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Anatomical and physiological modifications in water hyacinth under cadmium contamination

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Summary

The pollution of water bodies with heavy metals is generating increasing concern worldwide, and among those heavy metals, cadmium is one of the most toxic elements released into the environment. The present study aimed to evaluate the anatomical and physiological modifications adopted by the water hyacinth (*Eichhornia crassipes*) under cadmium contamination. The plants were grown in Hoagland solution in a greenhouse at five cadmium levels: 0.00, 3.5, 7.0, 14.0, and 28.0 μM. The net photosynthesis, stomatal conductance, transpiration rate, Ci/Ca ratio, antioxidant system enzymes activity, and anatomical traits in plant roots and leaves were evaluated. The plants exhibited increased photosynthesis, stomatal conductance, transpiration, and Ci/Ca ratios in all treatments containing cadmium. Antioxidant system enzymes displayed increased activity in the roots and leaves of plants treated with cadmium. Plants exhibited higher stomatal density and spongy parenchyma thickness under Cd contamination. The anatomical traits of the roots exhibited no evidence of toxicity or improved vascular system traits. Thus, *Eichhornia crassipes* demonstrated an ability to tolerate Cd by adopting changes in the anatomy, gas exchange and antioxidant system.

Keywords: *Eichhornia crassipes*. Phytoremediation. Antioxidant system. Ecological anatomy. Ecophysiology.

Introduction

Recent anthropogenic activities such as industrialization, mining, and agricultural practices have led to the high production and accumulation of toxic elements in the soil, food chains, and aquatic environments (Gratão et al., 2005). Cadmium (Cd) is a toxic element that can negatively influence plant growth and development and is released into the environment by power stations, heating systems, metallurgical industries, and vehicular traffic (Benavides et al., 2005). Phytoremediation is a cleaning process that can be used to remove toxic elements from the soil and water (Gratão et al., 2005). Cadmium phytoremediation has been the subject of recent studies because it is a relatively inexpensive and clean process; however, some studies have shown evidence of high Cd toxicity in plants, thus making the identification of species that hyperaccumulate Cd difficult (Mangkoedihardjo, 2008; Krämer, 2010).

Native macrophyte species are used in the phytoremediation of contaminated water. The water hyacinth (*Eichhornia crassipes*) is a native Brazilian macrophyte that belongs to the family Pontederiaceae and can potentially be used in phytoremediation systems. This species has shown a potential ability to phytoremediate chromium that had accumulated at high levels (Faisal and Hasnain, 2003). Oliveira et al. (2001) studied potential Cd hyperaccumulation in *E. crassipes* and found that this species has a high capacity to uptake Cd, with greater accumulation observed in the roots and accumulation occurring proportionally to exposure time and element concentration. This species may accumulate about 80% of the cadmium in the solution, depending on the concentration of this element (Mishra et al., 2007; Wu et al., 2008). Absorbed Cd *E. crassipes* is readily associated to low molecular weight complex and further a high molecular weight complex, containing PC_2, PC_3 and PC_4 phytochelatins is developed as the final storage Cd form (Wu et al., 2008). However, chelation Cd in *E. crassipes* may also be related to S-containing compounds and vacuolar accumulation (Puzon et al., 2008).

This species also exhibited a significant ability to accumulate Cu and Zn and was able to remove these metals from biofertilizer solutions (Mondardo et al., 2006). The *E. crassipes* is able to hyperaccumulate arsenic (Dhankher et al., 2002; Pereira et al., 2011) and lead (Gonçalves Júnior et al., 2008).

Physiological traits of plants exposed to Cd are generally altered. For example, Schützendübel et al. (2001) found that Cd concentrations of 50 μM stimulated superoxide dismutase at the expense of catalase and peroxidases, promoting H_2O_2 accumulation in necrotic *Pinus sylvestris*. Pietrini et al. (2003) reported a 30% inhibition in chlorophyll content, photosynthesis, and Rubisco activity as well as stimulated antioxidant system activity in *Phragmites australis* plants exposed to Cd; these alterations enabled the maintenance of chloroplast structure at the Cd levels evaluated and protected the photosynthetic system of this species. In *E. crassipes* plants, Cd decreased chlorophyll content of leaves (Mishra et al., 2007). The antioxidant system in *E. crassipes* plants was stimulated by the increased enzymatic activity of catalase, superoxide dismutase, and guaiacol peroxidase in the presence of cadmium; furthermore, the increases in activity occurred proportionally to Cd concentrations (Odjegba and Fasidi, 2007). However, *E. crassipes* show Cd stimulated superoxide dismutase in roots, but decreased the catalase, glutathione peroxidase, glutatione reductase and peroxidase activities (Vestena et al., 2011). *Pistia stratiotes* plants exposed to Cd also showed anatomical and reproductive modifications (Silva et al., 2013).

The anatomy of *E. crassipes* showed plasticity in the presence of textile industry effluents, with reductions in cell size in the leaf tissue observed (Mahmood et al., 2005). Moreover, *E. crassipes* plants developed enhanced leaf and root structure under arsenic and lead contamination, increasing stomatal density, mesophyll thickness, number of xylem vessels between another favorable anatomical modifications (Pereira et al., 2011; Pereira et al., 2014). Cadmium is capable of influencing the foliar anatomy of *Alternanthera philoxeroides* and *Polygonum ferrugineum*, resulting in reduced amounts of aerenchyma and crystal idoblasts, thicker mesophyll, and increased numbers of glandular trichomes, although with no effect on photosynthesis (Souza et al., 2009).

Given the possible effects of Cd on the anatomical and physiological traits of plants with phytoremediation potential, the present study aimed to evaluate the effect of Cd on these traits in *E. crassipes*.

Materials and methods

Water hyacinth (*Eichhornia crassipes* Mart.) plants were collected from a set of ponds in Alfenas, MG, Brazil, that were apparently

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free of cadmium contamination and that originated from a spring located approximately 60 meters away. The collected plants were washed with running water and grown in a greenhouse in Hoagland and Arnon nutrient solution (HOAGLAND and ARNON, 1940) at 40% ionic strength for 30 days to obtain clonal generations acclimatized to the greenhouse and endogenously cadmium-free.

To obtain uniform experimental units, plants were selected based on the number of leaves, the absence of stolons, the viability of daughter plants, and size. Six-L polypropylene vases containing 4 L of Hoagland and Arnon nutrient solution at 20% ionic strength supplemented with increasing cadmium levels (0.0, 3.5, 7.0, 14.0, and 28.0 μM) were used to grow the plants under the treatment conditions. The cadmium levels corresponded to the control (0 mg L^{-1} concentration) and the treatment levels corresponded to 100, 200, 400, and 800 times the maximum concentration permitted, starting at 3.5 μM, determined as the maximum concentration permitted by CONAMA Resolution no. 357 (WOLFF et al., 2009). The plants were kept under these conditions for 20 days.

After 15 days, the plant gas exchange characteristics were evaluated using an infrared gas analyzer (IRGA), LI-6400 model (Li-COR Biosciences, Lincoln-USA) and a 6 cm^2 cuvette with a red/blue LED light source (LI-6400-02B). The stomatal conductance (g_s), transpiration rate (E), photosynthetic rate (A), and the ratio between internal and external carbon (Ci/Ca) were evaluated. To measure these variables, fully expanded leaves from five plants per treatment were selected, starting at 10 a.m., when the photosynthetic photon flux density was fixed in the device chamber at 1000 μmol m^2 s^{-1}, the pump flow was 500 μmol s^{-1}, the vapor pressure deficit was 2.6 KPa. The measurements were conducted only in pathogen-free leaves.

The anatomical analyses were performed on daughter plants with fully developed leaves and roots grown during the 20-day experimental period. The daughter plants were fixed in a solution of formaldehyde, acetic acid, and 70% ethanol (F.A.A. 70) for 72 hours and then stored in 70% ethanol until further analysis. Paradermal leaf sections were obtained using steel blades on abaxial and adaxial sides. Next, the sections were cleared with 50% sodium hypochlorite, rinsed in distilled water twice for 10 minutes, stained with 1% safranin solution, and mounted on slides with coverslips with 50% glycerol (JOHANSEN, 1940). The 2-cm leaf fragments taken from the median region of leaves. In roots, the maturation zone region, 2 cm away from the root apex, was sampled. All of the sections were performed using a LPC table microtome. Sections were cleared with sodium hypochlorite, rinsed in distilled water twice for 10 minutes, stained with safrablau solution (1% safranin and 0.1% astra blue at 7:3 ratio), and mounted on slides with coverslips with 50% glycerol. The slides were photographed under an Olympus BX 60 microscope (Olympus, Tokyo, Japan) coupled to a Canon A630 digital camera (Canon Inc., Tokyo, Japan).

The UTHSCSA-Imagetool software was used for image analysis and the following parameters were measured: AbE = abaxial epidermis thickness, AdE = adaxial epidermis thickness, MF = mesophyll thickness, PP = palisade parenchyma thickness, SP = spongy parenchyma thickness, PP/SP ratio = palisade and spongy parenchyma ratio, DV = distance between vascular bundles in the mesophyll, BSC = diameter of the bundle sheath cells, PAE = proportion of aerenchyma in the leaves (area/area), NS = number of stomata per field: NC = number of epidermal cells per field; POL = polar diameter of the stomata; EQU = equatorial diameter of the stomata, SD = stomatal density (stomata per mm^2), FUN = stomatal functionality (ratio POL/EQU), SI = stomatal index, PAR = proportion of aerenchyma in the cortex, CC = diameter of cortical cells, EP = epidermis thickness, ED = endodermis thickness, CVI = vascular system vulnerability index (Carlquist vulnerability index), MP = diameter of pith parenchyma cells, EX = exodermis thickness, and PHL = phloem thickness. The CVI was calculated according to the method described by CARLQUIST (1975) and the proportions of the root aerenchyma area were calculated according to the method described by PEREIRA et al. (2008).

Twenty days after the onset of the experiment, leaf and root samples were collected in the morning for biochemical analysis and then were frozen in liquid nitrogen and preserved in a freezer at -80 ºC until further analysis. To obtain the analyzed enzymes, protein was extracted from 0.5 g of roots or leaves to which were added 2.0 mL of extraction buffer consisting of 1.924 μL of 0.1 M potassium phosphate buffer at pH 7, 20 μL of 0.1 M EDTA [ethylenediaminetetraacetic acid], 8 μL of 0.5 M DTT [dithiothreitol], 16 μL of 0.1 M PMSF [phenylmethanesulfonyl fluoride], and 40 mg of PVPP [polyvinylpolypyrrolidone], as adapted from the method described by BOR et al. (2003). After homogenization, the enzyme extract was centrifuged at 14,000 g for 20 minutes at 4 ºC. The supernatant was then collected and used to determine the activity of the following enzymes: ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD).

SOD activity was assessed according to the method proposed by GIANNOPOLITIS and RIES (1977). Ascorbate peroxidase (APX) activity was evaluated by adding 160 μL of 0.1 M potassium phosphate buffer pH 7, 100 μL of 0.005 M ascorbic acid, 100 μL of 0.005 M H_2O_2, and 605 μL of distilled water to 35 μL of the enzyme extract, as adapted from the method described by NAKANO and ASADA (1981). Enzymatic activity was determined by monitoring decreasing absorbance at 290 nm for 2 minutes and calculated based on the extinction factor of 2.8 $mM^{-1}cm^{-1}$. To evaluate catalase (CAT) activity, we added 35 μL of enzyme extract to 160 μL of 0.1 M potassium phosphate buffer at pH 7, and then added 125 μL of 0.01 M H_2O_2 dissolved in buffer and 680 μL of distilled water, as adapted from the method described by MADHUSUDHAN et al. (2003). Enzyme activity was determined by monitoring decreasing absorbance at 240 nm for 1 minute and calculated based on the extinction factor of 36 $mM^{-1}cm^{-1}$.

The experiment consisted of a completely randomized design with five treatments and five replications with the experimental units composed by one tray with one *E. crassipes* plant. The data were subjected to analysis of variance and comparison of means by Scott-Knott test at $P<0.05$ or regression analysis using Sisvar statistical software.

Results

For all Cd levels applied, the *E. crassipes* plants experienced changes in gas exchange characteristics (Fig. 1). Photosynthesis increased proportionally to Cd concentrations on nutrient solutions (Fig. 1A). The stomatal conductance increased with higher concentrations of Cd, however, dropped under concentrations higher than 14 μM (Fig. 1B). The transpiration rate increased proportionally to Cd concentrations (Fig. 1C) and the Ci/Ca ratio were increased with higher Cd levels, but were decreased under concentrations higher than 7 μM (Fig. 1D).

E. crassipes leaves display uniseriate epidermis with reduced cuticle on both sides. The palisade parenchyma consists of three to four layers of cells facing the adaxial side. The spongy parenchyma is composed by large aerenchymal chambers filled with trabeculae formed by bundles of parenchyma cells; parenchyma occurs in some of the chambers, and idioblasts can be observed. The vascular bundles are collateral, have developed bundle sheath, and exhibit the xylem toward the adaxial side in some cases and towards the abaxial side in other cases. Sclereids and idioblasts containing raphides in the palisade and spongy parenchyma can be seen, and the leaves are amphistomatic display uniseriate epidermis with reduced cuticle on both sides. The palisade parenchyma consists of three to four layers of cells facing the adaxial side. The spongy parenchyma is composed

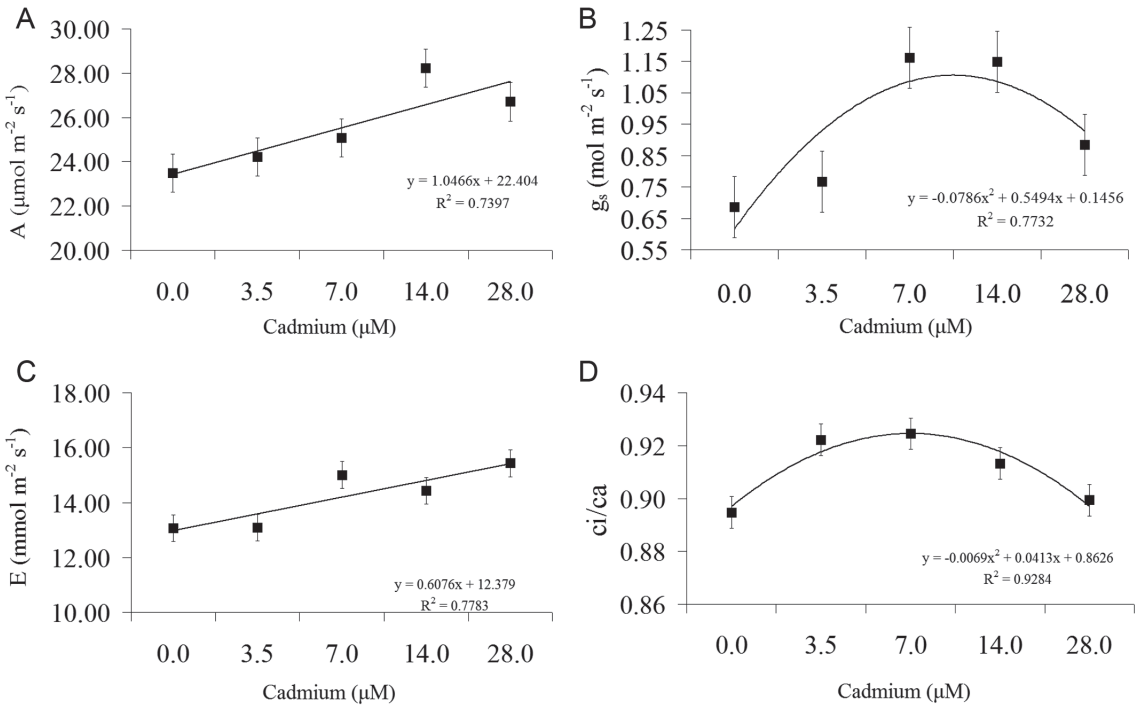

Fig. 1: Gas exchange characteristics of *E. crassipes* plants grown under different cadmium levels. A = photosynthetic rate, B = stomatal conductance, C = transpiration rate, D = Ci/Ca ratio. The bars represent standard error.

by large aerenchymal chambers filled with trabeculae formed by bundles of parenchyma cells; parenchyma occurs in some of the chambers, and idioblasts and angular collenchyma can be observed. The vascular bundles are collateral, have developed sheathes with some bundles, and exhibit the xylem toward the adaxial side (the palisade parenchyma) in some cases and towards the abaxial side in other cases. Sclereids and idioblasts containing raphides in the palisade and spongy parenchyma can be seen, and the leaves are amphistomatic (Fig. 2).

Changes in anatomical traits in *E. crassipes* leaves were observed in the different treatments; in particular, all tissues from treated leaves were thicker than those of control leaves (Fig. 2). The epidermis of the abaxial side was already 99.57% thicker at the first Cd concentration (3.5 μM); the thickness increased another 12.17% at the 14 μM concentration then decreased at the 28 μM concentration (Tab. 1). The thickness of the epidermis increased up to 124% on the adaxial side in all treatments containing Cd and was significantly higher than in the control group, although not significantly different between Cd levels (Tab. 1). Mesophyll thickness increased by 154.65% in treatments containing Cd and did not differ between Cd levels (Tab. 1 and Fig. 2). Palisade parenchyma was 138.20% thicker at the 3.5 and 7 μM levels, increased further in thickness

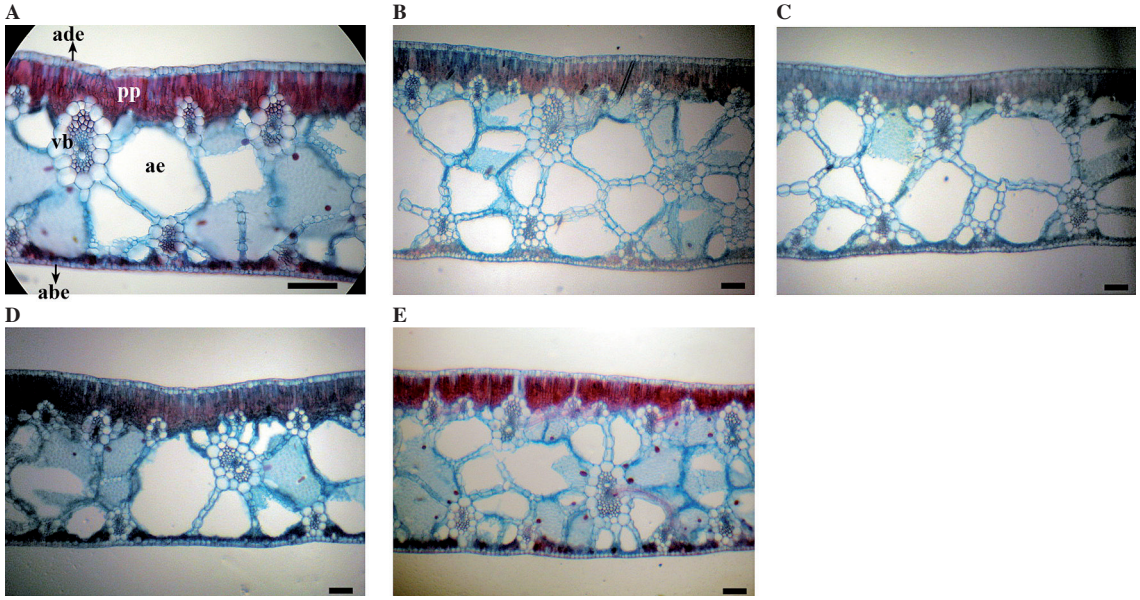

Fig. 2: Cross-sections of leaves from *E. crassipes* grown under different cadmium levels. ade = adaxial epidermis, abe = abaxial epidermis, pp = palisade parenchyma, ae = aerenchyma, vb = vascular bundle. A = 0.0; B = 3.5; C = 7.0; D = 4.0 and E = 28.0 μM of cadmium. bars = 100 μm

Tab. 1: Leaf tissue characteristics in cross-sections of leaves of *E. crassipes* grown under different cadmium levels (μM)

Cd (μM)	AbE (μm)	AdE (μm)	MF (μm)	PP (μm)	SP (μm)	DV (μm)
0.0	09.4±0.7c*	13.1±1.4b	320.4±31.7b	062.9±9.6c	260.3±39.3b	104.0±10.1b
3.5	18.7±1.7b	24.5±3.8a	815.8±33.8a	149.8±18.8b	700.2±63.4a	118.9±8.9b
7.0	18.9±2.5b	25.5±2.7a	761.1±46.7a	142.9±9.5b	618.3±56.9a	151.4±11.2a
14.0	21.0±1.8a	27.0±2.6a	752.5±39.0a	181.0±2.3a	591.6±42.7a	159.8±24.9a
28.0	16.8±1.6b	29.3±2.2a	784.7±67.6a	141.6±24.6b	660.9±92.9a	124.4±9.6b

*Means followed by the same letter in the column do not differ (Scott-Knott, 5%). Data is shown as mean ± standard deviation. AbE = abaxial epidermis thickness, AdE = adaxial epidermis thickness, MF = mesophyll thickness, PP = palisade parenchyma thickness, SP = spongy parenchyma thickness, PP/SP = palisade and spongy parenchyma ratio, DV = distance between vascular bundles in the mesophyll, PAE = proportion of aerenchyma in the leaf (area/area)

by 26.59% at 14 μM, and then decreased again at the 28 μM concentration. Spongy parenchyma thickness increased by 168.98% in all treatments with Cd but did not differ between the different levels (Tab. 1). The palisade/spongy parenchyma ratio remained unchanged between the different treatments (P = 0.29; mean value was 0.25). The distance between the vascular bundles increased by 53.60% at the 7 and 14 μM Cd levels then decreased again at the 28 μM concentration. The proportion of aerenchyma in leaves was the same in all plants, including treated and control plants (P = 0.26; mean value was 27.8%).

In the paradermal section, changes in stomatal traits were observed only on the abaxial side of *E. crassipes* leaves. The stomatal density increased by 14.29% at the 7 μM concentration but remained unchanged at higher levels (Tab. 1). Cadmium treatment did not result in changes in stomata size on the abaxial side, as shown by the polar and equatorial diameter values (Tab. 2); in addition, there were no changes in stomatal functionality or in stomatal index on this leaf side. On the adaxial face, there were no significant changes in stomatal characteristics (Tab. 2).

The anatomical structure of *E. crassipes* roots shows uniseriate epidermis and exodermis formed by a layer of thick-walled cells. The cortex can be divided into three regions: the outer cortex, comprising three layers of large elongated parenchyma cells with few intercellular spaces, the median cortex, which exhibits aerenchymal chambers bounded by trabeculae of elongated parenchymal cells, and the inner cortex, composed of six layers of thin-walled isodiametric parenchymal cells and the endodermis. The intercellular spaces of the inner cortex cells are larger than those present in the outer cortex, and idioblasts are sometimes found among these cells; the inner cortex ends with the endodermis which displays small tabular-shaped cells. The vascular cylinder is composed of large metaxylem vessels alternating with phloem, pericycle was composed of one layer of cells, and the pith parenchyma in found in the center with isodiametric cells; some smaller metaxylem vessels may be associated with larger vessels or may form new metaxylem clusters.

There were no changes in the number of aerenchyma in roots treated with Cd (*P = 0.52*; mean value was 14.4%). However, there was an 18.12% reduction in the diameter of cortical cells at the 14 μM Cd concentrations that remained unaltered at the 28 μM concentration (Tab. 3). There were no significant changes in epidermis, endodermis, and exodermis thicknesses in Cd-treated plants (Tab. 3). The CVI decreased by 54.78% in treated plants at the 7 μM concentration and remained unaltered at the higher levels; this decrease was related to a higher number of metaxylem vessels in the treated plants (Fig. 3). The PHL increased by 36.15% only at the 28 μM concentration (Tab. 3).

Tab. 2: Anatomical characteristics of paradermal leaf sections of *E. crassipes* grown under different cadmium levels

ABAXIAL SIDE							
Cd (μM)	NS	NC	POL (μm)	EQU (μm)	SD	FUN	SI (%)
0.0	8.40±0.5b*	63.2±1.8b	43.7±1.7a	21.2±0.6a	107.9±7.0b	2.06±0.1a	13±1.0a
3.5	8.00±0.5b	61.4±1.6b	47.9±5.6a	23.5±1.5a	107.9±7.3b	2.04±0.2a	14±0.9a
7.0	9.40±0.4a	61.2±3.4b	48.4±3.2a	20.7±3.4a	120.7±10.7a	2.36±0.3a	15±0.7a
14.0	9.00±1.0a	63.4±7.4b	52.9±5.3a	23.9±1.4a	115.6±12.8a	2.21±0.2a	14±1.8a
28.0	9.60±0.5a	69.2±4.2a	46.9±5.4a	25.9±5.8a	123.3±9.7a	1.87±0.4a	14±0.6a
ADAXIAL SIDE							
Cd (μM)	NS	NC	POL (μm)	EQU (μm)	SD	FUN	SI (%)
0.0	8.20±0.4a*	65.0±2.2a	46.3±2.6a	24.8±1.5a	105.3±5.7a	1.87±0.2a	13±0.3a
3.5	8.60±0.5a	61.4±1.7a	46.3±3.0a	27.0±1.6a	110.5±7.3a	1.72±0.1a	14±0.7a
7.0	9.40±1.1a	64.4±5.5a	46.6±1.4a	23.7±2.1a	120.8±14.7a	1.97±0.1a	15±1.8a
14.0	9.80±2.0a	66.0±6.5a	42.3±6.6a	23.6±3.3a	125.9±26.3a	1.79±0.1a	15±2.2a
28.0	10.60±1.9a	67.4±5.7a	46.1±4.0a	25.4±1.3a	136.2±25.0a	1.82±0.1a	16±1.7a

*Means followed by the same letter in the columns do not differ (Scott-Knott, 5%). Data is shown as mean ± standard deviation. NS = number of stomata per field; NC = number of epidermal cells per field; POL = polar diameter of the stomata; EQU = equatorial diameter of the stomata, SD = stomatal density (stomata per mm^2); FUN = stomatal functionality (POL/EQU ratio); SI = stomatal index

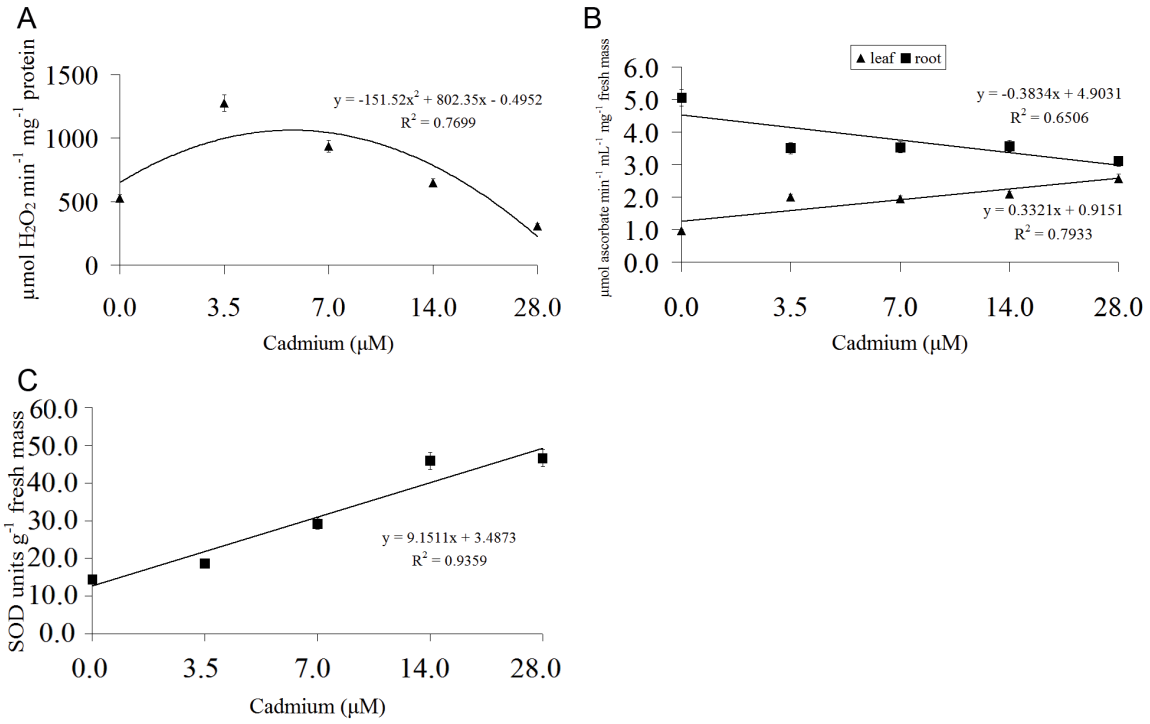

Fig. 3: Activity of the antioxidant system enzymes of *E. crassipes* plants grown under different cadmium levels. A = catalase activity in leaves, B = ascorbate peroxidase activity in roots and leaves C = superoxide dismutase activity in roots. Bars indicate standard error.

Tab. 3: Anatomical characteristics of cross-sections of roots from *E. crassipes* grown under different cadmium levels

Cd (µM)	CC (µm)	EP (µm)	ED (µm)	CVI	EX (µm)	PHL (µm)
0.0	115.9±8.2a	26.32±2.5a	19.51±1.6a	2.30±0.3a	39.58±3.5a	34.54±5.1b
3.5	108.0±5.5a	23.83±1.7a	18.59±0.7a	2.19±0.4a	31.04±3.6a	33.70±1.3b
7.0	109.8±15.4a	27.06±2.4a	17.86±1.2a	1.36±0.1b	41.03±1.7a	33.17±3.8b
14.0	099.4±1.9b	24.82±2.2a	18.09±1.9a	1.31±0.2b	39.50±2.2a	38.41±2.6b
28.0	094.9±8.4b	27.99±3.6a	19.64±2.4a	1.04±0.1b	38.26±6.5a	45.16±4.3a

*Means followed by the same letter in the columns do not differ (Scott-Knott, 5%). Data is shown as mean ± standard deviation. CC = diameter of cortical cells, EP = epidermis thickness, ED = endodermis thickness, CVI = vascular system vulnerability index (Carlquist vulnerability index), EX = exodermis thickness, PHL = phloem thickness

The antioxidant system of *E. crassipes* changed with cadmium treatment (Fig. 3). CAT activity in *E. crassipes* leaves increased by 141.60% at the 3.5 µM concentration and decreased under higher Cd concentrations (Fig. 3A). However, CAT activity in roots was not modified by Cd (P = 0.84). APX activity in the *E. crassipes* leaves increased proportionally to the Cd levels, but its activity decreased in roots with under higher Cd concentrations (Fig. 3B). SOD activity increased proportionally to Cd levels in roots of *E. crassipes* (Fig. 3C) but its activity in leaves was not affected (P = 0.12).

Discussion

The responses of the photosynthetic system of *E. crassipes* under the influence of Cd were different from those reported in the literature for sensitive species. According to Pietrini et al. (2003), the presence of Cd reduced the photosynthetic rate of *Phragmites australis* plants by up to 30%. In the presence of Cd, the deposition of reactive oxygen species such as H_2O_2 that can accumulate in cells was observed by Schützendübel et al. (2001); in these cases, reactive oxygen species can be harmful to the photosynthetic system by damaging chloroplast membranes, changing their structure and reducing photosynthetic efficiency (Stoeva and Bineva, 2003). Thus, in plants exposed to Cd, reduced photosynthetic rates are likely associated with damaged chloroplast membranes due to oxidative stress.

In the present study, *E. crassipes* plants did not exhibit inhibited photosynthesis rates (Fig. 1) but actually it increases under Cd influence. Likewise, increased photosynthesis has been recently reported to species under low Cd concentrations (Jia et al., 2015; Pereira et al., 2016). This ability of some species to maintain photosynthesis in the presence of Cd may be related to the activation of the antioxidant system, as the activities of APX, CAT, and SOD in leaves of this species increased with increasing Cd concentrations (Fig. 3). This modification may have enabled the maintenance of the chloroplast structure in *E. crassipes* leaves, thus preventing the inhibition of the photosynthetic rate. High Cd concentrations may reduce the chlorophyll concentration in *E. crassipes*, but at lower concentrations this element may increase chlorophyll content in this species (Mishra et al., 2007) and in *Robinia pseudoacacia* (Dezhban et al., 2015). This shows that the response of *E. crassipes* to Cd may be related to the concentrations of this element in the solution. Likewise, at the 28 µM or higher concentrations, stomatal

conductance and ci/ca ration tended to decrease (Fig. 1), which may be related to the decreased activities of APX and CAT and the unchanged activity of SOD (Fig. 4). These altered enzymatic activities may have led to H_2O_2 accumulation in the chlorenchymal cells and thereby a reduction in photosynthesis. These data are evidence of the relationship between the antioxidant system of this species and the maintenance of the photosynthetic rate in the presence of Cd.

While the photosynthetic rate can be regulated by different factors, radiation and CO_2 availability are the main factors that can increase photosynthesis when in higher quantities (Zhou and Han, 2005). Radiation was standardized for all treatments at the time of measurement; the increased of the photosynthetic rate is therefore likely associated with higher CO_2 availability in the leaves. This hypothesis is corroborated by the alterations in leaf anatomy that promoted uptake of this gas. Increased stomatal density on the abaxial side may be a factor contributing to higher CO_2 uptake under the experimental conditions, as this trait can increase when plants are under certain types of stress (Souza et al., 2010; Pereira et al., 2014) and under Cd contamination (Pereira et al., 2016). The absence of changes in stomatal functionality in plants from all treatments enabled the maintenance of the functional characteristics of *E. crasssipes* stomata under Cd stress.

Another anatomical factor that contributed to increased CO_2 uptake was the increased mesophyll and spongy parenchyma thickness in all treatments containing Cd. This increase in thickness may have enabled a higher capacity for CO_2 retention in the leaves, as CO_2 retention is one of the basic functions of the spongy parenchyma and aerenchyma. These effects were also described to *Schinus molle* trees under Cd contamination, increasing its leaf photosynthesis (Pereira et al., 2016). Thicker leaf mesophyll under Cd contamination was also reported to *Arachis hypogaea* (Shi and Cai, 2009). The unaltered proportion of leaf aerenchyma is an interesting result, as this tissue conserves the space for retaining gases (CO_2 and O_2), thus contributing to photosynthesis and respiration in the leaves of this species. These results are similar to those observed in other aquatic macrophytes (Souza et al., 2009) even though a lower number of leaf aerenchyma was found under conditions of Cd stress. Increased palisade thickness can promote a more efficient use of incident radiation in photosynthesis.

Other findings that corroborate the hypothesis that increased photosynthesis of *E. crassipes* is associated with CO_2 uptake are the observed increases in g_s and in the E ratio in the Cd-treated plants. An increase in these parameters results in favored CO_2 flow into the leaf, thus improving the uptake of this gas; this enhanced ability to uptake CO_2 may also be related to increased stomatal density, which promoted higher g_S and thereby increased transpiration. These results, combined with the maintained stomatal functionality and stomata size, indicate that a stress response, similar to that seen under conditions of water stress in which stomatal functionality tends to increase and stomatal diameter to decrease, occurred in the plant (Souza et al., 2010). Thus, CO_2 uptake and retention inside the leaves was favored, which may have increased the photosynthetic rate of the *E. crassipes* in the presence of Cd.

In aquatic or wetland environments, the percentage of aerenchyma in the roots is an essential component of the physiology of plant roots, and this percentage can grow when plants are grown under these conditions (Pereira et al., 2008; Pereira et al., 2010). The fact that this tissue remained unchanged in *E. crassipes* roots in the presence of Cd is a favorable factor. The endodermis, epidermis, and exodermis are considered apoplastic barriers to substance flow in the roots (Ribeiro et al., 2015). As these layers did not increase in the presence of Cd, a flow of metal to the shoots may have been promoted that can result in reduced photosynthesis which may explain why photosynthesis was inhibited at the highest Cd concentration (Benavides et al., 2005; Mangkoedihardjo, 2008).

The characteristics of the vascular system of *E. crassipes* roots were modified to increase their efficiency under conditions of stress. The CVI is a parameter that, when reduced, may indicate higher water conductivity in the roots, thus reducing embolism (Carlquist, 1975); this parameter typically decreases when the plants are under stress (Pereira et al., 2010). Thus, reduced CVI may indicate improved water conductivity in *E. crassipes*, which may have promoted increased nutrient flow and enhanced survival under these conditions; in addition, transpiration is not an issue because *E. crassipes* is an aquatic species. The thickening of the phloem observed at the highest concentration of Cd also contributed to the improvement of the vascular system. As this tissue is related to photoassimilation transport, it may allow better root system development and improved food in daughter plants.

The increased activity of the antioxidant system enzymes in *E. crassipes* corroborates the findings of Schützendübel et al. (2001), Pietrini et al. (2003), and Odjegba and Fasidi (2007), who found increased activity of these enzymes in plants subjected to Cd. However, Vestena et al. (2011) reported reduced activity for the antioxidant system enzymes of *E. crassipes* under Cd contamination. This different response may be related to the experimental conditions, in their work, Vestena et al. (2011) carried out experiments under growth chamber conditions, with lower radiation intensity and different Cd concentrations were applied. The high SOD activity in the root that occurred at the expense of APX and CAT corroborates the results of Schützendübel et al. (2001), who reported the same behavior in *Pinus sylvestris* plants. This alteration in enzymatic activity may have resulted in H_2O_2 accumulation in cells and led to oxidative stress. However, these effects are not evident in *E. crassipes,* as no signs of toxicity were observed at the levels of Cd studied.

The evaluated characteristics are evidence of the potential use of *E. crassipes* in Cd phytoremediation systems. The anatomical and physiological traits observed allowed us to elucidate the mechanisms by which this species demonstrate potential for hyperaccumulation and phytoremediation of several toxic elements, as reported by Dhankher et al. (2002), Oliveira et al. (2001), Mondardo et al. (2006), and Gonçalves Júnior et al. (2008). The absence of negative effects of Cd on physiology and anatomy of *E. crassipes* may be related to efficient vacuolar sequestration (Puzon et al., 2008) or the association of this element to phytochelatins (Wu et al., 2008), leading to a inactive form of this element. Thus, the *E. crassipes* is promising for use in Cd phytoremediation; indeed, this plant appears to commit significant resources to photosynthesis to meet the requirements for energy and carbon for daughter plants, which may promote its capacity for vegetative reproduction and high population growth.

Conclusion

E. crassipes plants exhibit physiological modifications in the presence of cadmium that contribute to maintaining photosynthetic rates and increasing the activity of antioxidant system enzymes. Changes in anatomical characteristics of *E. crassipes* promote higher CO_2 uptake and retention in leaves which may allow for higher photosynthesis. The changes in root anatomy of *E. crassipes* do not exhibit signs of toxicity. Antioxidant system enzymes are more active in the leaves than in the roots of *E. crassipes* in the presence of Cd.

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