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Tolerance mechanisms in *Cassia alata* exposed to cadmium toxicity – potential use for phytoremediation

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Abstract

Cadmium is often detected in areas contaminated by heavy metals and the incidence of this element in dangerous concentrations has been increasing due to anthropogenic activities. The aim of this research was to determine Cd concentrations in tissues, quantify compounds, pigments and enzymes, and to evaluate the gas exchange. Our aim was also to identify components that can modify and contribute to tolerance of *Cassia alata* against Cd toxicity. We used five Cd concentrations (0, 22, 44, 88, and 132 μM) to validate our hypothesis. The Cd concentrations in tissues of *C. alata* plants increased significantly, compared with the control treatment, in the following graduated sequence: root > leaf > stem. Progressive enhancement in glutathione (GSH) was verified in plants treated with all Cd concentrations used, when compared with treatment without Cd. Antioxidant enzyme activities presented similar patterns with progressive enhancements, being a desirable characteristic for plants with a potential to hyperaccumulate Cd. Our results suggest that *C. alata* plants can be used for phytoremediation programs. Their defense mechanism is based on Cd accumulation in roots, coupled with increase in GSH and the efficient activity of antioxidant enzymes that contribute to minimize the oxidative stress and consequently improve the protection of the metabolic machinery.

Additional key words: detoxification; gas exchange; heavy metal; reactive oxygen species; remediation.

Introduction

Cadmium is often detected in areas contaminated by heavy metals (Sun *et al.* 2010, Silva *et al.* 2014), and the incidence of this element in dangerous concentrations has been intensified as a result of increased anthropogenic activity (Pan *et al.* 2010). Cd is not considered a nutrient for plants, because it does not play any structural or physiological role. This metal, when absorbed in high concentrations, can affect various plant tissues, such as roots, stem, and leaves (Faller *et al.* 2005).

The absorption and accumulation of Cd in plants can cause reductions in root growth (Valentovičová *et al.* 2010) and restrict plant development (Liu *et al.* 2010, Riaz *et al.* 2014). Furthermore, Cd is an element which is easily translocated to shoots (Akhter *et al.* 2012, Wang *et al.* 2013, Su *et al.* 2014) and its accumulation in leaves normally promotes negative changes in metabolism and under extreme stress, it can provoke cell death.

Cd toxicity induces interferences in gas exchange, pigments, and growth. Deng *et al.* (2014) evaluated *Ceratopteris pteridoides* plants exposed to five Cd concentrations; they described a decrease in net photosynthetic and transpiration rates. Wan *et al.* (2011) detected significant reductions in chlorophyll (Chl) contents of two *Brassica napus* cultivars exposed to 500 μM Cd. Najeeb *et al.* (2011) reported that Cd altered morphological attributes of *Juncus effuses* plants as reflected by growth retardation.

A number of plants, such as *Dittrichia viscosa* and *Brassica juncea*, have specific mechanisms for growth and survival in areas contaminated by heavy metals (Mohamed

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Abbreviations: APX – ascorbate peroxidase; AsA – ascorbate; BCF – bioconcentration factor; Car – carotenoids; CAT – catalase; Cd_{ns} – Cd concentration in nutritive solution; Cd_{pt} – Cd concentration in plant tissue; Cd_s – Cd concentration in the shoot; Cd_r – Cd concentration in root; Chl – chlorophyll; C_i – intercellular CO_2 concentration; $CL_{50\%}$ – contamination level linked to reduction in dry matter by 50%; CTC – critical toxic concentration; DM_C – dry matter from the control treatment; E – transpiration rate; EL – electrolyte leakage; g_s – stomatal conductance; GSH – glutathione; MDA – malondialdehyde; P_N – net photosynthetic rate; P_N/C_i – instantaneous carboxylation efficiency; POX – guaiacol peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; TF – translocation factor; TSP – total soluble proteins; WUE – water-use efficiency.
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et al. 2012, Fernández *et al.* 2013). The main strategies employed are avoiding the absorption of metal and, where this is not possible, limiting accumulation in the root tissue supplemented by a lower translocation rate to shoots or absorption and accumulation in stems (Dai *et al.* 2011, Fan *et al.* 2011).

Plants used for phytoremediation programs must possess efficient detoxification mechanisms to prevent the stress (Zhou *et al.* 2015). Cd frequently interferes in the metabolism, inducing oxidative stress due to overproduction of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), but antioxidant systems based on compounds such as glutathione (GSH), and enzymes, such as ascorbate peroxidase (APX), guaiacol peroxidase (POX), and catalase (CAT), can mitigate the negative effects and improve the detoxification process (Fernández *et al.* 2013).

The identification of species with potential for phytoremediation is of a fundamental importance in order to reduce impacts in areas previously affected by Cd and other metals (Bech *et al.* 2012, Wanat *et al.* 2013). Study conducted by Silva *et al.* (2014) described large occurrence of *C. alata* plants in an area used to disposal of waste and contaminated with heavy metals, mainly cadmium, copper, zinc, and lead. Moreover, the presence of *C. alata* in an environment contaminated by heavy metals suggests that this species possesses an efficient antioxidant mechanism to keep the growth and development under unfavourable conditions.

Therefore, our hypothesis was that morphological, biochemical, and physiological approaches could explain the survival strategy of *C. alata* exposed to Cd. The study aimed to (*1*) determine the Cd accumulation in tissues, (*2*) quantify compounds, photosynthetic pigments, and enzymes, (*3*) evaluate the gas exchange, and (*4*) identify the components modified in order to contribute to the tolerance mechanism of *C. Alata* against Cd toxicity.

Materials and methods

Location and growth conditions: The experiment was carried out at the Instituto de Ciências Agrárias of the Universidade Federal Rural da Amazônia, Belém, Brazil (1°28'03''S, 48°29'18''W). The study was performed in a greenhouse with temperature and humidity controlled. The minimum, median, and maximum temperatures were 24, 27, and 35°C, respectively. Relative humidity during the experimental period varied between 72 and 91%.

Plants, pretreatment and acclimation: Seeds of *C. alata* L. were immersed in sulfuric acid of 98% for 60 min and subsequently washed with distilled water for 3 min, them with sodium hypochlorite of 2% for 10 min, and again washed with distilled water for 10 min, which is a procedure used to awaken from dormancy and accelerate germination (Braga *et al.* 2010). The seeds were placed into containers filled with substrate (*Plantmax*, *Eucatex*, Brazil) without nutrients for a period of 25 d under the conditions described above. The 25-d-old seedlings of similar sizes were selected and placed in 2-L containers containing a nutritive solution. The ionic force was initiated at 25%, then increased to 50 and 100% at a regular interval of 7 d. After this period, the nutritive solution remained at the total ionic force level. Subsequently, 50-d-old young plants were subjected to Cd treatment.

Experimental design: The experiment was entirely randomized with five Cd concentrations (0, 22, 44, 88, and 132 μM) with five replicates making a total of 25 experimental units, with one plant in each unit. These concentrations were chosen according to our preliminary study and research of Mohamed *et al.* (2012) with *B. juncea*.

Plant growth and Cd treatments: During plant growth, one young plant was placed in each pot. The plants received the following macro- and micronutrients contained in the nutrient solution (*Sigma-Aldrich*, USA):

KNO_3	8.75 mM
$Ca(NO_3)_2 \cdot 4H_2O$	7.50 mM
$NH_4H_2PO_4$	3.25 mM
$MgSO_4 \cdot 7\ H_2O$	1.50 mM
KCl	62.50 μM
H_3BO_3	31.25 μM
$MnSO_4 \cdot H_2O$	2.50 μM
$ZnSO_4 \cdot 7H_2O$	2.50 μM
$CuSO_4 \cdot 5H_2O$	0.63 μM
$NaMoO_4 \cdot 5H_2O$	0.63 μM
$NaEDTAFe \cdot 3H_2O$	250 μM

(*Sigma-Aldrich*, USA). $CdCl_2$ was used in oder to simulate Cd toxicity. The Cd concentrations were applied to young plants for 30 d. During the cultivation process, the solutions were changed at 07:00 h at 3-d intervals, with the pH adjusted to 5.5 using HCl or NaOH. After 30 d of treatment, all plants were tested for their physiology and morphology, and tissues (roots, stem, and leaves) were harvested for nutritional and biochemical analyses.

Evaluation of gas exchange: Stomatal conductance (g_s), net photosynthetic rate (P_N), and transpiration rate (E) were evaluated using an infrared gas analyser (*LCPro*$^+$, *ADC BioScientific*, UK). These parameters were measured on the adaxial surface of fully expanded leaves, located in the middle region of the plant. Water-use efficiency (WUE) was estimated according to Ma *et al.* (2004). Gas

exchange was evaluated in all plants under constant conditions of CO_2 concentration, PAR, air-flow rate, and temperature in a chamber at 360 μmol mol^{-1} CO_2, 900 μmol(photon) m^{-2} s^{-1}, 300 μmol s^{-1}, and 28ºC, respectively, between 9:00 and 12:00 h.

Morphological parameters: Growth of roots, stems, and leaves was measured by their constant dry mass (DM) after drying in a forced-air ventilation oven at 65°C.

Extraction and determination of Cd in plant tissues: Samples of 250 mg(leaf DM) were predigested in 2 mL of H_2O_2 (30% v/v) for 120 min. Subsequently, 2 mL of 65% HNO_3 (*Merck*, USA) were added (sub-boiling) to each of the samples which were then left to stand for 12 h in 50-mL conic tubes (*Falcon*, *BD Biosciences*, USA). The mixtures were transferred to a PFA digestion vessel and digested in a microwave system (*Mars Xpression*, *CEM Corporation*, UK) according to the following heating parameters: (1) 80°C for 3 min; (2) 150°C for 5 min; (3) 180°C for 10 min; (4) and then left to cool (Melo *et al.* 2011). Next, the volume was topped up to 30 mL with ultrapure water and rhodium was added as the internal standard (10 μg L^{-1}). The analyses were carried out using an inductively coupled plasma mass spectrometer (*SpectrAA 220*, *Varian*, USA).

Critical toxic concentration and contamination level: For the determination of critical toxic concentration (CTC), a regression line between the Cd concentration in the solution and the DM of each tissue was constructed. Calculation were based on the Cd concentration in the solution required to reduce DM by 10%, as determined by the equation: $CTC = (0.9\ DM_C - b)/a$, described by Soares *et al.* (2001), where DM_C represents the DM from the control treatment (0 μM Cd) and a and b are regression coefficients (Table 1). To measure the contamination level linked to a reduction in DM by 50% ($CL_{50\%}$), another regression line was constructed between the Cd concentration in the solution required to decrease DM by 50% and the calculated 50% of DM for each tissue as determined by the equation: $CL_{50\%} = (0.5\ DM_C - b)/a$, where DM_C represents the DM from the control treatment, as well as a and b being the regression coefficients (Soares *et al.* 2001).

Bioconcentration and translocation factors: The bioconcentration factor (BCF) was calculated by the equation: $BCF = Cd_{pt}/Cd_{ns}$ as described by Yoon *et al.* (2006), where Cd_{pt} represents the Cd concentration in plant tissue and Cd_{ns} represents the Cd concentration in the nutritive solution. The translocation factor (TF) was obtained by the Cd_s/Cd_r, formula postulated by Yoon *et al.* (2006), where Cd_s represents the Cd concentration in the shoots (stem + leaves) and Cd_r represents the Cd concentration in roots.

Photosynthetic pigments: The determination of Chl and carotenoids (Car) was carried out using 40 mg of leaf tissue. The samples were homogenized in the dark and in the presence of 8 mL of 90% methanol (*Nuclear*). Subsequently, the homogenate was centrifuged at 6,000 × *g* for 10 min at a temperature of 5ºC. The supernatant was removed and Chl *a*, Chl *b*, Car, and total Chl contents were quantified using a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) and measuring the absorbance at 470, 652.2, and 665.2 nm, according to the methodology of Lichtenthaler and Buschmann (2001).

Extraction of nonenzymatic compounds: H_2O_2 and GSH were extracted as described by Wu *et al.* (2006). Briefly, a mixture for extraction of H_2O_2 and MDA was prepared by homogenising 500 mg of fresh leaf material in 5 mL of 5% (w/v) trichloroacetic acid. Then, the samples were centrifuged at 15,000 × *g* for 15 min at 3°C to collect the supernatant.

Hydrogen peroxide concentration: To measure H_2O_2, 200 μL of supernatant and 1,800 μL of reaction mixture [2.5 mM potassium phosphate buffer (pH 7.0) and 500 mM potassium iodide] were mixed, and the absorbance was measured at 390 nm by a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) (Velikova *et al.* 2000). The H_2O_2 concentration was expressed in μmol g^{-1}(FM).

Glutathione quantification: For GSH detection, 200 μL of supernatant, 1,800 μL of reaction mixture (containing 100 mM phosphate buffer [pH 7.6] and 0.60 mM 2-nitrobenzoic acid) were combined, and the absorbance was measured at 412 nm by a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) (Wu *et al.* 2006). The GSH concentration was expressed in μmol g^{-1}(FM).

Extraction of antioxidant enzymes and soluble proteins: CAT, APX, POX, and total soluble proteins (TSP) were extracted from leaf tissue following the method of Badawi *et al.* (2004). The extraction mixture was prepared by homogenising 500 mg of fresh matter in 5 mL of extraction mixure, which contained 50 mM phosphate buffer (pH 7.6), 1.0 mM ascorbate, and 1.0 mM EDTA. Subsequently, the samples were centrifuged at 14,000 × *g* for 4 min at 3°C, and the supernatant was collected. The TSP quantification used the method described by Bradford (1976). The absorbance was measured at 595 nm, using a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) and bovine albumin as a standard.

Catalase determination: For CAT assay (EC 1.11.1.6), 200 μL of supernatant and 1,800 μL of reaction mixture (containing 50 mM phosphate buffer [pH 7.0] and 12.5 mM hydrogen peroxide) were combined, and absorbance was measured at 240 nm by a spectrophotometer

(*UV-M51*, *Bel Photonics*, Italy) (Havir and McHale 1987). The CAT activity was expressed in μmol(H_2O_2) mg^{-1} (protein) min^{-1}.

Ascorbate peroxidase quantification: For APX assay (EC 1.11.1.11), 1,800 μL of reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 1.0 mM hydrogen peroxide was mixed with 200 μL of supernatant, and absorbance was measured at 290 nm by a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) (Nakano and Asada 1981). The APX activity was expressed in μmol(AsA) mg^{-1}(protein) min^{-1}.

Guaiacol peroxidase quantification: For POX assay (EC 1.11.1.7), 1,780 μL of reaction mixture [containing 50 mM phosphate buffer (pH 7.0) and 0.05% guaiacol] was mixed with 200 μL of supernatant. Subsequently, 20 μL of 10 mM hydrogen peroxide was added. Absorbance was then measured at 470 nm by a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy) (Cakmak and Marschner 1992). The POX activity was expressed in μmol(tetraguaiacol) mg^{-1}(protein) min^{-1}.

Data analysis: The data were subjected to an analysis of variance (*ANOVA*), and significant differences between the means were determined using the *Scott-Knott*'s test at a probability level of 5% (Steel *et al.* 2006). Standard deviations were calculated for each treatment. Statistical analyses were performed using *Assistat* software (Silva and Azevedo 2002). Regression analysis was performed with a significance level of 5%.

Results

Growth: The dry root, stem, and leaf matters showed reductions of 38.1, 46.9, and 31.1%, when subjected to a concentration of 132 μM Cd (Table 1), compared with the control treatment (0 μM). Total DM showed decreases of 14.7 and 38.7% at concentrations of 22 and 132 μM Cd, respectively, compared with the control plants. The DM production in *C. alata* plants had negative linear responses to treatments with Cd in all tissues evaluated (Table 1). The CTC, which caused the reduction of 10% in root dry matter, was 14.45 μM Cd, while for the stem and leaf CTC were 7.28 and 10.35 μM, respectively. However, the $CL_{50\%}$ of the leaf tissue was 201.48 μM Cd in solution, higher than that of $CL_{50\%}$ for the roots, which was 168.49 μM Cd in solution (Table 1).

Cd concentration in tissues increased significantly, compared with the control treatment (Table 2), in the following progression trend: root > leaf > stem. In the roots, an

Table 1. Dry matter, critical toxic concentration, and contamination level needed to reduce the dry matter to 50% in young *Cassia alata* plants treated with five cadmium concentrations. CTC – critical toxic concentration and CL50% [μM] – Cd concentration in the solution required to decrease dry matter by 50%. *Different letters* in each tissue indicate significant differences in *Scott-Knott* test (P<0.05). Means ± SD, n = 5, and regression α > 0.05.

Cd in solution [μM]	Dry matter [g]			
	Root	Stem	Leaf	Total
0	10.40 ± 0.61^{A}	7.39 ± 0.97^{A}	7.17 ± 0.32^{A}	24.96 ± 1.36^{A}
22	8.80 ± 0.52^{B}	6.38 ± 0.72^{A}	6.12 ± 0.41^{B}	21.29 ± 1.07^{B}
44	8.05 ± 0.55^{B}	4.80 ± 0.42^{B}	5.37 ± 0.28^{C}	18.22 ± 0.83^{C}
88	7.28 ± 0.31^{B}	4.74 ± 0.42^{B}	5.16 ± 0.24^{C}	17.17 ± 0.76^{C}
132	6.43 ± 0.22^{C}	3.92 ± 0.27^{C}	4.94 ± 0.20^{C}	15.29 ± 0.54^{D}
Equation	y = 9.75 – 0.027 x	y = 6.83 – 0.024 x	y = 6.61 – 0.015 x	y = 23.18 – 0.066 x
R^2	0.90	0.83	0.76	0.86
CTC [μM]	14.45	7.28	10.35	10.88
$CL_{50\%}$ [μM]	168.49	130.49	201.48	162.15

Table 2. Cadmiun concentration, bioconcentration factor (BCF), and translocation factor (TF) in young *Cassia alata* plants treated with five Cd concentrations. nd = not detected. *Different letters* in each tissue indicate significant differences in *Scott-Knott* test (P<0.05). Means ± SD, n = 5.

Cd in solution [μM]	Cd [mg kg^{-1}]			BCF			TF
	Root	Stem	Leaf	Root	Stem	Leaf	
0	nd	nd	nd	nd	nd	nd	nd
22	213.70 ± 33.04^{D}	nd	57.72 ± 9.32^{B}	86.52 ± 13.38^{B}	nd	23.37 ± 1.34^{A}	0.27 ± 0.02^{A}
44	391.51 ± 46.88^{C}	13.71 ± 1.61^{B}	80.44 ± 14.92^{B}	79.09 ± 9.49^{B}	1.83 ± 0.51^{B}	16.25 ± 1.11^{B}	0.23 ± 0.01^{B}
88	870.96 ± 89.29^{B}	31.61 ± 3.94^{A}	138.46 ± 20.63^{A}	88.06 ± 9.03^{B}	3.20 ± 0.40^{A}	14.00 ± 0.89^{C}	0.20 ± 0.03^{B}
132	1,741.27 ± 185.92^{A}	28.94 ± 2.36^{A}	159.62 ± 18.68^{A}	117.34 ± 12.53^{A}	1.95 ± 0.43^{B}	10.76 ± 0.76^{D}	0.11 ± 0.02^{C}

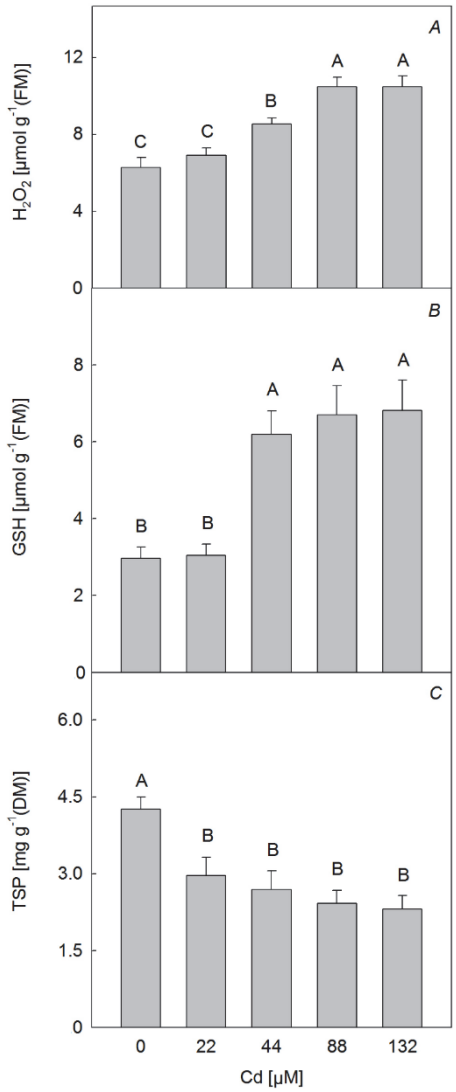

Fig. 1. *A*: Hydrogen peroxide (H_2O_2), *B*: total glutathione (GSH), and *C*: total soluble proteins (TSP) in young *Cassia alata* plants treated with five cadmium concentrations. *Different letters* to cadmium concentrations in solution indicate significant differences in *Scott-Knott* test (P<0.05). Means ± SD, n = 5.

increase of 99.9% after the treatment with 132 µM Cd was observed, compared to treatments under 88 µM Cd. The BCF in the roots was higher at concentrations of 132 µM Cd, while the BCF in leaves decreased with increases in Cd concentrations in solution (Table 2). Additionally, the TF presented values between 0.27 and 0.11, revealing reductions with rising Cd concentration.

Discussion

The lower DM of all tissues is related to reductions in growth due to interference of Cd in P_N, whereby it becomes a metal toxic to plants even in low quantities (Akhter *et al.* 2012, Wang *et al.* 2013, Su *et al.* 2014).

Modifications of H_2O_2, GSH and TSP: The H_2O_2 contents exhibited significant modifications after the application of Cd showing increases of 9.8, 35.9, 66.9, and 66.9% with concentrations of 22, 44, 88, and 132 µM Cd, respectively, when compared with the control (Fig. 1*A*). For GSH, the concentrations of 22, 44, 88, and 132 µM presented greater enhancements (Fig. 1*B*). TSP showed significant decreases in all treatments by Cd, in comparison with the control treatment (Fig. 1*C*).

Damage to photosynthetic pigments: The Chl and Car exhibited a similar behavior (Fig. 2); Chl *a* contents decreased by 18.7, 19.2, 31.5, and 39.1% after treatments with 22, 44, 88, and 132 µM Cd, respectively, compared with the control (Fig. 2*A*). Chl *b* exhibited reductions, with more severe repercussions with a concentration of 132 µM (Fig. 2*B*). The increase of Cd concentrations in solution produced declines by 19.4, 20.3, 32.7, and 39.7 % in total Chl contents, when compared with the control plants (Fig. 2*C*). The Car suffered reductions after all Cd treatments (Fig. 2*D*).

Gas exchange: The progressive increase in Cd concentrations caused a significant decrease in P_N, which were more intense under 132 µM Cd (Fig. 3*A*). Reductions of E were 2.8, 3.2, 19.3, and 20.6% in the plants treated with 22, 44, 88, and 132 µM Cd (Fig. 3*B*), respectively, when compared with the treatment without Cd. The g_s results showed insignificant increases under 22 and 44 µM Cd and a significant decrease when 88 and 132 µM Cd (Fig. 3*C*) were applied, in comparison with the control plants. The WUE presented a tendency to decrease after treatments with Cd, showing reductions of 26.7, 39.9, 40.8, and 50.8% in plants exposed to 22, 44, 88, and 132 µM Cd, respectively (Fig. 3*D*).

Antioxidant enzyme activities presented similar patterns, with progressive increases. CAT activities were significantly modified by the Cd action, and the concentrations of 22, 44, 88, and 132 µM Cd caused increases of 42.5, 49.1, 96.6, and 130.1% (Fig. 4*A*), respectively, compared with treatment without Cd. For the APX enzyme, the results indicated strong increases in plants subjected Cd toxicity, being more intense (104%) at concentration of 132 µM Cd (Fig. 4*B*). In relation to the POX, significant interferences were verified at concentrations of 44, 88, and 132 µM Cd (Fig. 4*C*), when compared to the control.

Growth reduction is frequently seen in plants exposed to Cd, since this metal interferes in CO_2 fixation, resulting in lower P_N. Concomitantly, Cd affects the photosynthetic apparatus, membrane permeability (Fan *et al.* 2011,

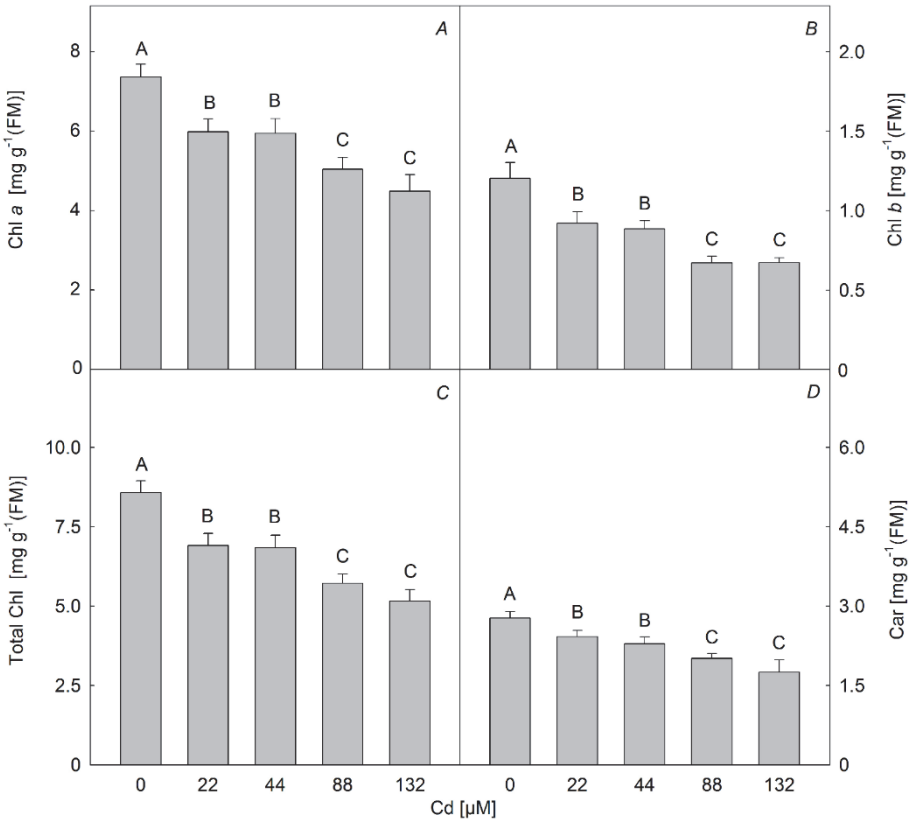

Fig. 2. *A*: Chlorophyll (Chl) *a*, *B*: Chl *b, C*: total Chl, and *D*: carotenoids (Car) in young *Cassia alata* plants treated with five cadmium concentrations. *Different letters* to Cd concentrations in solution indicate significant differences in *Scott-Knott* test ($P<0.05$). Means ± SD, $n = 5$.

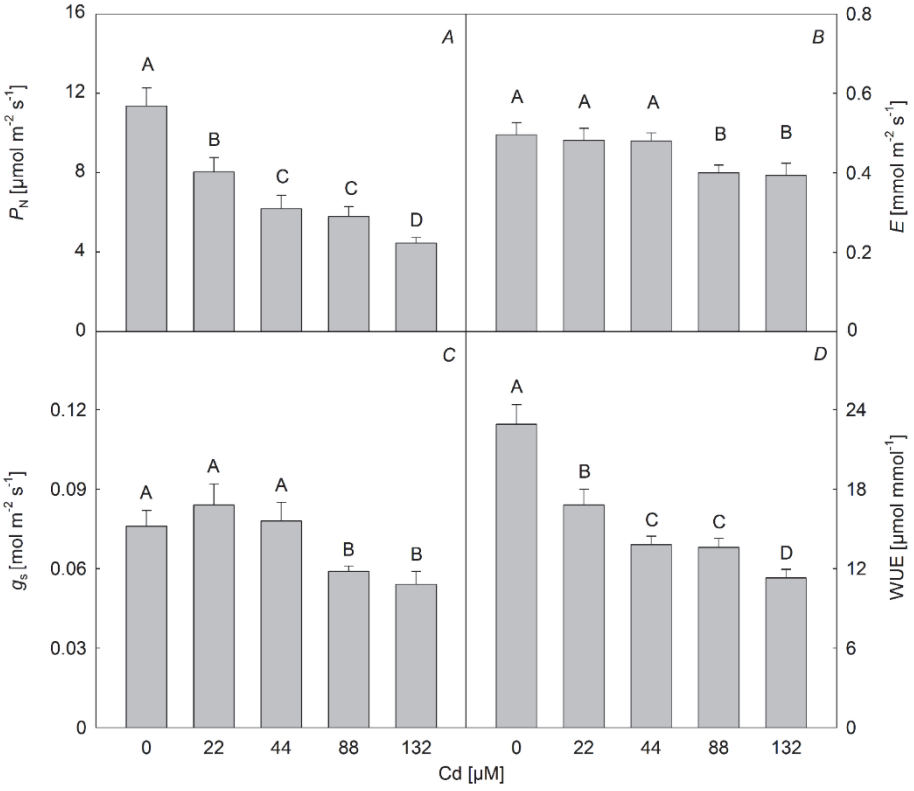

Fig. 3. *A*: Net photosynthetic rate (P_N), *B*: transpiration rate (*E*), *C*: stomatal conductance (g_s), and *D*: water-use efficiency (WUE) in young *Cassia alata* plants treated with five cadmium concentrations. *Different letters* to cadmium concentrations in solution indicate significant differences in *Scott-Knott* test ($P<0.05$). Means ± SD, $n = 5$.

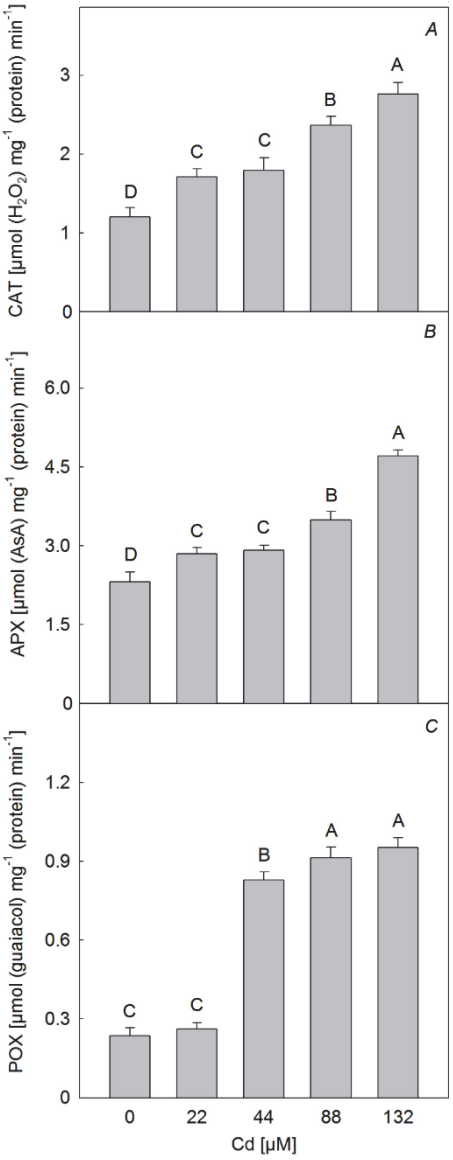

Fig. 4. *A*: Catalase (CAT), *B*: ascorbate peroxidase (APX), and *C*: guaiacol peroxidase activities (POX) in young *Cassia alata* plants treated with cadmium concentrations. *Different letters* to cadmium concentrations in solution indicate significant differences in *Scott-Knott* test (P<0.05). Means ± SD, n = 5.

Fernández *et al.* 2013, Deng *et al.* 2014), inducing lower development of the root system, which limits the capacity of absorption and transport of nutrients (Hartwig *et al.* 2007). Additionally, the survival of *C. alata* plants under different Cd concentrations, mainly under 132 µM Cd, suggested that this species is moderately tolerant to Cd (Fan *et al.* 2011).

A study conducted with *Dittrichia viscosa* clones subjected to Cd revealed decreases in plant dry matter and negative consequences for P_N, which were explained by the alteration in membrane permeability produced by Cd (Fernández *et al.* 2013). Fan *et al.* (2011) working with *Swietenia macrophylla* seedlings verified a decline in the rate of relative growth induced by increases in Cd concentration in solution. Cd toxicity in *Ricinus communis* and *B. juncea* caused reductions in fresh and dry matter (Bauddh and Singh 2012).

The higher CTC in the roots indicates that the stem and leaves are plant parts more sensitive to Cd, since oxidative stress frequently manifested itself in both tissues. However, the values are considered high, when compared to other species, indicating a greater tolerance to this metal in *C. alata*. The CTC in the shoots observed in *Eucalyptus maculata* and *Eucalyptus urophylla* were 2.4 and 1.5 µM Cd (Soares *et al.* 2005), lower than the values observed for *C. alata*, although the CTC found for *C. alata* was higher than the CTC estimated by Paiva *et al.* (2000) in *Cedrella fissilis*.

The $CL_{50\%}$ in leaves was higher than that in roots, suggesting that the tolerance to Cd toxicity was induced by the compartmentalization of metal ions into the vacuole in leaves (Liang *et al.* 2006, Wu *et al.* 2013). Independently of the tissue evaluated, the $CL_{50\%}$ was high, when compared to other species considered less sensitive to Cd, such as *Zea mays* (Macnicol and Beckett 1985). In *Zea mays* plants, 30% reduction was found in shoot dry matter with 8.7 mg dm^{-3} Cd available in the soil, equivalent to 77.39 µM Cd (Cunha *et al.* 2008), while the concentration needed to achieve the $CL_{50\%}$ in leaves and stem of *C. alata* were 201.48 and 130.49 µM Cd, respectively, indicating higher tolerance to Cd. Soares *et al.* (2005) found that the $CL_{50\%}$ in *E. maculata* was 14.5 mg(Cd) kg^{-1} in leaf dry matter, and the value found in *C. alata* was 118% higher, reinforcing the hypothesis that *C. alata* is more tolerant to Cd.

The high Cd concentration in the roots indicated that this species performed reduced translocation of this metal to other plant organs. This defense strategy suggests that the plant absorbs and accumulates Cd in the roots with the objective of reducing transportation to the shoots, and it, consequently, improves tolerance to Cd (Dai *et al.* 2011, Fan *et al.* 2011). Other fact related to increase in Cd concentration in roots is linked to decrease in E, reducing the Cd flux from roots to shoots due to Cd being translocated by the apoplastic route (Liu *et al.* 2010, Sterckeman *et al.* 2011). Furthermore, increases in Cd concentrations in the roots and solution probably promote negative effects on photosynthetic apparatus and respiration, because Cd transportation is dependent on ATP (Fernández *et al.* 2013).

C. alata is a plant considered a Cd-hyperaccumulator, because our results showed that Cd concentration in the shoot exceeded 100 mg kg^{-1}(DM) (Krämer 2010) and it presents BCF > 1 (Fan *et al.* 2011). Furthermore, this plant is a phytoextractor and phytostabilizer due to BCF values in the roots being higher than those found in the stem and the leaves. The lower TF can be explained by Cd adsorption at the root surface (Fernandes 2006). TF is dependent on species and Cd concentration in solutions which plants are exposed to (Sterckeman *et al.* 2011).

Results similar to those described by Deng *et al.* (2014) were found in relation to TF in *C. pteridoides* seedlings treated with Cd. On the other hand, Lu *et al.* (2013), working with five *Arachis hypogaea* cultivars, showed that the Cd concentration in the roots and shoots increased as a function of increasing the Cd in a solution. However, TF varied between cultivars. Studies of *Lactuca sativa* and *Hordeum vulgare* treated with Cd revealed that about 90% of this metal was found in the roots (Akhter *et al.* 2012).

The Cd concentrations induced progressive increase in GSH and initial increase of H_2O_2, responses linked to oxidative stress caused by Cd in *C. alata*. Frequently, plants, when exposed to inadequate conditions, present disbalance between production and scavenging of ROS, as there is an overproduction of O_2^- with consequent formation of H_2O_2 (Cho and Seo 2005, Hsu and Kao 2007) owing to a reaction catalyzed by the superoxide dismutase (SOD) enzyme (Gill and Tuteja 2010). Nakamura *et al.* (2013) described how roots treated with GSH showed lower Cd transport to the shoots. In another study, Pietrini *et al.* (2003) working with leaves of *Phragmites australis* under Cd stress reported an increase in antioxidant activity associated with large reserves of GSH, as well as Xiang *et al.* (2001) who suggested that higher concentrations of GSH contribute to minimizing the stress caused by Cd. Cadmium also promoted reductions in TSP of *Ricinus communis* and *B. juncea*, the effect being more intense in *B. juncea* (Bauddh and Singh 2012).

Chl and Car decreased as a result of the action of ROS generated by Cd intoxication. H_2O_2 is a form of ROS produced and frequently studied owing to the large quantity accumulated in plant tissue in situations of oxidative stress. Concomitantly, these reductions in photosynthetic pigments contribute to minor light absorption and interfere negatively in water photolysis and photosynthesis (Ekmekçi *et al.* 2008, Deng *et al.* 2014). Studies on *Pisum sativum*, *Vigna mungo,* and *B. juncea* plants also presented decreases in Chl and Car contents when plants were exposed to Cd (Singh *et al.* 2008, Hattab *et al.* 2009, Mohamed *et al.* 2012).

A reduction in P_N was caused by Cd accumulation in the leaves. Cadmium inhibits several enzymes of the photosynthetic apparatus, such as Rubisco (Parmar *et al.* 2013). This decrease in P_N and consequently in dry matter is related to stomatal limitation. In order to minimize the damage caused by Cd, the plants reduced *E* to limit Cd transporting from the roots to shoots (Liu *et al.* 2010, Gratão *et al.* 2015). In general, reduced *E* were found in *Lonicera japonica* (Jia *et al.* 2015) and *Lycopersicon esculentum* (Degl'Innocenti *et al.* 2014), being explained by the high temperature and humidity during the experiment.

The activity increases in antioxidant enzymes (CAT, APX, and POX) are related to protection of the metabolic machinery and membrane stabilization with the aim to avoid oxidative damage and consequent inefficient functioning of the cell. Our results contributed to explaining the survival and development of *C. alata* plants in soils with high Cd concentrations. Similar results in the behavior of antioxidant enzymes were obtained by Hasan *et al.* (2011) with *Solanum lycopersicum* plants. In *B. juncea*, the higher activities of antioxidant enzymes promoted a better detoxification process and consequent tolerance to oxidative stress induced by Cd (Mohamed *et al.* 2012). CAT and APX are two enzymes with their roles linked to the detoxification of ROS, such as H_2O_2 (Bhatt and Tripathi 2011), and CAT is extremely efficient in removing H_2O_2 (Sharma *et al.* 2012). Hasan *et al.* (2008) also found increases in CAT activity in *Cicer arietinum* when menaced by Cd toxicity. A study using *Arabidopsis thaliana* with differential tolerance to Cd revealed that CAT activity was higher in tolerant plants (Cho and Seo 2005). Similar results showing increases in APX activity were described by Singh *et al.* (2008) who evaluated *V. mungo* plants treated with Cd concentrations.

Conclusion: Our results suggest that *C. alata* plants can be used in phytoremediation programs. Their tolerance mechanism are based on Cd accumulation in the roots, coupled with increase in glutathione, and the efficient activities of antioxidant enzymes, which contribute to minimize oxidative stress and consequently improve the protection of the metabolic machinery.

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