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Anatomical characteristics and nutrient uptake and distribution associated with the Cd-phytoremediation capacity of *Eucalyptus camaldulenses* Dehnh

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Abstract

Cadmium (Cd) is a hazardous heavy metal whose concentrations have been increasing in Brazilian soils, largely due to mining activities. Eucalyptus species are widely planted in Brazil to produce raw materials, and the confirmation of their phytoremediation potential would link their economic and environmental roles. We examined the Cd-tolerance of *Eucaliptus camaldulenses* Dehnh and the anatomical and physiological features associated with that capacity. Plants were grown under greenhouse conditions in nutrient solutions with increasing concentrations of Cd (0, 15, 25, 45, 90 µmol m^{-3}). Shoot biomass production was less sensitive to the phytotoxic effects of cadmium than root biomass production due to low Cd transport rates from roots to shoots. Increases in epidermal and endodermal thickness, changes in the vascular conductive elements of the roots, as well as differential nutrient distributions between roots and shoots are features of Cd tolerance in this species. The Cd tolerance of *E. camaldulenses* and its high biomass production support its potential use in Cd phytoremediation programs.

Keywords: heavy metal; mineral nutrition; phytotoxicity; root anatomy; Cd-uptake.

1. Introduction

Phytoremediation is an environmentally friendly technique and often represents the most cost-effective treatment for metal-polluted soils – especially in cases of extensive pollution (Dary *et al*., 2010). The use of woody species in phytoremediation programs has been increasing, and a better understanding of the biological processes of heavy metal absorption, translocation, and accumulation, tolerance mechanisms, and phytotoxicity symptoms could improve this already useful technology.

Heavy metal contamination and its consequences for environmental quality and safety are now well-know global problems (Sharma and Agrawal, 2006). Among the hazardous metals, cadmium (Cd) is of particular concern because of its high toxicity to living organisms; Cd has no known biological function but shows high mobility in the soil – leading to Cd accumulation by plants and thus in the entire food chain (Yang, *et al*., 1996). In Brazil, particularly in Minas Gerais State (MG), background Cd concentrations in soils are increasing, principally due to mining and agricultural activities.

Uptake of heavy metals from soils depends on their concentrations, bioavailability, presence of organic matter, pH, redox potential, temperature, fertilizer cations, and other factors (Bernal *et al*., 2009). Much is already known about the bioaccumulation, toxicity, and Cd-adsorption by plants (Marchial *et al*., 1996; Sharma and Agrawal, 2006). Cd competition with macro- and micronutrients such as phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and nickel (Ni) for the same transmembrane carrier proteins (Rivetta *et al*., 1997) has been reported, but little information is available concerning how Cd affects nutrient acquisition and distribution within woody perennial plants. Cd provokes changes in both the physiological and morphological characteristics of plants, reducing growth and affecting their assimilatory capacity, water balance, and provoking structural changes (Lux *et al*., 2004; Wójcik *et al*., 2005) as well as disturbances of nutrient uptake and distribution (Sarwar *et al*., 2010).

Due to growing demands for raw materials such as paper, cellulose, charcoal and wood, plantings of exotic species such as eucalyptus have increased in Brazil. Their rapid growth, well-developed root systems and widespread distributions in Brazil give species such as *Eucalyptus maculata* Hook. and *E. urophilla* S. T. Blake (that are reportedly heavy metal tolerant) significant potential for use in restoration programs of contaminated soils (Soares *et al*., 2005).

In this context, the present study investigated Cd-tolerance in *E. camaldulenses* as a promising species for Cd-reclamation programs, the physiological and anatomical features associated with Cd uptake and bioaccumulation, as well as the mineral nutrition of this species.

2. Material and methods

2.1 Plant growth

E. camaldulenses seeds were acquired from the Seed Commercial Production Area of Açailandia (Maranhão, Brazil) and germinated in Styrofoam trays in a thermostat-controlled and darkened chamber (70% relative humidity, at 25°C). Forty-five-day-old seedlings having similar sizes and weights were transferred

to plastic beakers (6 m^{-3} capacity, four plants per beaker) containing Clark's nutrient solution (2.53 mmol Ca m^{-3}; 7.26 mmol N-NO_3 m^{-3}; 0.9 mmol N-NH_4^+ m^{-3}; 1.80 mmol K m^{-3}; 0.7 mmol Cl m^{-3}; 0.07 mmol P m^{-3}; 0.6 mmol Mg m^{-3}; 0.5 mmol S m^{-3}; 7 mmol Mn m^{-3}; 19 mmol B m^{-3}; 2 mmol Zn m^{-3}; 0.6 mmol Mo m^{-3}; 0.5 mmol Cu m^{-3}; 38 mmol Fe m^{-3}, Clark, 1975) and maintained under greenhouse conditions (temperature 15-31 °C; average photosynthetic photon flux density 825 µmol m^{-2} s^{-1}). After an additional growth period of 15 days, Cd was added (as $CdSO_4$) at different concentrations (0, 15, 25, 45 and 90 µmol m^{-3}) to the culture media of plants selected for their apparent vigor. The nutrient solutions were continuously aerated and renewed weekly. The pH of the medium was checked and adjusted on a daily basis to 5.5 $\pm$ 0.1. After 20 days of Cd-treatment, the plants were harvested and divided into root and shoot fractions, placed in paper bags and dried in an air circulation oven at 70 °C to a constant weight to determine biomass production.

2.2 Chemical analyses

Plant samples (0.1 g) were digested in 5 ml of a strong acid solution (HNO_3/$HClO_4$, 3:1, v v^{-1}) (Silva, 1999) and their Ca, Cd, Cu, K, Fe, Mg, Mn, and Zn concentrations were determined using flame atomic absorption spectrometry (Perkin-Elmer AAnalyst 400, Norwalk, CT). Nitrogen was determined by titration after Kjeldahl digestion; sulfur by turbidimetry (Malavolta *et al*., 1989); and phosphorous concentrations in solution were measured by colorimetry following the phosphorus-molybdate method.

The mineral contents (Cd and nutrients) of shoots, roots, and whole plants were calculated considering dry weight (DW) productions (element concentration x dry matter production). The primary transport index (PTI) was calculated as the ratio of mineral contents in the shoots to those of the roots (Moral *et al*., 1994). The Cd-critical dose solution values of CDS_{10} and CDS_{50} (Cd-concentrations of solutions that promoted decreases of 10% and 50% in plant yields respectively) and Cd-toxicity levels (TCL: Cd-concentrations in shoots that promoted 10% decreases in shoot yields) were assessed. To study Cd uptake and bioconcentration behavior, the following indices were used: bioconcentration factor (BCF), calculated by dividing the Cd-concentration in plant tissues (mg g^{-1} DW) at harvest by the metal concentration in the solution (Sharma and Agrawal, 2006); total accumulation rate (TAR, mg $plant^{-1}$ d^{-1}) was determined following Zhu *et al*. (1999) calculated by (root Cd content + shoot Cd content)/(total dry matter x T), where T is the experimental period (20 days).

2.3 Light microscopy

Anatomical analyses were performed at the end of the experimental period (20 days after initiating the Cd-treatment) to verify possible tolerance features induced by Cd that allowed plants to survive. Root samples were collected and fixed for 48 hrs in Karnovsky's fixative solution (2.5 % glutaraldehyde and 2.5% paraformaldehyde), dehydrated in a graded ethanol series, and included in butanol/plastic resin (Historesin, Laica). Semi-thin sections (5 to 8 mm thick) were prepared using a Jung AG rotary microtome and stained with toluidine blue (equal volumes of 0.3% of basic toluidine and 1% sodium tetraborate). All slides were examined and photographed using a Ken-a-Vision TT18 light microscope equipped with a Canon Power Shot A620 digital camera. Measurements of the anatomical characteristics were made using Sigma Scan software. The items assessed in the root system included: thick-

ness of the epidermis and endodermis, and the numbers and diameters of the tracheary elements. The evaluation of Cd effects on hydraulic limitations considered the relationship between the diameters and numbers of root tracheary elements (Carlquist vulnerability index - CVI) according to Carlquist (1975) (CVI = vessel diameter/vessel frequency). A minimum of five samples were tested for each Cd treatment.

2.4 Statistical analyses

The results are expressed as the averages of five replicates. The data were statistically evaluated using analysis of variance run on the SAS software program (SAS Institute Ins., 1996). Regression and correlation analyses were also performed to test for relationships between the variables. The percentage data relating to the mineral element contents of Cd-treated plants were divided by the concentrations of the same mineral in the control plants and arc-sine transformed before being submitted to Scott-Knott testing at a 5% probability level.

3. Results

3.1 Plant growth and visual symptoms

Dry weight (DW) production was negatively affected by Cd concentrations (Figure 1). Cd had inhibiting effects on growth up to concentrations of 45 μmol m^{-3} Cd; at higher concentrations, dry biomass production of both shoots and roots showed only slight changes. The Cd-critical dose solution (Cd-CDS_{10}) concentration for shoots was 6.6 μmol m^{-3}, and 5.5 μmol m^{-3} for roots. CDS_{50} was 71.24 μmol m^{-3} for roots; 50% of shoot-yield reductions were not observed. The Cd-toxicity level (TCL) in the shoot was approximately 32 mg g^{-1} DW ($y = 1.2567 - 0.0042x$; $r^2 = 0.72$).

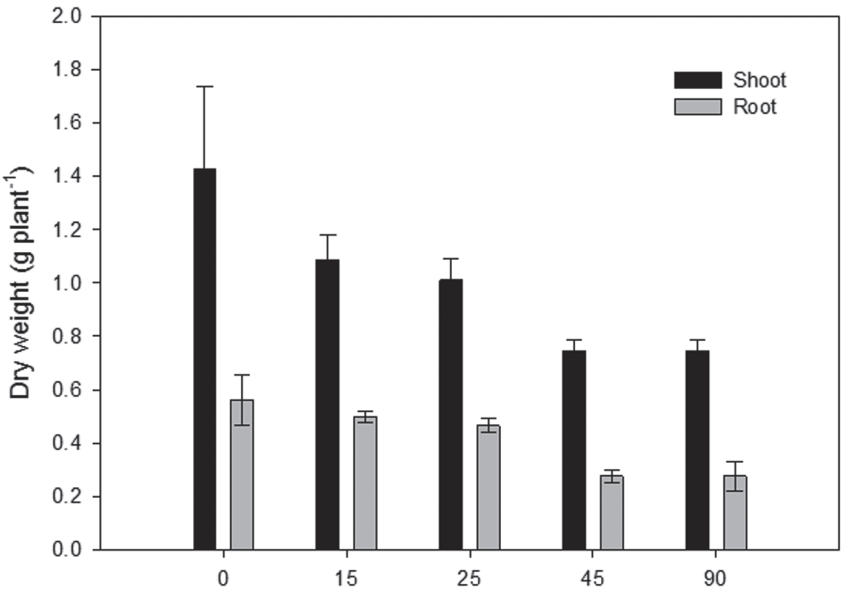

Figure 1. Shoot and root dry weight (DW) production of *E. camaldulenses* plants cultivated in nutritive solution with added 0, 15, 25, 45 and 90 μmol Cd m^{-3}. Each point is the mean of five measurements.

Plants growing in the solutions with the highest Cd concentrations showed symptoms of Cd phytotoxicity; at the end of the experimental period the plants appeared wilted and had yellowed leaves as well as blackened and thickened roots.

3.2 Cadmium content, uptake, and bioaccumulation

Except in the controls, the Cd contents of roots were higher than those of the shoots (Table 1). The greatest increases in Cd content were observed in plants growing in the highest Cd concentrations (Table 1). Although plants grown in 45 μmol m^{-3} Cd showed significant increases in both their root (+450%) and shoot (+350%) Cd-contents as compared to the control, when compared to the others Cd treatments these plants demonstrated the lowest increases in Cd contents of their tissues (p<0.05) (Table 2). The increment on total content of Cd per plant over the control did not differ in plants of treatments ranging from 15 to 25 mmol m^{-3} Cd and was lowest in 45 mmol m^{-3} Cd (p<0.05) (Table 2).

Table 1. Shoot, root, and total plant Cd content, primary transport index (PTI), shoot and root bioconcentration factors (BCF), and total accumulation rate (TAR), in *Eucalyptus camaldulenses* plants cultivated in nutritive solution with added 0, 15, 25, 45 and 90 μmol Cd m^{-3}.

	Cd concentrations (μmol m^{-3})				
	0	**15**	**25**	**45**	**90**
Shoot Cd-content (mg $shoot^{-1}$)	14.0d	77.6b	84.4b	63.2c	126.0a
Root Cd-content (mg $root^{-1}$)	0.6c	353.8b	381.5b	250.9b	530.9a
Total Cd-content (mg $plant^{-1}$)	14.6d	431.4b	465.9b	314.1c	656.9a
Cd PTI	24.8a	0.2b	0.2b	0.25b	0.3b
Shoot BCF	*	0.4a	0.3b	0.1c	0.2c
Root BCF	*	0.4a	0.3b	0.1c	0.2c
TAR (μg d^{-1})	0.4c	13.6b	15.8b	15.3b	31.8a

Different letters within the same line indicate significant differences after arc-sine transformation (Scott-Knott, p<0.05). *Not applicable

Control plants had low Cd contents in their tissues, but with higher Cd contents in the shoots than the roots (Table 1). The Cd primary transport index (PTI) did not differ among the Cd-treated plants, and was higher in control plants (Table 1). The bioconcentration factors (BCF) of both shoots and roots decreased up to 45 μmol Cd m^{-3}. Total accumulation rate (TAR) was higher in the presence of Cd, and increased in plants exposed to highest Cd concentrations (Table 1).

3.3 Nutrient contents

Shoot, root, and total plant mineral element contents are shown in Table 2. The ratios of the element contents in each Cd treatment in relation to the control were calculated (in percentage) for shoots, roots, and total plants (Table 1).

Table 2. Shoot, root, and total plant content of mineral elements in *Eucalyptus camaldulenses* plants cultivated in nutritive solution with added 0, 15, 25, 45 and 90 µmol Cd m^{-3}.

	Cd levels (µmol m^{-3})				
	0	**15**	**25**	**45**	**90**
Shoot					
N (mg $shoot^{-1}$)	58.7	-34d	-54c	-65b	-72a
P (mg $shoot^{-1}$)	4.5	+6a	-32b	-48c	-45d
K (mg $shoot^{-1}$)	21.8	-38a	-46b	-65c	-51c
Ca (mg $shoot^{-1}$)	15.5	+2a	-6b	-35c	-26b
Mg (mg $shoot^{-1}$)	3.6	-14a	-24a	-38b	-37b
S (mg $shoot^{-1}$)	1.0	+66a	+71b	+11c	-61d
Cd (µg $shoot^{-1}$)	14.0	+453b	+501b	+350c	+798a
Fe (µg $shoot^{-1}$)	348.5	-68a	-76b	-81c	-82c
Zn (µg $shoot^{-1}$)	79.9	-45a	-61b	-66b	-73b
Cu (µg $shoot^{-1}$)	17.0	-71a	-85b	-85b	-86c
Mn (µg $shoot^{-1}$)	217.3	-26a	-47b	-57b	-58b
Roots					
N (mg $root^{-1}$)	14.0	-11.2a	-7.0a	-51.7b	-49.8b
P (mg $root^{-1}$)	1.0	+34.3b	+116.3a	+27.9b	+21.9b
K (mg $root^{-1}$)	12.9	-12.1a	-25.7b	-53.7c	-60.0c
Ca (mg $root^{-1}$)	4.8	-8.7a	-6.8a	-44.5b	-47.4b
Mg (mg $root^{-1}$)	1.5	-14.8b	+10.3a	-45.1c	-39.4c
S (mg $root^{-1}$)	1.0	+62.0a	+32.7a	-27.3b	-51.6b
Cd (µg $root^{-1}$)	0.6	+59972.4b	+644670.7b	+42496.4b	+90023.5a
Fe (mg $root^{-1}$)	1.0	+8.6b	+73.6a	+11.8b	-18.3b
Zn (µg $root^{-1}$)	75.5	-15.3a	-22.4a	+49.4b	-45.1b
Cu (µg $root^{-1}$)	15.4	+53.5b	+116.0a	+25.9c	+30.4c
Mn (µg $root^{-1}$)	29.75	-72.44b	-65.79a	-79.16b	-74.3b
Total					
N (mg $plant^{-1}$)	72.7	-28.6a	-45.0b	-62.5c	-68.4d
P (mg $plant^{-1}$)	5.5	+11.6a	-4.7b	-34.4c	-32.6c
K (mg $plant^{-1}$)	34.6	-28.8a	-39.0b	-60.9c	-54.4c
Ca (mg $plant^{-1}$)	20.3	-0.3a	-6.8b	-37.8c	-31.7c
Mg (mg $plant^{-1}$)	5.1	-14.5a	-14.7a	-40.7b	-38.7b
S (mg $plant^{-1}$)	2.0	+64.8a	+52.9a	-7.1b	-56.9b
Cd (µg $plant^{-1}$)	14.6	+2852.1b	+3088.0b	+2049.7c	+4395.0a
Fe (mg $plant^{-1}$)	1.4	-10.8a	+35.3a	-11.8b	-34.7c
Zn (µg $plant^{-1}$)	155.4	-30.8a	-42.6b	-58.0b	-59.5c
Cu (µg $plant^{-1}$)	32.4	-12.0b	+10.5a	-32.6c	-31.0c
Mn (µg $plant^{-1}$)	247.0	-32.0a	-49.8b	-59.8c	-60.7c

Data for treatments with 15, 25, 45 and 90 µmol Cd m^{-3} represent the percent changes (+, increase; -, decrease) in relation to the control. Different letters within the same line indicate significant differences after arc-sine transformation (Scott-Knott, $p<0.05$).

Decreased shoot contents of N, P, K, Ca, Mg, Fe, Zn, Cu, and Mn were observed in Cd-treated plants. Root contents of N, K, Ca, Zn, and Mn decreased and P and Cu contents increased in the presence of Cd. Root Mg content increased at 25 μmol m^{-3} Cd and decreased in relation to the control in the other Cd treatments; S content increased up to 25 μmol m^{-3} Cd and decreased at higher Cd concentrations. Increasing root Fe contents at Cd treatments up to 45 μmol m^{-3} were followed by decreases in plants exposed to the highest Cd treatment.

The primary transport index of each nutrient is shown in Table 3. The PTI of N, P, Zn, and Cu were reduced in relation to the control, while that of Mn increased in the presence of Cd. The PTI of K decreased at Cd concentrations up to 45 μmol m^{-3}, and increased at the highest Cd concentrations; the inverse was seen with S. The PTI of Ca increased above control levels in Cd-treated plants, except under 25 μmol m^{-3}. The PTI of Mg decreased at 15 and 25 μmol m^{-3} Cd, and increased at higher Cd concentrations.

Table 3. Primary transport index (PTI) of mineral nutrients in *Eucalyptus camaldulenses* plants cultivated in nutritive solution with added 0, 15, 25, 45 and 90 μmol Cd m^{-3}.

PTI	Cd levels (μmol m^{-3})				
	0	**15**	**25**	**45**	**90**
N	4.31	-32.7b	-52.0d	-27.4a	-45.9c
P	4.42	-20.3a	-68.8b	-60.0b	-53.9b
K	1.73	-31.0b	-30.2b	-25.9b	+24.4a
Ca	3,35	+10.2b	-1.8b	+12.4b	+43.2a
Mg	2.55	-0.3	-33.1	+10.9	+9.9ns
S	1.08	+0.3c	+27.9b	+62.0a	-1.0c
Cd	24.84	-99.1	-99.1	-99.0	-98.9ns
Fe	0.35	-70.6	-86.6	-83.8	-78.8ns
Zn	1.07	-36.3a	-50.3b	-33.3b	-50.2b
Cu	1.17	-82.8a	-93.6b	-89.2b	-90.2c
Mn	7.55	+159.8b	+52.0a	+98.6b	+57.0b

Data for treatments with 15, 25, 45 and 90 μmol Cd m^{-3} represent the percent changes (+, increase; -, decrease) in relation to the control. Different letters within the same line indicate significant differences after arc-sine transformation (Scott-Knott, $p<0.05$. ns – not significant).

3.4 Anatomical features

Quantitative anatomical changes were observed in root tissues in response to Cd exposure. Root epidermal and endodermal thicknesses increased as Cd treatment doses increased (Figure 2A). Moreover, these tissues showed thickened cell walls. The relationships between endodermal and epidermal thicknesses and shoot and root Cd contents are presented in Figure 2B. This data suggests a possible role of the endodermis in limiting Cd translocation to shoots at Cd-exposure levels above ~19 μmol m^{-3}, as well as an additional epidermal contribution observed principally at Cd-exposure levels above ~49 μmol m^{-3}; above these exposure levels, the slope of the curve relating shoot Cd content and Cd treatments deceased (Figure 2a), further corroborating this hypothesis. The vascular cylinders of Cd-treat-

ed roots showed small xylem vessels with thickened cell walls, but they did not differ in numbers from the controls (P>0.05). The Carlquist vulnerability index (CVI) therefore decreased as Cd treatment doses increased (Figure 3). An inverse relationship between endodermal thickness and CVI was observed on exposure to increasing Cd concentrations (Figure 3).

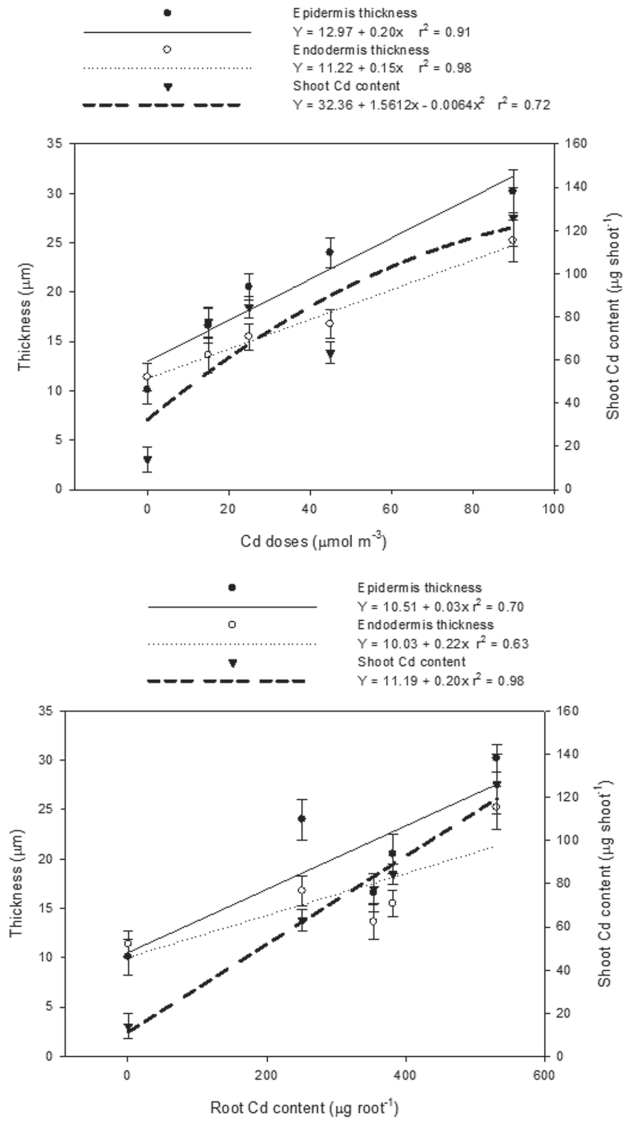

Figure 2. (A) Relationships between epidermal and endodermal thicknesses, shoot Cd contents, root Cd contents, and substrate Cd concentrations; (B) relationships between epidermal and endodermal thicknesses, shoot Cd contents, and root Cd content. Each point is the mean of five measurements.

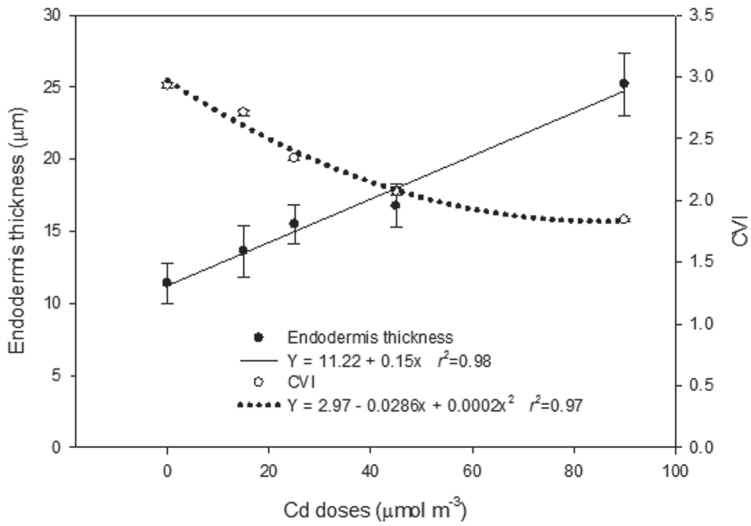

Figure 3. Relationships between endodermal thickness/Carlquist vulnerability index (CVI) and substrate Cd concentrations in Eucalyptus camaldulenses plants cultivated in nutritive solution with added 0, 15, 25, 45 and 90 µmol Cd m^{-3}.

4. Discussion

Our results demonstrated that Cd exposure provoked deleterious effects on *E. camaldulenses* plants, including growth reductions and visual symptoms of toxicity. Similar phytotoxic symptoms were reported by Soares *et al*. (2005) in *E. maculata* and *E. urophilla* grown in nutrient solutions with increasing Cd concentrations (mainly at treatments of 90 µmol m^{-3} Cd and higher). These symptoms may have been associated with multiple deficiencies of several nutrients essential for the formation, expansion, and operation of chloroplasts and with Cd-phytotoxic effects on the extensibility or synthesis of cell wall material (Barceló *et al*., 1990).

Cd phytotoxicity was noticeable in the roots, which showed greater decreases in biomass production than the shoots (Figure 1). In addition to coming directly in contact with substrate Cd, roots showed higher Cd contents than shoots (Table 1) and were presumably more subject to toxic heavy metal effects. The CDS_{10} of *E. camaldulenses* shoots was higher (6.6 µmol m^{-3}) than that reported by Soares *et al*. (2005) for *E. maculata* (2.4 µmol m^{-3}) or *E. urophylla* (1.5 mmol m^{-3}). Additionally, 50% reductions in shoot yields in these species were seen at 63.1 and 54.4 µmol m^{-3}, which was not observed in the present study. However, while the CDS_{10} of the roots (5.5 µmol m^{-3}) was lower in *E. camaldulenses* than in *E maculata* (CDS_{10} = 9.0 µmol m-3) and *E. urophylla* (CDS_{10} = 6.4), its CDS_{50} (71.24 µmol m^{-3}) was higher than either species (CDS_{50} = 62.8 µmol m^{-3} and CDS_{50} = 44.7 µmol m^{-3} respectively), demonstrating the wide variations in Cd-responses in different species and plant tissues. Likewise, Paiva *et al*. (2000) found higher shoot CDS_{10} in the native Brazilian species *Cedrela fissilis* Vell. and *Tabebuia impetiginosa* (Mart. Ex DC.) Standl. (6.7 and 49.8 µmol m^{-3} respectively). According to Marchial *et al*. (1996), the differences in the behaviors of different species maybe due to ge-

netic or physiological features such as the existence of blockers in roots promoting Cd allocation to the cell walls and apoplastic structures. The above-mentioned concentrations of Cd that inhibited growth exceeded the concentrations of soluble Cd found in the vast majority of contaminated soils.

In addition to the relatively small decreases in shoot DW production, the Cd-tolerance of *E. camaldulenses* could be seen in the elevated TCL of the species (32 mg g^{-1} DW). The TCL of *E. camaldulenses* was also higher than that reported by Soares *et al*. (2005) for *E. maculata* and *E. urophylla* (14.5 and 10.8 mg g^{-1} DW respectively). In species that are highly sensitive to this heavy metal, TCL ranged from 5 to 10 mg g^{-1} Cd (Macnicol and Beckett, 1985), supporting the observed Cd-tolerance of *E. camaldulenses*. When plants are grown on contaminated substrates, Cd is most likely to be concentrated in the roots (Kabatta-Pendias, 2000), and when the Cd concentration in the growth medium increases, the concentrations of this metal in the roots can exceed its shoot content by more than a factor of 100 (Kabatta-Pendias, 2000). Although root growth was negatively affected in *E. camaldulenses*, Cd-TAR was not (Table 1). TAR is a measure of heavy metal uptake by plants and this index has been widely used in bioaccumulation studies (Zhu *et al*., 1999). The highest Cd-TAR was seen in plants grown at the highest media Cd concentrations – with the highest Cd contents and lowest Cd-PTIs – demonstrating a positive relationship between Cd media concentrations and the uptake and root retention capacities of *E. camaldulenses*.

One of the mechanisms associated with heavy metal tolerance in plants involves decreasing metal translocation to the shoots (Shi and Cai, 2009), which was seen in the present study. A mean reduction of 99% was seen in the Cd-PTI of Cd-treated plants, showing the preferential retention of this heavy metal in the roots (Table 3). By employing this strategy plants can avoid negative growth effects by reducing heavy metal interference in their photosynthetic processes (including chlorophyll synthesis, chloroplast organization, and PSII activity) (Sandalio *et al*., 2001).

The restrictions of Cd transport to shoots can also be related to morphological and anatomical responses of the plants, as was seen in the roots of Cd-treated plants. Epidermal and endodermal thickening, decreases in xylem vessel diameters, and CVI are all related to Cd-tolerance in *E. camalduenses*. Epidermal thickening increases negative charge accumulation and biological filtering of metal ions (Gomes *et al*., 2011a). Additionally, increasing endodermal and cell wall thicknesses may also reflect heavy metal tolerance mechanisms (Gomes *et al*., 2011a). Cell walls have been found to be one of the most important sites of heavy metal allocation (Wójcik *et al*., 2005) and the endodermis is one the most important accumulation locations in roots (Lux *et al*., 2004; Wójcik *et al*., 2005). As such, cell wall thickening in the root endodermis provides greater areas for Cd retention and decreases its translocation to the shoot (Gomes *et al*., 2011a).

In terms of physiological processes, Cd appears to interfere with the plant water status (Barceló *et al*., 1990). As under salt stress, the development of apoplastic barriers (such as the endodermis) have been correlated with increasing resistance to the radial flow of water and solutes in roots, resulting in the reduced ion uptakes (i.e. Na^+) into shoots – and better survival under subsequent acute stress (Krishnamurthy *et al*., 2011). As a result of decreased water flow and water status, plants may experience interrupted water conductivity in their xylem vessels (Dickson, 2000). As such, morphological changes in the conductive system of plants may represent an additional tolerance mechanism to deal with heavy metal exposure (Lux *et al*., 2004; Gomes *et al*., 2011a), and the inverse relationship between endodermal thickness and CVI appears to support this view. While endodermal thick-

ness will lead to reduced water flow into the xylem vessels, the reductions in vessel diameters (and so CVI) will maintain water conductivity. Moreover, the reductions of tracheal element diameters were related to the increased thicknesses of their cell walls. In addition to ensuring water conductivity (Wójcik *et al*., 2005), the availability of thicker cell walls increases Cd-binding and limits heavy metal translocation to the shoots. Decreases in xylem diameters (as well as their increased cell wall thicknesses) in response to heavy metal exposure was reported in brachiaria grass and willows by Gomes *et al*. (2011a,b). These morphological alterations are related to heavy metal-induced imbalances in phytohormones (Barceló *et al*., 1990).

BAF/BCF is more important than shoot concentrations *per se* when considering the phytoremediation potential of a given species (Zhao *et al*., 2003). The BAC/BCF is typically lower than 1 in metal-excluder species, as seen in the present study (Table 1). The tendency towards decreasing BAC/BCF as substrate metal concentrations increase has been reported in some studies (Zhao *et al*., 2003), indicating a diminishing efficiency of metal accumulation with increasing metal availability (Zhao *et al*., 2003). According to Pence *et al*. (2000), this decrease may be due to the saturation of metal uptake and/or root to shoot transport when internal metal concentrations are high. The lack of any difference between the BCFs ($p<0.05$) at 45 and 90 µmol Cd m^{-3} may be explained by the higher uptake of Cd by plants exposed to 90 mmol Cd m^{-3} but the absence of any difference between their PTIs. This data suggests that Cd-uptake was not restricted or saturated in *E. camaldulenses* at substrate concentrations up to 90 µmol Cd m^{-3}, which supports its use in phytoremediation programs.

A plant's ability to survive in a stressful environment is correlated with its nutritional status, which can be affected by Cd uptake. Cd-induced changes in nutrient contents of eucalyptus species were reported by Soares *et al*. (2005). However, in contrast to the more common practice of evaluating nutrient levels, we evaluated the nutrient contents of the plants and, to assess the Cd effects, compared these values to the controls (Table 3) - thus avoiding dilution effects due to plant growth.

The deleterious effects of Cd on mineral absorption have been attributed to alterations in transpiration rates (Chaffei *et al*., 2004), and growth reductions in roots will likewise directly contribute to reduced mineral uptakes. Changes in N metabolism in Cd-stressed plants are similar to the changes observed during senescence (Masclaux *et al*., 2000), as was confirmed by the visual symptoms of *E. camaldulenses* leaves. Decreased nitrate reductase and nitrite reductase activities reduce nitrate assimilation by plants (Chaffei *et al*., 2004), and decrease photosynthetic rates and the chlorophyll contents of plants (Hernandez *et al*., 1997; Campbell, 1999). Nitrogen translocation from leaves to roots was seen in Cd-treated plants (Chaffei *et al*., 2004), and a positive correlation was observed between root Cd and N contents in 15 and 90 µmol Cd m^{-3} substrate concentrations (r=0.958 and r=0.967 respectively, P=0.05). According to these authors, this strategy may preserve roots as a nutritional safeguard organ to ensure future recovery, and also explain the lower N-PTI seen in Cd-treated plants.

Although N and P contents decreased in Cd-treated plants (as reported by Paiva *et al*., 2000; Soares *et al*., 2005), the contents of these elements remain higher in shoots than in roots. N is an important component of many structural, genetic, and metabolic compounds (Hassan *et al*., 2005) and P is indispensable for energy transfer and protein metabolism. The maintenance of these elements in shoots may contribute to Cd-tolerance and result in biomass production and the lessening of the deleterious effects of this element on shoot biomass. At the highest Cd concentrations, positive correlations were observed between shoot N

content and biomass production (r=0.955, P=0.01) as well as Cd and N/P content (r=0.916 and r=0.931 respectively, P=0.05). Balanced nutrient distributions are important under environmental stress. The PTI of both N and P decreased in the presence of Cd, showing a tendency of their retention in roots where Cd content was becoming higher, and a positive correlation was observed between the N and P contents of roots (r=0.997, P=0.01) as well as between root biomass and N content (r=0.955, P=0.01) with increasing Cd substrate concentrations. Plant growth can decrease the toxic effect of Cd by dilution.

The S contents of shoots were higher in Cd-treated plants than in controls except at the highest Cd concentrations. The S content of roots were also higher in Cd-treated plants than in the controls up to 25 μmol Cd m^{-3}; at higher Cd concentrations root S content decreased (including total S content). The lowest shoot S contents of the highest Cd concentrations may be related to the decreasing S-PTI seen in these plants, in contrast to the others. Gomes *et al*. (2011b) also report decreases in the S-PTI of willow plants grown in heavy metal-contaminated soils; S uptake, however was not affected. Our data, to the contrary, suggested that S uptake was restricted in *E. camaldulenses* plants grown in Cd substrate concentrations at and above 45 μmol Cd m^{-3}. Sulfur is an important component of amino acids and is required for plant growth (Wiedenhoeft, 2006). A positive correlation was observed between S content and biomass production in shoots at Cd concentrations up to 25 μmol m^{-3} (r=0.961, P=0.05) while a negative correlation was seen between both factors at the highest Cd treatment (r=-0.944, P=0.05), reinforcing the importance of S in promoting plant growth. N, P, and S, are also involved in the synthesis of Cd-detoxifying chelator molecules such as gluthatione and phytochelatins (Rosen, 2002) as well as the proliferation of antioxidant systems (i.e. superoxide dismutase, ascorbate peroxidase, and catalase) that can mitigate oxidative stress and prevent membrane damage (Wang *et al*., 2009). The higher S contents of roots at the highest Cd concentrations may be linked to this factor. The effective distribution of these elements may be one of the main physiological features related to Cd tolerance in *E. camaldulenses*.

The K, Ca, and Mg contents decreased in Cd-treated plants but remained higher in shoots than roots. Decreases in total K, Ca, and Mg contents probably indicated competition for bivalent ion binding sites by Cd (Kabata-Pendias, 2000). K is preferentially transported to the shoots and has close relationships with protein synthesis, cytokinin supplies, and plant growth, and also serves as an important cation for counterbalancing anions in plants. The increased K-PTI of plants at the highest Cd treatment indicates that this element was necessary in shoots where the Cd contents were highest, which is supported by the positive correlations between shoot Cd and K contents (r=0.975, P=0.05). Changes in shoot translocation of K and other nutrients have also been attributed to alterations in the vascular system and to reductions in the numbers and diameters of the xylem elements (Ouzounidou, 1994), as were seen here. The positive effects of K on growth in the presence of Cd were particularly visible in the positive correlation of amount of this element with biomass production (r=0.945, P=0.05; r=0.998, P=0.01) and Cd content (r=0.969; r=0.046, P=0.05) in the roots of plants exposed to 45 and 90 μmol Cd m^{-3}. The increased Ca-PTI of Cd-treated plants may be explained by its effects in alleviating Cd toxicity due to the fact high Ca concentrations near ion channels would decrease Cd influx (Sarwar *et al*., 2010) through competition for absorption sites (Clemens *et al*., 1998). As Mg serves as the central atom of the chlorophyll molecule and as a co-factor in many enzymes activating phosphorylation processes (Tu and Ma, 2005), the increased Mg-PTI in plants at the highest Cd concentrations may

ensure the maintenance of chlorophyll biosynthesis and avoid further damage to the photosynthetic system, which is supported by the lesser restrictions of shoot growth.

Micronutrient (Fe, Zn, Cu and Mn) contents were higher in roots than shoots, and Fe and Cu had similar uptake and distribution patterns. Fe and Cu metabolism appear to be associated in plants, and it has been suggested that Fe modulates Cu uptake (Lesuisse and Labbe, 1992). Decreasing micronutrient transport to shoots has been reported upon exposure to Cd (Sandalio *et al*., 2001), and the alleviation of Cd-phytotoxic effects by Fe (Shao *et al*., 2007), Zn (Aravind *et al*., 2009), and Mn (Baszynski et al., 1980) have been reported. Fe is an integral cofactor of antioxidant enzymes such as catalase and ascorbate peroxidase (Sharma et al., 2004), and the high Cd contents seen in roots may lead to increased production of phytochelatins that could sequester Fe as well as Cd. The Fe and Cd contents were positively correlated in roots at both of the highest Cd treatment (r=0.934; r=0,919, p=0.05). Likewise, Zn can reduce the production of free radicals released in response to Cd exposure (Aravind et al., 2009), and Mn is associated with lignin synthesis (Humphries et al., 2007) and therefore with heavy metal retention in the roots (Lux *et al*., 2004; Gomes *et al*., 2011b).

5. Conclusions

The eucalyptus species examined (*E. camaldulenses*) demonstrated tolerance to high soluble Cd-concentrations that was associated with changes in both anatomical and physiological features of these plants. In addition to the high biomass production of this species, it shows significant potential for use in Cd phytoremediation programs.

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