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ORIGINAL ARTICLE

Anatomical and physiological characteristics of tanner grass exposed to arsenic

Características anatômicas e fisiológicas de plantas de braquiária-do-brejo expostas ao arsênio

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ABSTRACT: Phytoremediation is a biological process that stands out as an effective and economic alternative for the removal of toxic elements; however, its success depends on previous studies of plant species and the species' tolerance of certain pollutants. The aim was at evaluating the anatomy and physiology of Tanner grass (*Brachiaria arrecta)* to determine its anatomical and physiological plasticity characteristics that potentially enable arsenic tolerance. *B. arrecta* plants were cultivated in a greenhouse in a Hoagland-Arnon nutritive solution with the following As concentrations: 0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}. Gas exchange, anatomical characteristics, DNA quantification and arsenic absorption were evaluated at 18 and 21 days. An evaluation of plant growth was conducted to compare the selected plants at the beginning and after 21 days of the experiment. The plants absorbed the arsenic contained in the solution and showed an increase in the leaf stomatal density, mesophyll and leaf blade thickness and root endodermis and exodermis, and the anatomical modifications showed no evidence of stress in the plant structure. The gas exchange, growth and DNA content were not modified by arsenic in *B. arrecta* plants. The *B. arrecta* plants have anatomical and physiological characteristics that contribute to their survival in the presence of arsenic, possibly helped by the tolerance of this species of arsenic contamination from the nutrient solution and the lack of anatomical and physiological changes resulting in damage to this species.

RESUMO: *A fitorremediação é um processo biológico, que se destaca como alternativa eficaz e econômica à remoção de elementos tóxicos; porém, depende de estudos prévios de espécies vegetais e de suas características relacionadas à tolerância a esses poluentes. O objetivo deste trabalho foi avaliar a anatomia e a fisiologia de plantas de braquiária-do-brejo (Brachiaria arrecta), no intuito de detectar características de plasticidade anatômica e fisiológica, relacionadas à potencial tolerância dessa espécie ao arsênio. As plantas de B. arrecta foram cultivadas em casa de vegetação, em solução nutritiva de Hoagland-Arnon contendo as seguintes concentrações de arsênio: 0,0; 0,25; 0,5; 1,0; 2,0 e 4,0 mg L^{-1}. Características de trocas gasosas, anatômicas, quantificação do DNA e absorção de arsênio foram avaliadas aos 18 e 21 dias. A avaliação do crescimento foi conduzida comparando-se plantas utilizadas no início do experimento e após 21 dias. As plantas absorveram o arsênio contido em solução e demonstraram aumento na densidade estomática, na espessura do mesofilo e no limbo foliar, sendo que as modificações anatômicas não indicaram evidências de estresse na estrutura das plantas. As trocas gasosas, o crescimento e o conteúdo de DNA não foram modificados pelo As nas plantas de B. arrecta. As plantas de B. arrecta apresentam características anatômicas e fisiológicas que contribuem para a sobrevivência da espécie, possivelmente por auxiliar na potencial tolerância dessas plantas à contaminação pelo arsênio em solução nutritiva, sem que as modificações anatômicas e fisiológicas resultem em prejuízos para essa espécie.*

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1 Introduction

Arsenic (As) is a serious aquatic contaminant that is often released by rocks through sedimentation and/or in industrial and agricultural activities that cause its accumulation in aquatic ecosystems. The presence of pollutants such as arsenic puts the environment at risk because they generally have a negative effect on the growth and development of exposed organisms. Furthermore, this element can be bioaccumulated by aquatic organisms and transferred to all trophic levels, including animals that are not present in these ecosystems, thereby affecting ecosystem and human health (MELO et al., 2009).

It is necessary to find plants that can survive in the presence of As for future phytoremediation programs that may serve an alternative method for the removal of pollutants other than by physical and chemical means and as an accessible low-cost option in areas with large workforces. Such programs may include plants that can absorb and accumulate toxic elements such as arsenic, without which growth and development are affected in this stressful situation. To succeed at phytoremediation, these plants must possess anatomical and physiological plasticity characteristics that allow them to survive in the presence of arsenic, thus requiring species' tolerance of fluoride (GRATÃO et al., 2005).

However, many plant species subjected to contamination by heavy metals present a number of structural and physiological disorders that adversely affect their growth and development; therefore, they cannot be characterized as potential phytoremediators (MUFARREGE; HADAD; MAINE, 2010).

Nevertheless, the toxic effects of As on the physiological characteristics of plants is still poorly studied (PEREIRA et al., 2011). Recent studies of water hyacinth (*Eichhornia crassipes*) confirm its tolerance, leading to an increase in photosynthesis that is likely to be associated with enhanced uptake of CO_2 and changes in anatomical features of leaves and roots (PEREIRA et al., 2011).

In addition to these features, to study the various adaptive mechanisms that result in species tolerance to arsenic, molecular factors such as changes to the amount of DNA should be further analyzed. Toxic elements such as Cr may incite damage to the DNA of plants and affect their growth characteristics (RODRIGUEZ et al., 2011).

In many studies of phytoremediation, aquatic macrophytes have been used as extractors of pollutants in aquatic environments (SKINNER; WRIGHT; PORTER-GOFF, 2007). Many species of macrophytes can be used in these studies, but the *Brachiaria arrecta* (Hack.) Stent. (Poaceae) has the potential to display rapid propagation and high biomass production (MARTINS et al., 2008); these features are among the main essential characteristics for plants used in phytoremediation, besides belonging to the genus Brachiaria, which dominates the tolerant species (ARAÚJO et al., 2011). However, no studies have described the anatomical and physiological characteristics of this species in the presence of toxic elements such as As, thereby elucidating what contributes to metalloid tolerance in this species.

The aim was to evaluate the anatomical characteristics, gas exchange, growth and DNA content in *Brachiaria arrecta* grown under increasing concentrations of As.

2 Materials and Methods

Plants (*Brachiaria arrecta*) were collected from the banks of the Funnel Dam, located in the municipality of Lavras (44° 55' W, 21° 05' S). Subsequently, the plants were washed in running water to eliminate impurities and were selected by plant and number of leaves; the selected plants had five fully expanded leaves with average roots of approximately 25±1 cm and were acclimatized in a greenhouse. The plants were propagated in 20 L plastic trays containing Hoagland and Arnon (1950), modified to reach half ionic strength.Daughter plants were used in the experiment and were selected by the number of leaves and the average size of their roots. To standardize the experimental plants, those plants containing five leaves and roots of approximately 25 cm ± 1 were selected. The plants were grown in polypropylene pots of up to 8 G, containing 6 L of Hoagland-Arnon with 40% ionic strength (HOAGLAND; ARNON, 1950); increasing concentrations of sodium arsenate heptahydrate were incorporated: 0, 0.25, 0.50, 1.00, 2.00 and 4.00 U mg^{-1}. The pH was maintained at approximately 5.5 and was adjusted with the addition of sodium hydroxide or hydrochloric acid, after which it was measured every 2 days. The arsenic concentrations were based on the maximum allowable concentration for freshwater (Class 1) from Resolution 357 of Conama (BRASIL, 2005), corresponding to the control (0 mg L^{-1}) and 2,500, 5,000, 10,000, 20,000 and 40,000 times the maximum allowable concentration. The maximum allowable concentration for effluent discharge from Resolution 357 of Conama (BRASIL, 2005) corresponded to the control (0 mg L^{-1}) and 50, 100, 200, 400 and 800 times the maximum allowable concentration at concentrations of 0.25 mg L^{-1}. The plants were left in these conditions for 21 days, during which time two exchanges were made possible nutrient solution plus's, and the growth and development of plants were assessed. The experimental design was completely randomized and was characterized by six treatments and six replications; the experimental plot consisted of a pot with three plants.

The roots and the second fully expanded leaf were collected at the end of the experiment for anatomical analysis and were fixed in formaldehyde, glacial acetic acid and 70% ethanol (FAA70%) for a period of 72 h and then stored in ethanol 70º GL (JENSEN, 1962).

Samples of the pilifera roots and the middle region of the leaves were embedded in Historesin according to the methods of O'Brien, Feder and McCULLY (1964). Cross-sections of pilifera roots and leaves from the median region were removed with the aid of a rotary microtome. After the cuts, the sections were stained with toluidine blue. Paradermic sections were also obtained from the abaxial and adaxial median region of the leaves and were stained with Safranin (JOHANSEN 1940; BUKATSCH, 1972; BURGER; RICHTER, 1991). Appropriately colored semi-permanent slides were made with the material in 50% glycerol and were held between the blades and the colorless nitrocellulose sealing resin.

To study the quantitative anatomy, photomicrographs were created with the aid of an Olympus U-TV1X-2 digital camera coupled to an Olympus CX41 microscope and with the aid of BEL View software. The obtained images were subsequently analyzed with image analysis software - the ImageTool UTHSCSA. For this analysis, five cuts were counted per slide, with a blade mounted for each experimental replicate and four fields per section.

The percentage of aerenchyma in the root cortex was determined using the methods of Pereira et al. (2008). The Carlquist vulnerability index (CVI = diameter of the tracheal elements/number of tracheal elements) was calculated according to the methods of Carlquist (1975). We also analyzed the root tissue thicknesses. The stomatal density (number of stomata per mm^2) of the leaves and the polar and equatorial diameters of the stomata, as well as the thickness of the tissue constituents of the leaf, were analyzed.

On the eighteenth day of the experiment, the fully expanded leaves were evaluated, along with the following characteristics of leaf gas exchange, i.e., the photosynthetic rate (A), stomatal conductance (gs) and transpiration (E), using an infrared gas analyzer (IRGA), model ADC Model - LCA - 4. Analyses were performed beginning at 10 am, with a flux density photosynthetic photon setting the device to 1.000 mol m^{-2} s^{-1}.

We also evaluated the relative growth rate (RGR), leaf area ratio (LAR), net assimilation rate (NAR) and specific leaf area (SLA) using the methods proposed by Hunt, Causton and Shipley (2002). The leaf area was measured from photographs of all of the leaves and was analyzed using image analysis software (UTHSCSA-ImageTool).

An assessment of the nuclear DNA was accomplished using flow cytometry. To determine the DNA content, approximately 20-30 mg of young leaf *Brachiaria arrecta* and *Pisum sativum* (internal reference standard) were crushed in a petri dish containing 1.0 mL of LB01 buffer at 4 °C to release the cores (DOLEZEL; BARTOS, 2005). The nuclei suspension was aspirated through two layers of gauze with a pipette and filtered through 50-micron mesh. Nuclei were stained by adding 25.0 µL of a solution of 1 mg mL^{-1} propidium iodide and were added with 5.0 µL RNase for each sample. The samples were analyzed immediately after preparation. For each sample, 5000 nuclei were analyzed using a logarithmic scale. The analysis was performed on a FACSCalibur cytometer; the histograms were obtained using the Cell Quest software and were analyzed using he WinMDI 2.8 software. The nuclear DNA content (pg) of the plants was estimated using the ratio of the fluorescence intensities of the G1 nuclei (nuclei that are in the G1 phase of Interphase) reference standard (*P. sativum*) and Sample G1 nuclei and by multiplying this ratio by the DNA amount of the reference standard (9.09 pg). Analyses were performed on three sheets for each of the six replicates for each treatment for a total of eighteen assessments.

To confirm the absorption of the solution by the plants, the location of arsenic in the *B. arrecta* root tissue and leaves was evaluated using Scanning Electron Microscopy with EDS and analyzed in a Scanning Electron Microscope (LEO Evo 40 PVX) with Bruker's fluorescence spectrometry energy dispersive X-ray detectors coupled with the protocols specific to this type of analysis proposed by Alves (2006).

The data were subjected to an analysis of variance, and the means were compared using the Scott-Knott test at $p < 0.05$ using the statistical software Sisvar.

3 Results and Discussion

Arsenic absorption was confirmed by the location of the element in the roots and leaves of *B. arrecta* by mapping its distribution in plant tissues cultured in the presence of arsenic, and the absence of the treatment element in the culture solution (control, 0 mg L^{-1}) (Figure 1).

Plants that are exposed to toxic elements such as arsenic may undergo changes in their anatomy and physiology, which characterize their plasticity under stress and allow for their survival in the presence of pollutants or, in contrast, adversely affect their growth and development due to the toxicity caused by these elements. Studies by Singh et al. (2007) with *Phaseolus aureus* in the presence of As demonstrated anatomical changes in the plant structure when subjected to this contaminant, such as its plasticity response.

No differences were found in the thickness of the epidermis on the abaxial and adaxial of the *B. arrecta* plant in the various the concentrations of As (Table 1). The leaf blade thickness increased by 32.72% at concentrations from 0.25 to 1.00 mg L^{-1} compared to the control and to the increases observed at concentrations of 2.00 and 4.00 mg L^{-1} (Table 1). There was also a similar increase in the number of clusters and the area of bulliform cells (36.20%), which significantly increased at concentrations up to 1.00 mg L^{-1} (Table 1) and began falling again under higher concentrations, becoming similar to the control treatment (Table 1). The number of mesophyll cells present in the vascular bundles increased in the presence of As at concentrations of 2.00 and 4.00 mg L^{-1} (Table 1).

When working with *Salix humboldtiana* contaminated by zinc (Zn) in the soil, found that the soil with the highest level of contamination had a reduction in mesophyll thickness, resulting in a lower leaf thickness. This anatomical modification was attributed to the relatively smaller size of the mesophyll cells and to the collapse of the parenchyma cells (GOMES et al., 2011a). The same was observed by Gomes et al. (2011b) in a study of *Brachiaria decumbens* exposed to cadmium (Cd) in soil and by Marques et al. (2011) in a study of *Eucalyptus camaldulensis* exposed to Cd contamination in a nutrient solution. These studies suggest that the metal could have prompted the collapse of cells due to induction in the extensibility cell, indicating that the metal caused toxicity symptoms in these plants. This phenomenon was not observed for *B. arrecta* because it maintained the average size of the mesophyll cells and their integrity (Figure 2).

The size of the mesophyll cells of *B. arrecta* and their integrity could have been maintained due to a lack of influence from arsenic on the plant hormones responsible for cell extensibility, such as auxins. Unlike Cd, auxin requires a competitive inhibitor of the biosynthetic pathway cofactors, for example, indoleacetic acid, which may not have affected the function of these phytohormones in cell expansion. Moreover, Cd competitively inhibits the action of Zn, which, in turn, acts as a cofactor in the biosynthetic

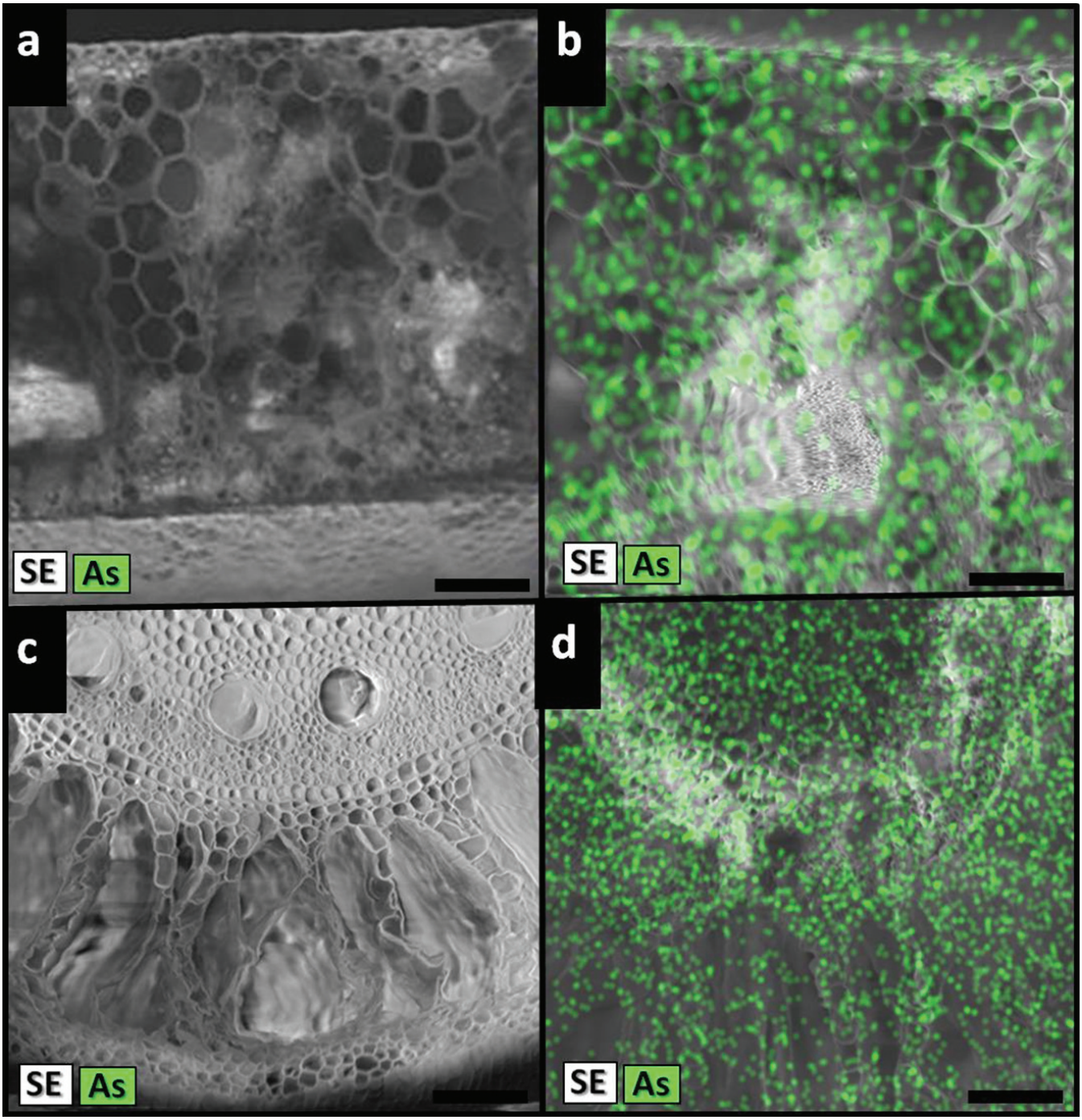

Figure 1. Electron micrographs of leaf blade cross-sections and roots of *Brachiaria arrecta* under increasing concentrations of As in the nutrient solution. (a and c - 0.0 mg L^{-1}, b and d - 4.0 mg L^{-1}, SE - Scanning Electron, As - Arsenic). a and b: bar = 20 μm; c and d: bar = 100 micron.

pathways of these metabolites. Its absence results in a loss of production capacity for indoleacetic acid, which promotes the extensibility cell, allowing it to change the physiological processes of the plants, thereby causing toxicity. Because these features were not observed in this study, there is an indication that *B. arrecta* exposed to As maintains its vital cellular processes, as indicated by the absence of toxicity caused by this metalloid.

In a study of *S. humboldtiana* (GOMES et al., 2011a), the greatest concentration of Zn caused lower leaf mesophyll thickness due to the smaller size and rupturing of its cells, suggesting toxicity caused by this pollutant. Though zinc is an essential micronutrient for plants and is required in low concentrations to maintain life processes, in excess, this element causes toxicity in plants. This toxicity is caused by the role of Zn as a competitive inhibitor of other cofactors of metabolic pathways that are essential to plants, as in the case of Fe, which is directly linked to redox reactions and may alter cell function, causing lower growth or breaks that hamper cell maintenance.

Table 1. The mean values of the anatomical characteristics of *Brachiaria arrecta* leaves under increasing concentrations of As in the nutrient solution.

As (mg L^{-1})	EP FAD (µm)	EP FAB (µm)	Mesofilo (µm)	LL (µm)	NCB	ABCA	NVB
0.00	11.20 a	7.18 a	113.80 b	132.18 b	5.91 b	4316.80 b	6.46 b
0.25	11.00 a	7.16 a	136.04 a	154.20 a	7.97 a	6954.60 a	6.17 b
0.50	10.60 a	7.00 a	145.40 a	163.00 a	7.89 a	7972.20 a	6.69 b
1.00	11.00 a	7.26 a	145.14 a	163.40 a	8.05 a	8075.60 a	6.36 b
2.00	10.80 a	7.18 a	121.42 a	139.40 b	6.04 b	4230.60 b	7.65 a
4.00	11.25 a	7.20 a	109.55 b	128.00 b	6.17 b	4247.80 b	8.11 a

Means that are followed by the same letter in a column do not differ based on the Scott-Knott test at $p < 0.05$. EP = FAD on the adaxial epidermis; FAB = EP on the abaxial epidermis; LL = Limbo Leaf; NCB = number of cells per mm bulliform sheet; ABCA = area of bulliform cell assemblies; NVB = number of vascular bundles per section.

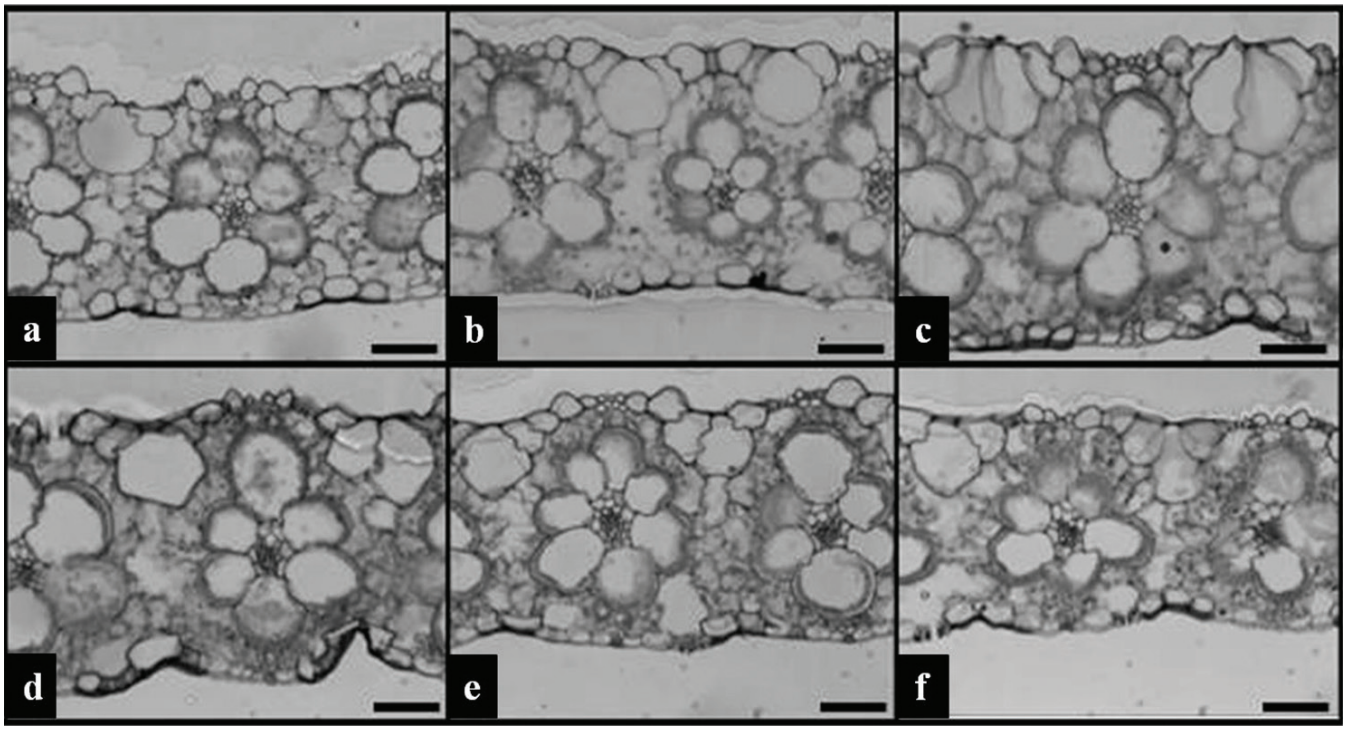

Figure 2. Photomicrographs of cross-sections of the leaf blades of Brachiaria arrecta under increasing concentrations of As in the nutrient solution. (a - 0.0 mg L^{-1} and b - 0.25 mg L^{-1}, c - 0.50 mg L^{-1} and d - 1.0 mg L^{-1} and e - 2.0 mg L^{-1} and f - 4.0 mg L^{-1}'s). Bar = 50 µm.

Policy chains of bulliform cells were found to be present in *B. decumbens* that was grown in soil contaminated by cadmium. The levels were greater in leaves that were exposed to higher levels of contamination, minimizing water loss through transpiration and preventing an increase in the degree of plant stress under higher metal contamination, also implying a lower absorption of Cd due to a decrease in the transpiration rate. Because these cells are responsible for winding the sheath on this Poaceae species, they minimize the area exposed to radiation energy, thereby preventing excessive sweating (GOMES et al., 2011b). However, this study showed that after a gradual increase in the area of the bulliform cell sets, up to a concentration of 1.00 mg L^{-1} the area of bulliform cell sets did not differ statistically from plants grown in the absence of As (control treatment). This result was possibly due to these plants not being exposed to conditions requiring limits to excessive sweating, having been grown in a nutrient solution and thus promoting the greater uptake of metal ions by the transpiration stream.

The increase that was observed in the number of vascular bundles present in mesophyll indicated that the transport of assimilates, phytohormones and nutrients in the leaves were not adversely affected by As, allowing for the distribution of these metabolites throughout the plant body and allowing the species to survive this stress condition. The effect of As was studied in the species *Eichhornia crassipes* and showed the shortest distance between the vascular bundles present in higher concentrations in the As-tested mesophyll (1 and 2 mg L^{-1}) (PEREIRA et al., 2011). Many of these concentrations indicated that the plant organs maintain the distribution of assimilates, confirming the data found in this work.

There was a significant increase (25.14%) in the stomatal density on the upper side of the leaves starting at a concentration of 1.00 mg L^{-1}. There was also a significant decrease in the

polar diameter at these concentrations (Table 2). On the abaxial surface of the leaves of *B. arrecta* under different concentrations of As, the epidermis showed no significant changes in relation to the stomatal density. However, the polar diameter of the stomata decreased significantly starting with a concentration of 1.00 mg L^{-1} of As, compared with lower concentrations (Table 2).

Heavy metals influence the process of cell expansion by decreasing the cell size (GOMES et al., 2011a; GOMES et al., 2011b; MARQUES et al., 2011). Therefore, the observed increase in stomatal density in plants grown under contamination with heavy metals compensated for the smaller diameter stomata found in these conditions, confirming the data found in this study for *B. arrecta* cultivated under As contamination. Therefore, the increase in stomatal density found in higher concentrations of As (1, 2 and 4 mg L^{-1}) on the adaxial surface was aimed at compensating for the input of CO_2, which is essential to the photosynthetic process. The lower polar diameter of the stomata in these conditions increased the efficiency of water use, indicating that the plants have a plastic response to the stress caused by higher concentrations of As. This behavior indicates the anatomical plasticity of the *B. arrecta* leaves exposed to As of various concentrations, which are characteristics that may support the species' tolerance to As and provide for the growth and development of the species under this stress condition.

Thus, chlorenchyma thickness and stomatal density are associated with characteristics directly related to photosynthesis in plants (CASTRO; PEREIRA; PAIVA, 2009; PEREIRA et al., 2011). The increase in the number of stomata can cause the CO_2 uptake capacity to increase, which is favorable for plant photosynthesis, especially in conditions where perspiration is not a limiting factor, such as plant aquatic environments. In *Podocarpus lambertii* contaminated by oil (MARANHO et al., 2006), and in a water-stress resistant coffee cultivar (GRISI et al., 2008) the greater stomatal density aims to maintain CO_2 capture when the polar diameter is smaller, supporting the conditions required for maintaining photosynthetic rates under stress conditions. This research showed that increasing the thickness of the mesophyll and stomatal density might have contributed to the maintenance of the photosynthetic rate in B. arrecta, allowing the species to survive and develop in the presence of As.

With respect to the root anatomy, the number of metaxylem vessels reduced in concentration beginning at 2.00 mg L^{-1} (Table 3). The Carlquist vulnerability index (CVI), showed no significant differences at any of the concentrations tested. The percentage of aerenchyma related to the root bark was also not significantly different at various tested concentrations of As (Table 3).

A smaller number of vessels were present in metaxylem of *Salix humboldtiana* exposed to Zn and reported that heavy metals can reduce the number of xylem vessels, confirming the data found this study (GOMES et al., 2011a). In other words, toxic elements can alter the balance of the hormones that are directly related to the morphogenesis of the plant tissues.

However, the Carlquist vulnerability index (CVI), which allows for the verification of the sensitivity of the

Table 2. The mean values of stomatal characteristics of the leaf epidermis of *Brachiaria arrecta* under increasing concentrations of As in the nutrient solution.

As (mg L^{-1})	Face Adaxial			Face Abaxial		
	DP (µm)	DEQ (µm)	SD (µm)	DP (µm)	DEQ (µm)	SD (µm)
0.00	29.75 a	7.13 a	117.17 b	32.83 a	7.25 b	124.71 a
0.25	28.50 a	7.17 a	123.42 b	32.21 a	7.88 a	114.54 a
0.50	29.42 a	7.86 a	126.75 b	32.58 a	8.29 a	108.33 a
1.00	27.92 b	7.79 a	139.84 a	31.29 b	8.46 a	110.42 a
2.00	27.63 b	7.29 a	145.04 a	30.79 b	8.29 a	109.33 a
4.00	26.83 b	7.03 a	146.63 a	30.63 b	8.29 a	119.00 a

Table 3. The mean values of anatomical root characteristics of *Brachiaria arrecta* under increasing concentrations of As in the nutrient solution.

As (mg L^{-1})	EPID (µm)	EXOD (µm)	END (µm)	DV (µm)	PA (%)	NV	CVI (%)
0.00	16.60 a	12.40 d	5.20 f	52.00 a	31.20 a	8.00 a	6.50 a
0.25	13.40 b	18.60 c	9.20 e	52.40 a	32.40 a	8.00 a	6.55 a
0.50	9.80 c	26.20 b	10.80 d	51.60 a	28.60 a	8.00 a	6.45 a
1.00	6.80 d	27.00 b	13.60 c	50.80 a	30.60 a	8.00 a	6.35 a
2.00	6.60 d	28.40 b	16.00 b	41.40 b	29.20 a	6.00 b	6.90 a
4.00	0.00 e	35.00 a	19.60 a	38.60 c	28.60 a	6.00 b	6.43 a

Means that are followed by the same letter in a column do not differ based on the Scott-Knott test at $p < 0.05$. EPID = Epidermis; EXOD = exodermis; END = Endodermis; PA = Percentage of aerenchyma in relation to the cortex; NV = Number of metaxylem vessels; DV = diameter of metaxylem vessels; CVI = Carlquist Vulnerability Index.

plant's vascular system to cavitation, showed a favorable reduction (CARLQUIST, 1975; PEREIRA et al., 2008; CASTRO; PEREIRA; PAIVA, 2009). According to Denardi and Marchiori (2005), a higher CVI indicates a greater vulnerability of the conduction system to crude sap in the plant; however, great laugh from embolism. Therefore, maintaining the CVI conditions tested in this study contributes to the flow of water and nutrients in the xylem, even in the presence of a characteristic that is conducive to the survival of plants of *B. arrecta* exposed to As.

The absence of significant differences in the proportions of aerenchyma present in the root cortex indicated that the ability of the plants to survive in an aquatic environment was not affected by As, thus allowing the plants to grow and develop in this environment. In *E. crassipes* exposed to As, differences in the proportion of aerenchyma present in the root cortex were not perceived; therefore, the As did not affect the survivability of water hyacinth in this condition (PEREIRA et al., 2011). The formation of aerenchyma in rice exposed to As is affected by root aeration and different genotypes (WU et al., 2011). However, the absence of aerenchyma in the proportion present in the roots of *B. arrecta* grown under different As concentrations indicates that As absorption is not affected by this parameter.

With respect to the exodermis and endodermis, the thickness significantly increased with increasing concentrations of As in the solution. However, in the case of the exodermis, the results obtained using average concentrations of 0.5 to 2.00 mg L^{-1} did not differ statistically. In addition, treatment with 4.00 mg L^{-1} showed that the organization of the cells of the endoderm did not exhibit similar behavior to the treatments with lower doses of arsenic and the absence of As; the breakdown of endoderm cells may indicate early toxicity that is caused by As (Figure 3). The epidermis, in turn, was reduced significantly by increasing concentrations of As; with the highest (4 mg L^{-1}'s) concentration, the epidermis was totally replaced by the exodermis (Table 3), with some walls remaining adhered to the exodermis (Figure 4).

In studies of *E. camaldulensis* exposed to Cd, Marques et al. (2011) found that Cd significantly affected the thickness of the root tissue and that there was an increase in the thickness of the epidermis and endoderm root proportionate to the increase in the concentration of Cd, thus confirming the data for *B. arrecta* in the presence of As.

The exodermis and endodermis apoplastic barriers are effective in curbing the flow of contaminants and pathogens from the soil to the shoots (CASTRO; PEREIRA; PAIVA, 2009) and can be modified to increase their thickness in the presence of toxic elements (RAMOS et al., 2009; PEREIRA et al., 2011). These barriers are one of the main avenues for the local retention and allocation of the same roots, contributing to a reduction in the translocation of pollutants and protecting the plant from pollutants. These characteristics are favorable to the survival of the species in the presence of toxic elements and are directly related to the mechanisms of tolerance. Therefore, *B. arrecta* demonstrated favorable tolerance characteristics by presenting apoplastic barrier thickening.

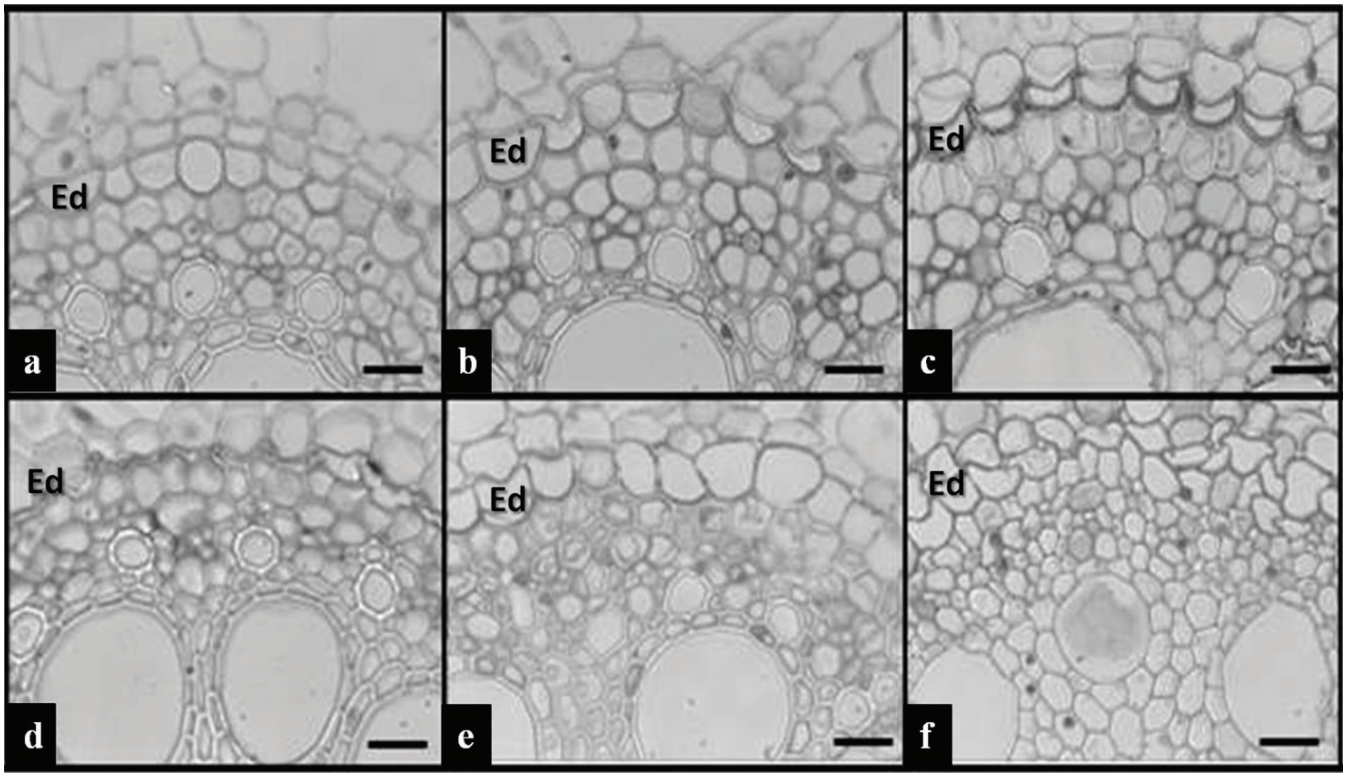

Figure 3. Photomicrographs of transverse sections of the roots of *Brachiaria arrecta* under increasing concentrations of As in the nutrient solution in the endoderm region (Ed). (a - 0.0 mg L^{-1} and b - 0.25 mg L^{-1}, c - 0.50 mg L^{-1} and d - 1.0 mg L^{-1} and e - 2.0 mg L^{-1} and f - 4.0 mg L^{-1}'s). Bar = 20 µm.

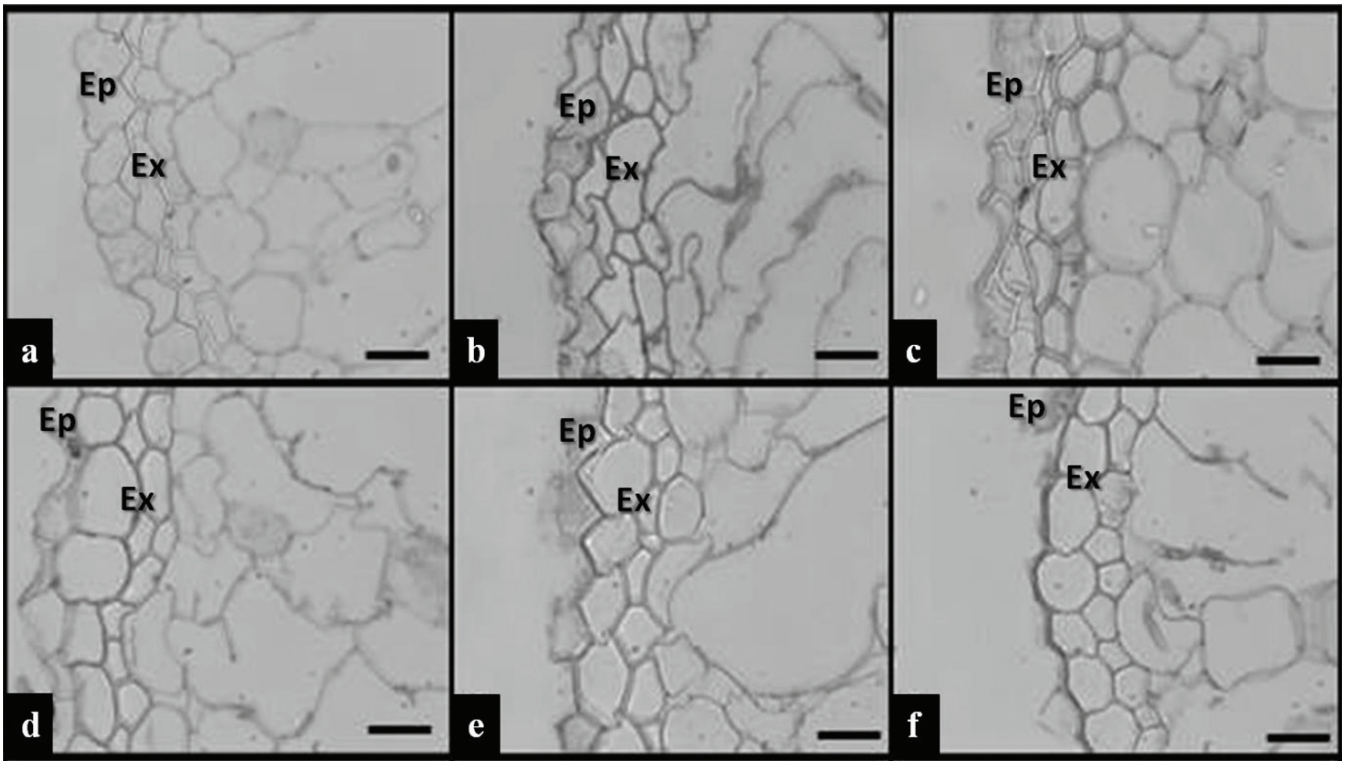

Figure 4. Photomicrographs of transverse sections of the roots of *Brachiaria arrecta* under different doses of arsenic in the As solution in the epidermis region (Ep) and exodermis (Ex). (a - 0.0 mg L^{-1} and b - 0.25 mg L^{-1}, c - 0.50 mg L^{-1} and d - 1.0 mg L^{-1} and e - 2.0 mg L^{-1} and f - 4.0 mg L^{-1}'s). Bar = 20 µm.

In the case of the *B. arrecta* epidermis, which suffered drastic reductions in its thickness with increased concentrations of As in the solution, the data are consistent with Gomes et al. (2011b) findings for *B. decumbens*. In the presence of Cd, the epidermis of the root was completely degraded with increased exposure to the metal. In both cases, the epidermis thickened exodermis was replaced and assumed the role of mechanical protection against the external environment to which the root was exposed. The epidermal cells of the root were more negatively affected by higher concentrations of pollutants because they are the first cells of the plant to have contact with the pollutants and may undergo oxidative stress, thus reducing their ability to control the removal of reactive oxygen species generated and causing the disruption and death of cells. In the presence of arsenic *Phaseolus aureus* showed a drastic reduction of trichomes and root turgidity, resulting in severe damage to the epidermal cells of the roots, with signs of lost turgidity and disintegration due to the oxidative stress resulting from lipid peroxidation from toxic exposure (SINGH et al., 2007).

The results of the gas exchange analysis revealed no significant differences for the photosynthetic rate, stomatal conductance and transpiration in the presence of As (Table 4). With respect to the growth characteristics at the highest concentration tested (4.00 mg L^{-1} As), the leaf area was significantly lower than that obtained using other concentrations, as was the number of sheaths produced during the experimental period, which consequently led to a decrease in the total dry mass found at this concentration (Table 5).

Table 4. The average gas exchange values in of *Brachiaria arrecta* plants submitted to increasing concentrations of As in the nutrient solution.

As (mg L^{-1})	A (µmol m^{-2} s^{-1})	Gs (µmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})
0.00	5.71 a	26.96 a	0.50 a
0.25	4.72 a	31.45 a	0.60 a
0.50	5.21 a	44.94 a	0.79 a
1.00	3.64 a	33.70 a	0.65 a
2.00	4.11 a	24.71 a	0.51 a
4.00	6.20 a	22.47 a	0.51 a

Means that are followed by the same letter in a column do not differ based on the Scott-Knott test at $p < 0.05$. A = photosynthetic rate, gs = stomatal conductance, E = transpiration.

However, the relationship among the dry leaf weight, leaf area and the specific leaf area was not significantly different at concentrations beginning at 4.00 mg L^{-1} As. The tested concentrations also caused no significant changes in the relative growth rate of the plants, the leaf area ratio and the net assimilation rate of the plants (Table 5).

The reduction in leaf area at a concentration of 4.00 mg L^{-1} and the number of leaves produced during the experimental period (Table 5) were compensated for by the increase in stomatal density on the adaxial (Table 2). At this concentration, which allowed for the gas exchange to be maintained statistically equal to the other concentrations (Table 4), the plasticity of the species under the adverse conditions was indicated.

Table 5. The mean values of *Brachiaria arrecta* growth traits under increasing concentrations of As in the nutrient solution.

As (mg L^{-1})	DM (g)	LA (cm^2)	LP	RGR (g dia^{-1})	LAR (g cm^{-2})	NAR (g dia^{-1} cm^{-2})	SLA (g cm^{-2})
0.00	1.676 a	153.26 a	13.50 a	0.043 a	65.63 a	2.897 a	220.295 a
0.25	1.915 a	162.89 a	16.00 a	0.049 a	65.63 a	3.285 a	227.321 a
0.50	1.524 a	125.01 a	15.00 a	0.038 a	60.07 a	2.330 a	213.804 a
1.00	1.850 a	157.72 a	20.17 a	0.047 a	67.54 a	3.208 a	246.025 a
2.00	1.650 a	134.61 a	13.67 a	0.042 a	64.38 a	2.942 a	246.359 a
4.00	1.027 b	97.31 b	08.17 b	0.021 a	70.76 a	1.641 a	269.708 a

Means that are followed by the same letter in a column do not differ based on the Scott-Knott test at $p < 0.05$. DM = dry mass, LA = leaf area, LP = number of leaves produced; RGR = relative growth rate; LAR = leaf area ratio; NAR = net assimilation rate; SLA = specific leaf area.

Table 6. The mean values of DNA quantities in *Brachiaria arrecta* subjected to As in the nutrient solution.

As (mg L^{-1})	DNA quanties (pg)
0.00	2.21 a
0.25	2.14 a
0.50	2.11 a
1.00	2.15 a
2.00	2.10 a
4.00	2.09 a

Means that are followed by the same letter in a column do not differ based on the Scott-Knott test at $p < 0.05$.

Plant growth was most susceptible to contamination by toxic elements. In studies with Salvinia and water hyacinth under cadmium contamination, Oliveira et al. (2001) found that the relative growth rate decreased with increasing Cd concentrations in the nutrient solution. There was a dramatic reduction in the relative growth rate to leaf area ratio and the net assimilation rate of water hyacinth in a solution contaminated with copper (Cu), illustrating the deleterious effects on plant growth in the presence of this element (ALVES et al., 2003). However, *B. arrecta* showed no significant changes in growth indices in the presence of the nutrient solution, which demonstrated its physiological plasticity under this stress condition.

The results of DNA quantification by flow cytometry showed no significant differences between the treatments (Table 6).

The amount of DNA in plants is directly related to the plants' ability to produce proteins by primary and secondary metabolism and is essential for plant survival. The amount of DNA may vary due to different environmental factors, which can be reduced drastically by toxic elements (RODRIGUEZ et al., 2011) and are an efficient tool to signal a type of tolerance for contamination by toxic elements. *B. arrecta* do not demonstrate significant changes in their DNA content, and this feature suggests that this species is able to survive As contamination without toxicity by keeping the amount of DNA without significant losses in genetic content.

Plants that are not tolerant to heavy metals or other toxic elements can demonstrate serious physiological disorders because of their lack of anatomical and physiological plasticity in stressful situations (MUFARREGE; HADAD; MAINE, 2010). *B. arrecta* showed no clear signs of physiological or structural deficiencies, which can be classified as plastic contamination under the As conditions tested in this study. The macrophytes used in phytoremediation programs, which possess anatomical and physiological plasticity contribute to their tolerance to the pollutants they are subjected to (SKINNER; WRIGHT; PORTER-GOFF, 2007); these characteristics are directly dependent on the structure and proper physiological functioning of the plants and, therefore, require intense growth and biomass production (MARTINS et al., 2008).

4 Conclusions

B. arrecta have characteristics that contribute to their survival under As contamination of a nutrient solution and have the potential to be studied for application in phytoremediation programs for As contamination.

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