peixoto et al., 1999).

Enzyme activities were determined by adding 0.1 mL of the crude enzyme extract to: a) POX: 2.9 mL of a reaction medium consisting of 0.1 M potassium phosphate, pH 6.8, 20 mM pyrogallol and 20 mM H_2O_2 (Chance and Maehly, 1955); b) CAT: 2.9 mL of a reaction medium consisting of 50 mM potassium phosphate, pH 7.0 and 12.5 mM H_2O_2

(Havir and McHale, 1987). Enzyme activities were estimated using the following molar extinction coefficients: POX (240 nm; ε: 2.47 mM^{-1} cm^{-1}) (Nakano and Asada, 1981) and CAT (240 nm, ε: 36 M^{-1} cm^{-1}) (Anderson et al., 1995).

Activity of SOD was determined by adding crude enzymatic extract to a reaction mixture (50 mM potassium phosphate buffer, pH 7.8, 13 mM methionine, 0.1 mM EDTA, 75 μM nitroblue tetrazolium (NBT) and 2 μM riboflavin). The reaction was conducted in a chamber with a fluorescent lamp at 25 °C. After 5 min of illumination the blue formazana was measured at 560 nm (Giannopolitis and Ries, 1977). One unit of SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction.

2.5. Amino acid

Amino acid content was determined by the method of Moore and Stein (1948). 0.5 g of plant sample was homogenised in 10 mL of 80% ethanol. The homogenate was centrifuged at 800 rpm for 10 min. One millilitre of the extract was taken in the test tube and 1 mL of 0.1 N HCl was added to neutralise the sample. To this, 1 mL of ninhydrine reagent was added and heated for 20 min in a boiling water bath. Later 5 mL of the diluent solution was added and heated again in water bath for 10 min. The absorbance was read at 570 nm.

2.6. Morphoanatomical analyses of plant tissues

Anatomical sections were performed on plants exposed to 0, 15 and 20 μM of As. The samples consisted of roots collected two centimetres below the insertion point on the stem, and the insertion point and leaf fragments were removed from the median portion of leaves emerging after treatment initiation. The leaf and root samples were fixed in 50% FAA (formalin, acetic acid and ethyl alcohol), dehydrated in up to 70% alcohol and embedded in paraffin. Transverse and longitudinal sections were obtained with a manual rotary microtome (Leica model 2035 Biocut). The sections were stained with a 9:1 ratio of 1% astra blue and 1% safranin. Slides were mounted in Canada balsam and analysed under a light microscope coupled to a U-photo system.

2.7. Statistical analyses

The experiments were conducted with five replicates and a randomised experimental design. The results were analysed by ANOVA. For the factor "quantification of As absorbed", cubic polynomial regression was performed, and linear regression was used for the remaining data. The means of enzymatic activities, MDA and amino acids content activities were statistically compared by Tukey's test at 5% probability. The statistical analyses were conducted with the statistical program SAS (Cary, NC, United States), which analyses if the data are parametric or nonparametric before proceeding with the analysis.

3. Results

3.1. Arsenate uptake

Arsenic uptake increased with increasing concentrations of the metalloid in the solution (see Figure 1). Root As content increased up to a concentration of 15 μM and then declined in subsequent concentrations. On the other hand, there was no decrease in As absorbtion observed in aerial plant parts, and it was even possible to detect an increase in leaf metalloid content.

3.2. Membrane damage

MDA is the decomposition product of polysaturated fatty acids of membranes and its increase shows plants are under high-level oxidative stress. As shown in Figure 2, MDA content showed increased in presence of As in *P. stratiotes* leaves and roots over the control values. There was no difference between the damage generated in the roots and leaves (p=0.05), despite the elevated As concentration in the roots.

3.3. Effect of arsenic on photosynthetic pigments

The As accumulation resulted in a sharp decrease in the rate of plant growth (see Figure 1 and 6) and in a accentuated fall in the roots. The photosynthetic pigments (see Figure 3)

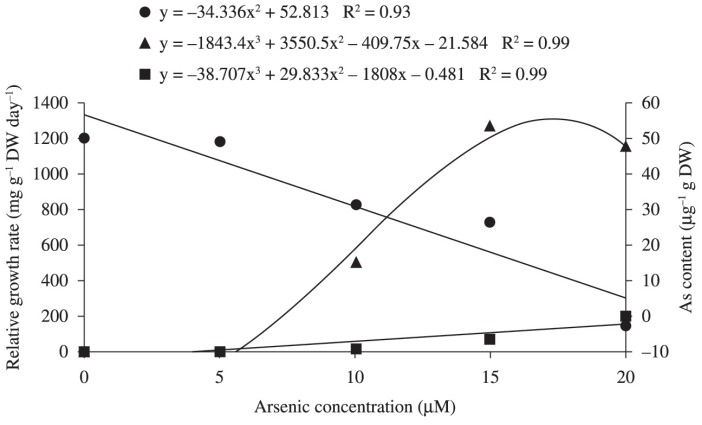

Figure 1. Relative growth rate ( ) and As concentration in the aerial parts ( ) and roots ( ) of *P. stratiotes* exposed to arsenate.

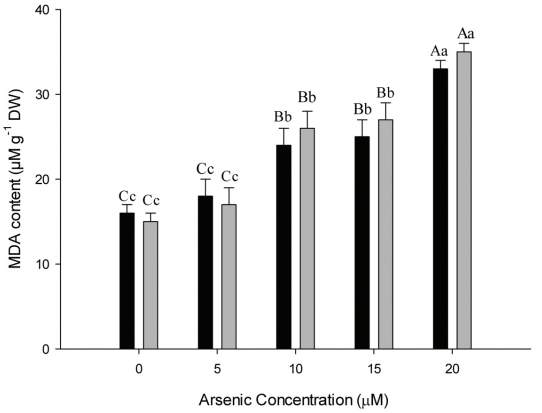

Figure 2. Content of MDA in the aerial parts ( ) and roots ( ) of *P. stratiotes* exposed to arsenate. Bars represent standard deviation. Means followed by the same upper-case letter, between treatments for the same organ, and by the same lower-case letter, between organs for the same treatment, were not significantly different according to Tukey's test at 5% probability.

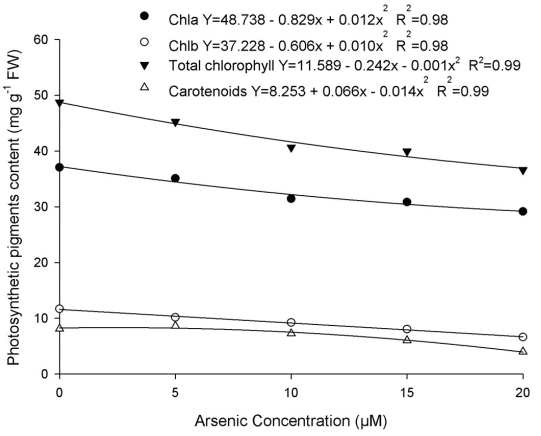

Figure 3. Concentration of chla (chlorophyll a), chb (chlorophyll b), total chlorophyll and carotenoids in *P. stratiotes* under As stress.

were also negatively affected by As concentration, and increase in metalloid concentration was accompanied by decreases in chla, chlb, total chlorophyll and carotenoids levels. The level of chlorophyll a, however, was more affected than the level of chlorophyll b. In this way, the chlorophyll a/chlorophyll b ratio increased in 47.6% at the highest As concentration.

3.4. Effect of As on enzymatic activities and amino acids content

The SOD activity was greatly increased, both in the leaves and roots of water lettuce, at an As concentration of 10 μM. At higher concentrations, however, the activity of the enzyme decreased. POX activity in roots increased significantly at a concentration of 15 μM, whereas in plant aerial parts this activity significantly decreased at concentrations equal to or above 10 μM. CAT activity in water lettuce roots was extremely low, and in aerial parts the activity of this enzyme increased at a concentration of 5 μM, subsequently declining at higher concentrations (see Figure 4).

The content of amino acids increased both in the leaves and roots of water lettuce with increasing As concentration up to a concentration of 15 μM. A gradual decrease in amino acid content was observed with the increase in As concentrations (see Figure 5).

3.5. Effects of As on morphoanatomical changes of plant tissues

Morphological changes due to As toxicity were observed in both the aerial plant parts and roots of water lettuce, especially at As concentrations of 15 and 20 μM. In aerial parts, symptoms of toxicity such as chlorosis and necrosis occurred and were the most prominent symptoms that emerged in leaves after treatment initiation (see Figure 6). Transverse sections of water lettuce leaves showed an increase in the number of layers in the palisade parenchyma and the amount of trichomes. Starch accumulation was also observed (see Figure 7).

In roots, at As concentrations of 15 and 20 μM, there was a decrease in the formation of secondary roots and a darkening of the root system. The roots acquired a gelatinous texture, and from the third day of treatment, the plants exposed to these concentrations began to lose their roots, resulting in a reduction of the root system. This decline continued until the last day of treatment. Structural changes were not observed in transverse sections. Longitudinal sections of water lettuce roots, in turn, showed that at an As concentration of 15 μM, approximately five to seven layers of cells formed at the insertion point of the root on the stem (see Figure 8).

4. Discussion

In several plant species, increase in As concentration in solution results in a higher accumulation of this metalloid in plants (Gusman et al., 2013). The preferential accumulation of As in *P. stratiotes* was in the root system, which is a typical behaviour of aquatic plants and also one of the defense mechanisms of these plants (Päivöke and Simola, 2001). However, a decrease of the metalloid was observed in the root at the higher concentration used. This reduction in As accumulation may be due to the sharp root decline at this concentration, combined with the morphological changes observed in the root system, including the acquisition of a gelatinous texture and dark colour, probably resulting from cell wall damage (Pimenta et al., 2004).

The heavy metal exposure can promote lipid peroxidation via free radical generation. Lipid peroxidation causes the breakdown of functional and structural integrity of biological membranes, increases the permeability of plasma membrane, leakage of K^+ ions and eventually causes cell death (Upadhyay and Panda, 2009). The increase in MDA content in *P. stratiotes* exposed to arsenic indicates

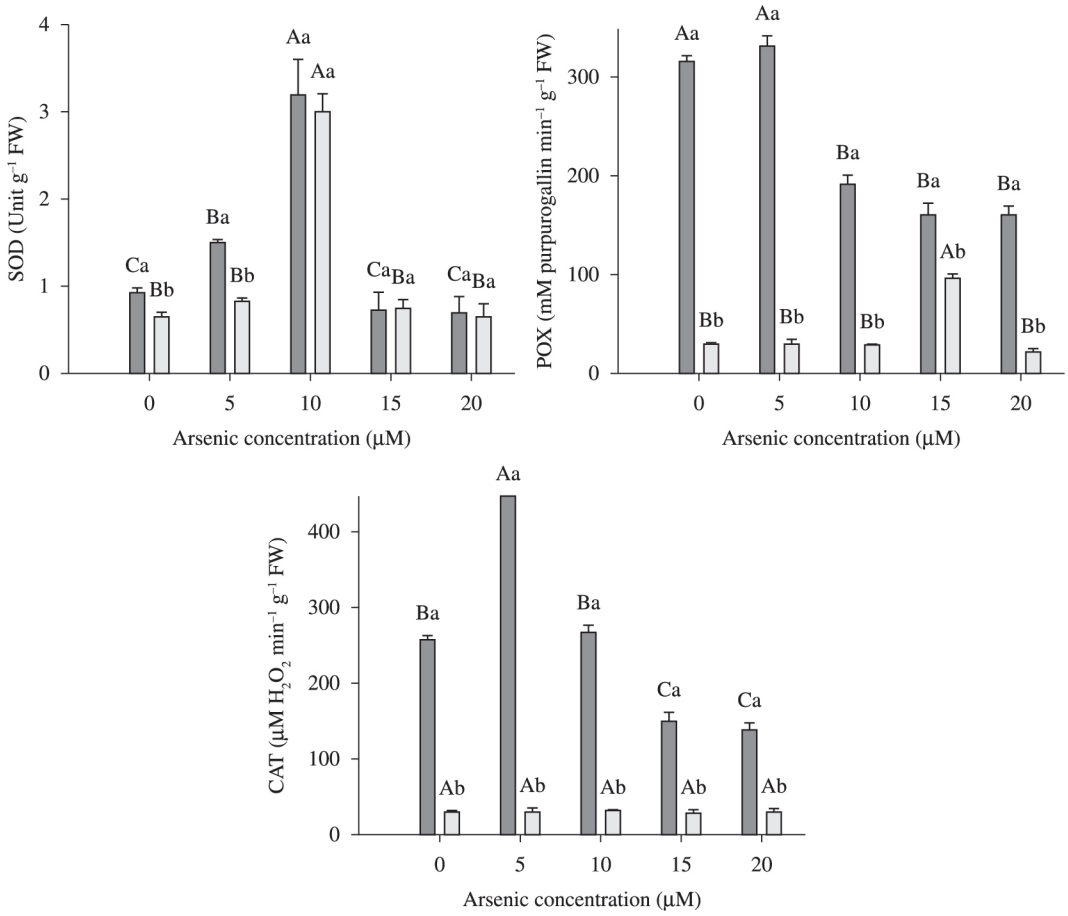

Figure 4. Enzimatic activity of SOD (superoxide dismutase; A), POX (peroxidases; B) and CAT (catalases; C) in the aerial parts ( ) and roots ( ) of *P. stratiotes* under As stress. Bars represent standard deviation. Means followed by the same upper-case letter, between treatments for the same organ, and by the same lower-case letter, between organs for the same treatment, were not significantly different according to Tukey's test at 5% probability.

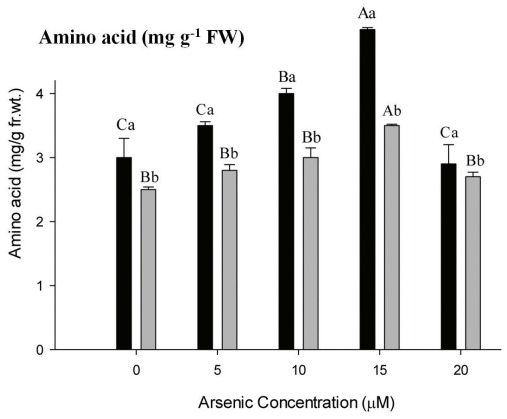

Figure 5. Amino acid content in the aerial parts ( ) and roots ( ) of *P. stratiotes* exposed to arsenate. Bars represent standard deviation. Means followed by the same upper-case letter, between treatments for the same organ, and by the same lower-case letter, between organs for the same treatment, were not significantly different according to Tukey's test at 5% probability.

the occurrence of lipid peroxidation, probably due to the increased production of ROIs.

The decrease in the growth rate of water lettuce is a typical plant response to As exposure at high concentrations (Päivöke and Simola, 2001) and may be related with increase in lipid peroxidation and decrease in the photosynthetic pigments. A decrease in total chlorophyll and carotenoid content is one of the first symptoms of plant toxicity after exposure to various stress agents, such as metals and metalloids (Guimarães et al., 2012), and this decrease is a parameter used in the bioindication of oxidative stress caused by heavy metals (Macfarlane and Burchett, 2001). Several studies have reported the toxic effect of arsenic on photosynthetic pigments (Farnese et al., 2014; Gusman et al., 2013). This provides evidence for the loss of the photosynthetic apparatus and changes in photosynthetic ability.

Chlorophyll *a* is the primary pigment involved in light absorption in Photosystem I (PSI), whereas both chlorophylls *a* and *b* are present in PSII. Thus, the high chlorophyll *a*/chlorophyll *b* ratio observed in the presence

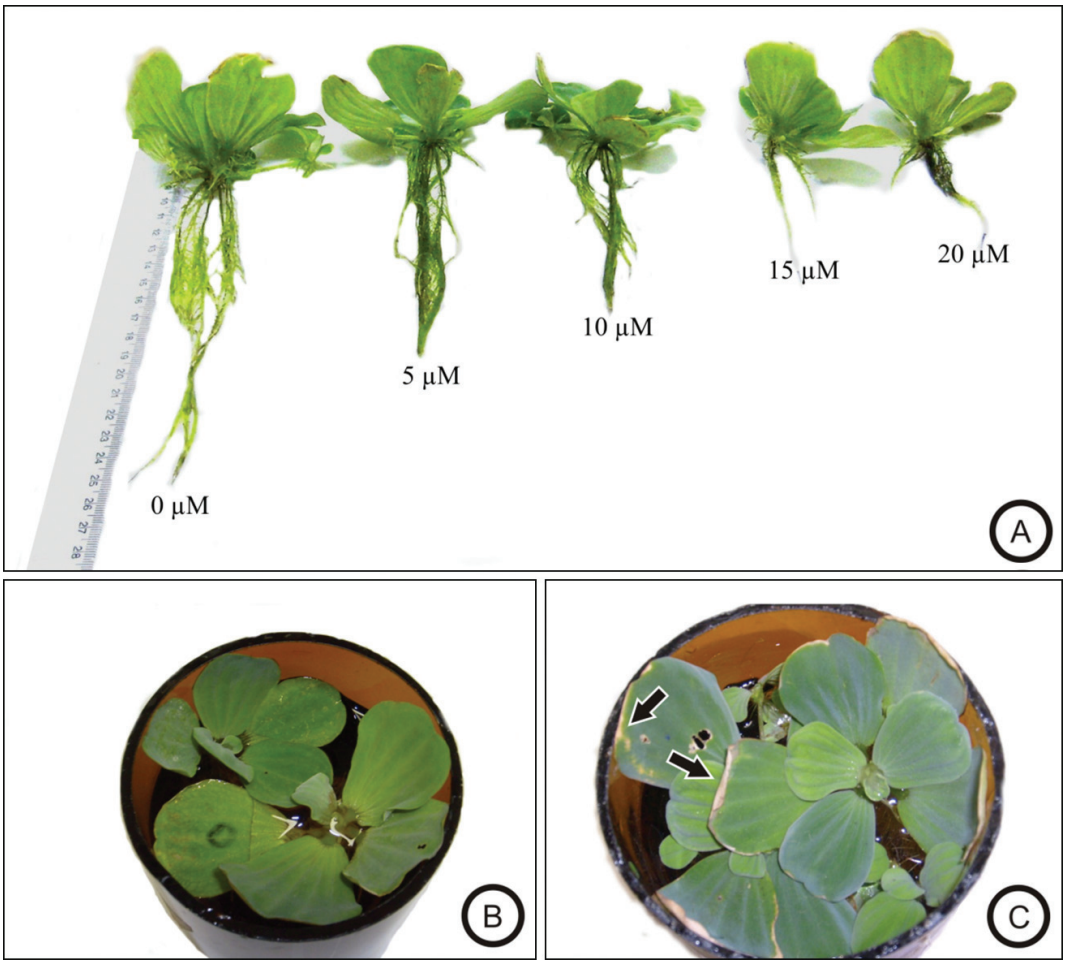

Figure 6. Effect of different As concentrations on *P. stratiotes* growth (A). Visible symptoms of toxicity: Control (B) and 20 µM As (C). Observed chlorosis and necrosis (arrows).

of As may suggest an increase in the PSI/PSII ratio due to the stress adaptation mechanisms of the chloroplasts (Takabayashi et al., 2005). Moreover, an increase is likely to occur in cyclic electron transport, which is involved in the dissipation of excess energy, thus avoiding further damage to PSII (Li et al., 2006).

SOD activity in water lettuce increased with increasing As concentration up to a certain point, above which the extent of the damage caused by the pollutant resulted in decreased enzyme activity. The decrease in SOD activity at high arsenic and heavy metal concentrations may be attributed to the inactivation of the enzyme by hydrogen peroxide due to conversion of the superoxide radicals, which is a reaction catalysed by SOD itself. SOD inactivation may also be due to the existence of other ROIs and the inactivation of other enzymes involved in the degradation of these compounds (Khan et al., 2009).

The POX activity is an important mechanism in defending against oxidative stress, mainly by eliminating H_2O_2 (Sinha et al., 2005). In the roots POX activity increased with increasing As concentration up to a concentration of 15 µM. Similar patterns were observed by Santos (2006), who studied aquatic plant species subjected to As. The reduction in POX activity may be a consequence of its inactivation due to the binding between the metalloid and thiol groups and longer As exposure (Meharg and Hartley-Whitaker, 2002).

The CAT enzyme shows increased activity in response to oxidative stress and participates in the elimination of H_2O_2, a product of SOD activity (Choi et al., 2004). In *P. stratiotes* this enzyme is located predominantly in leaves and seems to be effective in low concentrations of As.

Amino acid metabolism has the central role in abiotic stress resistance of plants. They act as precursors for a large array of metabolites with multiple functions in plant response to stresses, such as gluthathione (Less and Galili, 2008). The accumulation of amino acids up to a concentration of 15 µM of As is probably a defense strategy in *P. stratiotes*. The gradual reduction in amino acids content may be due to the fact that the nitrogen content of plants gets reduced by metal stress. The damage to roots at the higher arsenic concentration also may have compromised the nitrogen uptake.

The most conspicuous signs of toxicity observed in the aerial plant parts of water lettuce were chlorosis and

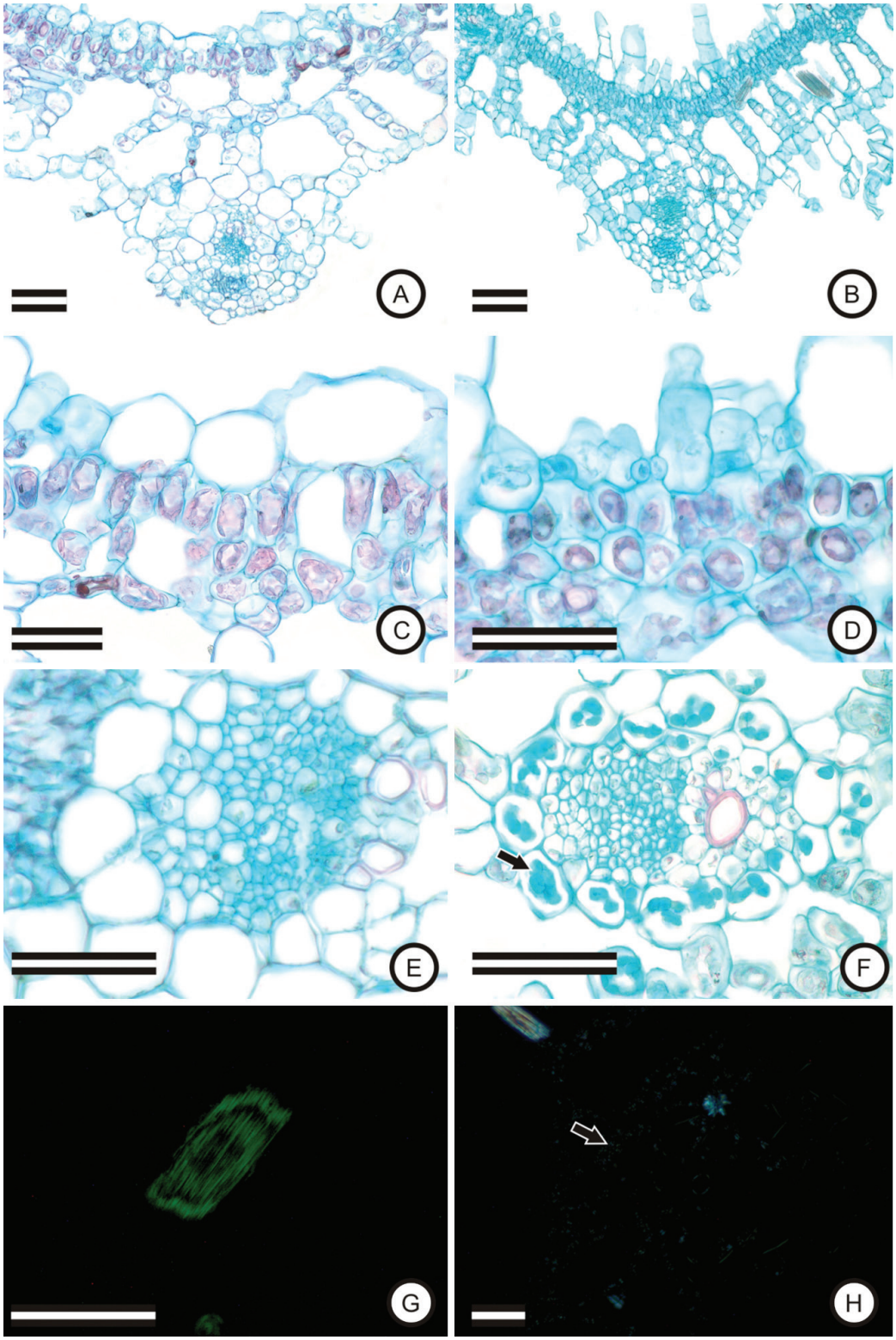

Figure 7. General appearance of the leaf in the control plant (A) and at As concentration of 20 µM (B), in which increased numbers of trichomes may be seen. Detail of the palisade parenchyma in the control plant (C) and treated specimen (D), showing increased numbers of layers. Accumulation of starch (arrow) in the bundle sheaths of plants treated with As (F) as compared to the control (E). Differential accumulation of starch (arrow) in the control (G) and treated specimen (H), visualized with polarized light. (Bar =100µm).

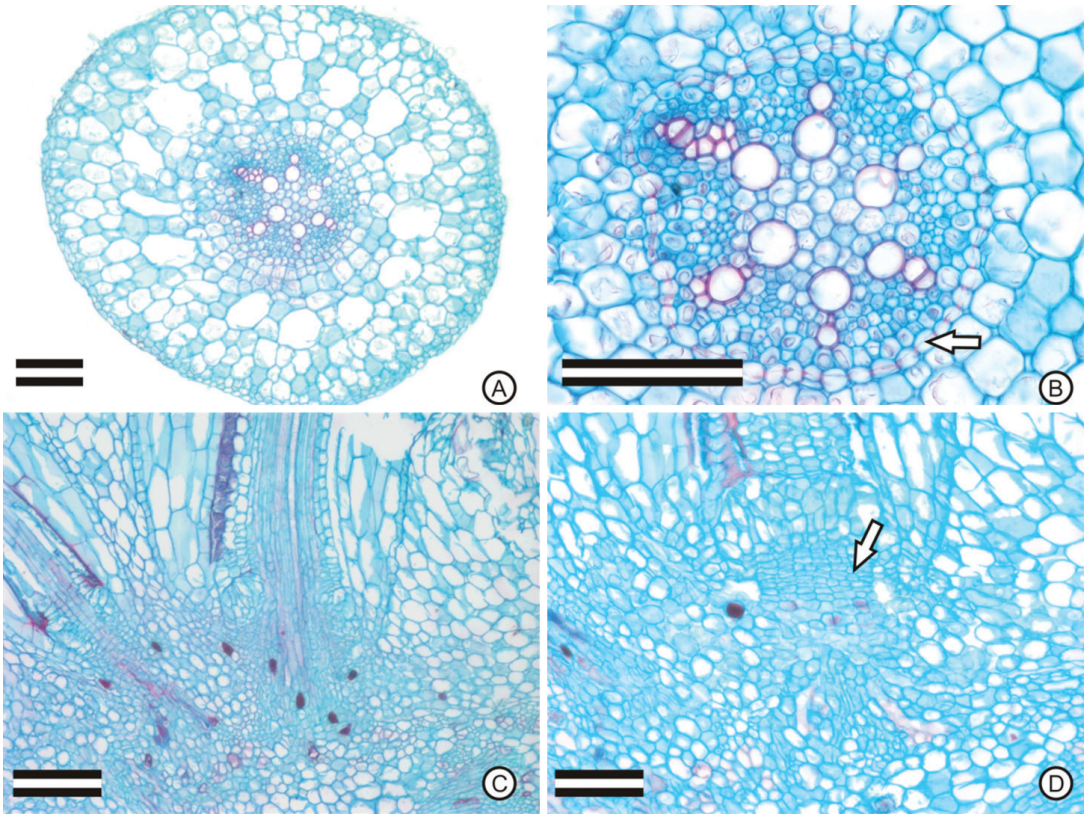

Figure 8. General appearance of the root (A) and detail of the vascular cylinder (B), where casparian ridges can be observed (arrow). Longitudinal section at the root insertion point on the stem in the control (C) at As concentration of 15 μM (D). Observe the formation of the abscission zone in D (arrow). (Bar=50μm).

necrosis. It was previously demonstrated that chlorosis and necrosis might be the result of arsenic stress, mainly due to the formation of reactive oxygen species in the region of the cell wall and within the cell (Mascher et al., 2002). These changes affect membrane permeability and enzyme and photosynthetic activity, and could cause chlorosis and necrosis. As can also have a damaging effect on micronutrient translocation. Thus, chlorosis symptoms would be the result of an As-induced nutrient deficiency, especially involving iron (Shaibur et al., 2009). Studies performed on water lettuce specimens subjected to stress as mediated by other metals, however, did not find any visible symptoms of toxicity (Sanità di Toppi et al., 2007).

Treatments with heavy metals usually result in the appearance of xerophilous features on the leaf structure, such as increased leaf thickness and abundant palisade parenchyma (Shi and Cai, 2009). A co-occurrence of metal contamination stress and hydric stress is commonly observed (Santala and Ryser, 2009) and is caused mostly by a reduction in root growth (Shi and Cai, 2009). In water lettuce, increases in the layers of palisade parenchyma and trichomes are probably related to the plasticity of the plant and its ability to withstand hydric stress, which is triggered either by a reduction in root growth or extreme root decline during the treatments. The exact reasons for the anatomical changes resulting from the accumulation of pollutants, however, remain unknown (Singh et al., 2007).

Morphological changes due to As toxicity were observed in water lettuce roots. The observed reduction in secondary root emission has also been described in bean specimens subjected to arsenic exposure (Singh et al., 2007). The gelatinous texture presented by the roots at a concentration of 20 μM may be due to changes in the cell wall (Pimenta et al., 2004), and further studies are required to verify this hypothesis. Root loss in the presence of As has already been reported for water lettuce after exposure to other pollutants (Mishra and Tripathi, 2008). In the case of As, this loss seems to be related to the formation of cambium during injury healing at the insertion point of the root stem, as observed in longitudinal anatomical sections. It is possible that this region corresponds to an abscission meristem, such as that which occurs in fruits and dehiscent leaves. The formation of this meristem abscission may be a strategy developed by these plants to pollutants, as a way of reducing damage to the plant as a whole.

Although a large amount of arsenic accumulated in the roots, in transverse sections the tissue structure remained virtually unchanged regardless of the arsenic concentration to which it was exposed. Barcelo et al. (1988) observed the same result when studying structural changes in bean specimens subjected to cadmium exposure. This absence

of change is probably related to the ability of roots to store the pollutant in an inactive form. A proposed mechanism for plant tolerance to As consists of its chelation with ligands, such as glutathione. These As complexes would be stored in the vacuoles, thereby preventing the development of cellular damage (Guo et al., 2012). Studies on the presence of these ligands in the roots of water lettuce subjected to As exposure would be of interest to verify this hypothesis.

5. Conclusions

The results obtained indicate that water lettuce has a high potential of being a bioindicator in As-contaminated aquatic environments. This is shown by the morphological, anatomical and physiological changes present in this species after treatment. It is interesting to note that these symptoms are not found in specimens of water lettuce exposed to other metals, such as cadmium, further demonstrating the validity of the use of this plant for As bioindication. Although only small concentrations of this pollutant are permitted in water, concentrations far above the permitted level are found worldwide, emphasising the importance of bioindicators that can signify possible contaminations, so that tests may be carried out and detoxification measures performed.

Under these experimental conditions, water lettuce was not effective in the phytoremediation of environments contaminated with high concentrations of As, since its metabolism was strongly affected by the metalloid, and phytoremediator species must be resistant to the damages caused by pollutants that exist in the environment. However, the period of plant exposure to the pollutant was relatively long, so studies using a shorter exposure time would contribute to the evaluation of the phytoremediation potential of water lettuce.

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References

ANDERSON, MD., PRASAD, TK. and STEWART, CR., 1995. Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotylus of maize seedlings. *Plant Physiology*, vol. 109, no. 4, p. 1247-1257. PMid:12228666.

ARNON, DI., 1949. Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, vol. 24, no. 1, p. 1-15. http://dx.doi.org/10.1104/pp.24.1.1. PMid:16654194

BARCELO, J., VÁZQUEZ, MD. and POSCHENRIEDER, C., 1988. Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris* L.). *The New Phytologist*, vol. 108, no. 1, p. 37-49. http://dx.doi.org/10.1111/j.1469-8137.1988.tb00202.x.

CHANCE, B. and MAEHLY, AC., 1955. Assay of catalases and peroxidases. *Methods in Enzymology*, vol. 2, p. 764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8.

CHOI, DG., YOO, NH., YU, CY., DE LOS REYES, B. and YUN, SJ., 2004. The activities of antioxidant enzymes in response to oxidative stresses and hormones in paraquat-tolerant Rehmannia glutinosa plants. *Journal of Biochemistry and Molecular Biology*, vol. 37, no. 5, p. 618-624. http://dx.doi.org/10.5483/BMBRep.2004.37.5.618. PMid:15479627

CLARK, RB., 1975. Characterization of phosphatase of intact maize roots. *Journal of Agricultural and Food Chemistry*, vol. 23, no. 3, p. 458-460. http://dx.doi.org/10.1021/jf60199a002. PMid:239039

FARNESE, FS., OLIVEIRA, JA., GUSMAN, GS., LEÃO, GA., SILVEIRA, NM., SILVA, PM., RIBEIRO, C. and CAMBRAIA, J., 2014. Effects of adding nitroprusside on arsenic stressed response of *Pistia stratiotes* L. under hydroponic conditions. *International Journal of Phytoremediation*, vol. 16, no. 2, p. 123-137. http://dx.doi.org/10.1080/15226514.2012.759532. PMid:24912205

FAVAS, PJ., PRATAS, J. and PRASAD, MNV., 2012. Accumulation of arsenic by aquatic plants in large-scale field conditions: opportunities for phytoremediation and bioindication. *The Science of the Total Environment*, vol. 433, p. 390-397. http://dx.doi.org/10.1016/j.scitotenv.2012.06.091. PMid:22820614

GIANNOPOLITIS, CN. and RIES, SK., 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, vol. 59, no. 2, p. 309-314. http://dx.doi.org/10.1104/pp.59.2.309. PMid:16659839

GUIMARÃES, FP., AGUIAR, R., OLIVEIRA, JA., SILVA, JAA. and KARAM, D., 2012. Potencial of macrophyte for removing arsenic from aqueous solution. *Planta Daninha*, vol. 30, no. 4, p. 683-696. http://dx.doi.org/10.1590/S0100-83582012000400001.

GUO, J., XU, W. and MA, M., 2012. The assembly of metals chelation by thiols and vacuolar compartmentalization conferred increased tolerance to and accumulation of cadmium and arsenic in transgenic Arabidopsis thaliana. *Journal of Hazardous Materials*, vol. 199-200, p. 309-313. http://dx.doi.org/10.1016/j.jhazmat.2011.11.008. PMid:22119299

GUSMAN, GS., OLIVEIRA, JA., FARNESE, FS. and CAMBRAIA, J., 2013. Arsenate and arsenite: the toxic effects on photosynthesis and growth of lettuce plants. *Acta Physiologiae Plantarum*, vol. 35, no. 4, p. 1201-1209. http://dx.doi.org/10.1007/s11738-012-1159-8.

HAN, FX., SRIDHAR, BBM., MONTS, DL. and SU, Y., 2004. Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea* L. *The New Phytologist*, vol. 162, no. 2, p. 489-499. http://dx.doi.org/10.1111/j.1469-8137.2004.01027.x.

HAVIR, EA. and MCHALE, NA., 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiology*, vol. 84, no. 2, p. 450-455. http://dx.doi.org/10.1104/pp.84.2.450. PMid:16665461

HUNT, R.,1978. *Plant growth analysis*. London: Edward Arnold Limited.

KAZI, TG., ARAIN, MB., BAIG, JA., JAMALI, MK., AFRIDI, HI., JALBANI, N., SARFRAZ, RA., SHAH, AQ. and NIAZ, A., 2009. The correlation of arsenic levels in drinking water with the biological samples of skin disorders. *The Science of the Total Environment*, vol. 407, no. 3, p. 1019-1026. PMid:19027142.

KHAN, I., AHMAD, A. and IQBAL, M., 2009. Modulation of antioxidant defence system for arsenic detoxification in Indian mustard. *Ecotoxicology and Environmental Safety*, vol. 72, no. 2, p. 626-634. http://dx.doi.org/10.1016/j.ecoenv.2007.11.016. PMid:18262648

LESS, H. and GALILI, G., 2008. Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic

stresses. *Plant Physiology*, vol. 147, no. 1, p. 316-330. http://dx.doi.org/10.1104/pp.108.115733. PMid:18375600

LI, X-G., ZHAO, J-P., XU, P-L., MENG, J-J. and HE, Q-W., 2006. Effects of cyclic electron flow inhibitor (antimycin a) on photosystem photoinhibition of sweet pepper leaves upon exposure to chilling stress under low irradianc‘. *Agricultural Sciences in China*, vol. 5, no. 7, p. 506-511. http://dx.doi.org/10.1016/S1671-2927(06)60084-9.

MACFARLANE, GR. and BURCHETT, MD., 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the Grey mangrove, Avicennia marina (Forsk.) Vierh. *Marine Pollution Bulletin*, vol. 42, no. 3, p. 233-240. http://dx.doi.org/10.1016/S0025-326X(00)00147-8. PMid:11381878

MARIN, AR., PEZESHKI, SR., MASSCHELEN, PH. and CHOI, HS., 1993. Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis in rice plants. *Journal of Plant Nutrition*, vol. 16, no. 5, p. 865-880. http://dx.doi.org/10.1080/01904169309364580.

MASCHER, R., LIPPMANN, B., HOLZINGER, S. and BERGMANN, H., 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. *Plant Science*, vol. 163, no. 5, p. 961-969. http://dx.doi.org/10.1016/S0168-9452(02)00245-5.

MCCLINTOCK, TR., CHEN, Y., BUNDSCHUH, J., OLIVER, JT., NAVONI, J., OLMOS, V., LEPORI, EV., AHSAN, H. and PARVEZ, F., 2012. Arsenic exposure in Latin America: biomarkers, risk assessments and related health effects. *The Science of the Total Environment*, vol. 429, p. 76-91. http://dx.doi.org/10.1016/j.scitotenv.2011.08.051. PMid:22119448

MEHARG, AA. and HARTLEY-WHITAKER, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *The New Phytologist*, vol. 154, no. 1, p. 29-43. http://dx.doi.org/10.1046/j.1469-8137.2002.00363.x.

MISHRA, VK. and TRIPATHI, BD., 2008. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. *Bioresource Technology*, vol. 99, no. 15, p. 7091-7097. http://dx.doi.org/10.1016/j.biortech.2008.01.002. PMid:18296043

MOORE, S. and STEIN, WH., 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *The Journal of Biological Chemistry*, vol. 176, no. 1, p. 367-388. PMid:18886175.

NAKANO, Y. and ASADA, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant & Cell Physiology*, vol. 22, no. 5, p. 867-880.

PÄIVÖKE, AEA. and SIMOLA, LK., 2001. Arsenate toxicity to Pisum sativum: mineral nutrients, chlorophyll content, and phytase activity. *Ecotoxicology and Environmental Safety*, vol. 49, no. 2, p. 111-121. http://dx.doi.org/10.1006/eesa.2001.2044. PMid:11386724

PEIXOTO, PHP., CAMBRAIA, J., SANT'ANNA, R., MOSQUIM, PR. and MOREIRA, MA., 1999. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. *Revista Brasileira de Fisiologia Vegetal*, vol. 11, no. 3, p. 137-143.

PIMENTA, CJ., VILELA, ER. and CARVALHO JUNIOR, C., 2004. Componentes de parede celular de grãos de frutos de café (*Coffea arabica* L.) submetidos a diferentes tempos à espera da secagem. *Acta Scientiarum Agronomy*, vol. 26, no. 2, p. 203-209. http://dx.doi.org/10.4025/actasciagron.v26i2.1884.

SANITÀ DI TOPPI, L., VURRO, E., ROSSI, L., MARABOTTINI, R., MUSETTI, R., CARERI, M., MAFFINI, M., MUCCHINO, C., CORRADINI, C. and BADIANI, M., 2007. Different compensatory mechanisms in two metal-accumulating aquatic macrophytes exposed to acute cadmium stress in outdoor artificial lakes. *Chemosphere*, vol. 68, no. 4, p. 769-780. http://dx.doi.org/10.1016/j.chemosphere.2006.12.092. PMid:17292445

SANTALA, KR. and RYSER, P., 2009. Influence of heavy-metal contamination on plant response to water availability in white birch, *Betula papyrifera*. *Environmental and Experimental Botany*, vol. 66, no. 2, p. 334-340. http://dx.doi.org/10.1016/j.envexpbot.2009.03.018.

SANTOS, GA., 2006. *Crescimento e respostas antioxidantes de macrófitas aquáticas submetidas ao arsênio*. Viçosa: Universidade Federal de Viçosa. 55 p. Dissertação de Mestrado em Fisiologia Vegetal.

SHAIBUR, MR., KITAJIMA, N., HUQ, SMI. and KAWAI, S., 2009. Arsenic–iron interaction: Effect of additional iron on arsenic-induced chlorosis in barley grown in water culture. *Soil Science and Plant Nutrition*, vol. 55, no. 6, p. 739-746. http://dx.doi.org/10.1111/j.1747-0765.2009.00414.x.

SHARMA, P., JHA, AB., DUBEY, RS. and PESSARAKLI, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Le Journal de Botanique*, vol. 2012, p. 1-26.

SHI, G. and CAI, Q., 2009. Leaf plasticity in peanut (*Arachis hypogaea* L.) in response to heavy metal stress. *Environmental and Experimental Botany*, vol. 67, no. 1, p. 112-117. http://dx.doi.org/10.1016/j.envexpbot.2009.02.009.

SINGH, HP., BATISH, DR., KOHLI, RK. and ARORA, K., 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regulation*, vol. 53, no. 1, p. 65-73. http://dx.doi.org/10.1007/s10725-007-9205-z.

SINHA, S., SAXENA, R. and SINGH, S., 2005. Chromium induced lipid peroxidation in the plants of *Pistia stratiotes L.*: role of antioxidants and antioxidant enzymes. *Chemosphere*, vol. 58, no. 5, p. 595-604. http://dx.doi.org/10.1016/j.chemosphere.2004.08.071. PMid:15620753

TAKABAYASHI, A., KISHINE, M., ASADA, K., ENDO, T. and SATO, F., 2005. Differential use of two cyclic electron flows around photosystem I for driving CO2-concentration mechanism in C4 photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 46, p. 16898-16903. http://dx.doi.org/10.1073/pnas.0507095102. PMid:16272223

TRIPATHI, RD., TRIPATHI, P., DWIVEDI, S., DUBEY, S., CHATTERJEE, S., CHAKRABARTY, D. and TRIVEDI, PK., 2012. Arsenomics: omics of arsenic metabolism in plants. *Frontiers in Physics*, vol. 3, p. 275. http://dx.doi.org/10.3389/fphys.2012.00275. PMid:22934029

UPADHYAY, RK. and PANDA, SK., 2009. Copper-induced growth inhibition, oxidative stress and ultrastructural alterations in freshly grown water lettuce (Pistia stratiotes L.). *Comptes Rendus Biologies*, vol. 332, no. 7, p. 623-632. http://dx.doi.org/10.1016/j.crvi.2009.03.001. PMid:19523602

WOLFF, G., PEREIRA, GC., CASTRO, EM., LOUZADA, J. and COELHO, FF., 2012. The use of Salvinia auriculata as a bioindicator in aquatic ecosystems: biomass and structure dependent on the cadmium concentration. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 72, no. 1, p. 71-77. PMid:22437387.

ZHANG, XZ.,1992. The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In ZHANG, XZ. (Ed.). *Research methodology of crop physiology*. Beijing: Agriculture Press. p. 208-211.