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COMPARATIVE TOLERANCE AND PHYTOSTABILIZATION POTENTIAL OF *Conocarpus erectus* AND *Eucalyptus camaldulensis* GROWN IN CADMIUM CONTAMINATED SOIL

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Phytoremediation is the most promising approach for the remediation of Cd contaminated soils. In the present study, Cd tolerance and phytoremediation potential of *Conocarpus erectus* and *Eucalyptus camaldulensis*, was evaluated in a pot experiment for a growth period of 6 months. Two-month-old plants of uniform size were transplanted in Cd- contaminated soil (0, 5, 10 and 15 mg kg^{-1}), and their growth attributes, chlorophyll contents, root and shoot Cd concentration, bioconcentration factor (BCF) and translocation factor (TF) were determined. With increasing soil Cd levels, shoot and root biomass, leaf water and chlorophyll contents (chl a, chl b and total chl) of *E. camaldulensis* were decreased more than *C. erectus*. Shoot and root Cd concentrations as well as Cd uptake were more in *C. erectus* than *E. camaldulensis*. The TF was less than one for both plant species, while BCF was more than one. It is concluded that due to relatively higher Cd tolerance and greater capacity to retain higher concentration of Cd in roots, *C. erectus* is a better species than *E. camaldulensis* for phytostabilization of Cd contaminated soils.

Keywords: Tree species, cadmium, phytostabilization, chlorophyll contents.

INTRODUCTION

Soil contamination with heavy metals is increasing worldwide due to various anthropogenic activities such as mining, pesticide manufacturing, industrial effluents and waste discharges (Ghosh *et al.,* 2016; Shabir *et al.,* 2017). Amongst various heavy metals, cadmium (Cd) is regarded as the fourth most toxic heavy metal to the higher plants (Ghosh and Singh, 2005) due to its higher mobility in soil-plant system (Kabata-Pendias and Dudka, 1990; Qayyum *et al.,* 2017).

The normal concentration of Cd in uncontaminated soil ranges between 0.1 and 0.5 mg kg^{-1} (Liu *et al.,* 2012), but it can reach up to 3 mg kg^{-1} depending on the soil parent materials (Nazar *et al.,* 2012). In the case of high Cd contamination, concentration up to 8 mg Cd kg^{-1} soil had been reported for some soils whereas soluble levels as low as 0.001 mg kg^{-1} have been found toxic for many plants (Kabata-Pendias and Dudka, 1990). Being a very mobile element, Cd is easily taken up by plants roots (Wang *et al.,* 2016).

The important factors affecting Cd availability in soil include; organic matter, soil pH, hydrous metal oxide content, clay content and type, other metal cations, presence of organic and inorganic ligands and soil salinity (He *et al.,* 2015; Shabir *et al.,* 2017). Plants growing on Cd contaminated soils show limited growth and biomass production due to disturbance in nutrient uptake and water balance, stomatal closure, changes in antioxidant and carbohydrate metabolism, and injuries to the photosynthetic apparatus (Gomes *et al.,* 2012).

The remediation of Cd contaminated soils can be done by various physical, chemical and biological approaches (Pietrini *et al.,* 2015; Agnello *et al.,* 2016; Sarwar *et al.,* 2017). Currently available physical and chemical approaches have many limitations including high cost, rigorous labor, small scale application and disturbance to soil structure (Ali *et al.,* 2013). In contrast, phytoremediation is the cost-effective, solar-driven, efficient, eco-friendly and socially accepted approach for remediation of polluted soils (Bhargava *et al.,* 2012; Ali *et al.,* 2013; He *et al.,* 2015; Sarwar *et al.,* 2017). Phytoremediation is further divided into different categories such as phytoextraction, phytovolatilization, phytostimulation, rhizofilteration, phytotransformation, and phytostabilization (Sarwar *et al.,* 2017). In phytostabilization, the major proportion of metals is accumulated in plant roots, and its further spreading to the surrounding environment is greatly reduced (Pilon-Smits, 2005; Hussain *et al.,* 2017). The mechanisms contributing to immobilization and sequestration of Cd in roots include the chelation of Cd by thiol-containing peptides such as metallothioneins and phytochelatins, Cd

compartmentalization in the vacuoles of root cells, and adsorption to the root cell walls (Nocito *et al.,* 2011; Fine *et al.,* 2013).

Metal uptake and biomass production potential of plants are the main considerations for selecting the plants for phytoremediation purpose (Manousaki *et al.,* 2009; Niazi *et al.,* 2017). Plant species having high metal tolerance and accumulation potential along with high biomass productivity are excellent choice for restoration of contaminated sites (Pulford and Watson, 2003; Pietrini *et al.,* 2015; Shabir *et al.,* 2017). The use of different tree species for phytoremediation of metal polluted soils is regarded as the most promising approach (Pulford and Watson, 2003; Fine *et al.,* 2013). Even though trees accumulate comparatively small quantities of metals, yet they are the most economical type of plants having deep root system and high biomass productivity with very low input. Moreover, they provide economic return from contaminated barren lands, and can remediate marginal quality soils having low fertility and poor structure (Pulford and Watson, 2003; Qados *et al.,* 2015; Pietrini *et al.,* 2015; Abbas *et al.,* 2016).

Phytoremediation potential of different species of Eucalyptus (Fine *et al.,* 2013; Pietrini *et al.,* 2015) and Conocarpus (Qados *et al.,* 2015) has been investigated for Cd in soils under different climates. However, to our knowledge, no study has explored the comparative Cd phytoremediation potential of *Eucalyptus camaldulensis* and *Conocarpus erectus* under similar soil and climatic conditions. Both of these tree species are commonly grown in Pakistan due to their fast growth, high biomass production, tolerance to harsh growth conditions, and adaptability to local climate. The present study has been planned to investigate the comparative Cd tolerance potential of both these species in terms of growth, leaf water contents and pigment contents. Moreover, their comparative phytoremediation potential in a Cd contaminated sandy loam soil has been explored.

MATERIALS AND METHODS

***Site and weather conditions*:** A pot experiment was conducted in a wire house at COMSATS University Islamabad (CUI), Vehari Campus - Pakistan (latitude 30.02º N, longitude 72.21º E) during 2015-2016. The Vehari district consists of plain areas located at an altitude of 446 ft (135 m) with very hot summer (April-August, up to 50 ºC temperature) and a very cold winter (November-January, up to 4 ºC temperature). Average annual rain fall in Vehari is about 127 mm which pours mainly during monsoon season (July-August). Land is semi-arid and dusty due to very little rain around the year.

***Growth conditions*:** The average weather conditions during the experiment were as: sunshine; 8 hours and 24 minutes, minimum temperature; 12.5 ºC, maximum temperature; 28.3 ºC, minimum relative humidity; 46% and maximum relative humidity; 78%.

***Soil and water analyses*:** Surface soil (0-15 cm depth) was collected from the experimental field of CUI-Vehari Campus. The soil was air-dried, ground and passed through a 2-mm sieve for the removal of plant parts, debris and gravels. The soil was analyzed for various physico-chemical properties as shown in Table 1. Hydrometer method (Bouyoucos, 1962) was followed for soil textural class determination using USDA textural triangle. Soil saturated paste was used for pH_s measurement and soil saturation extract was used for electrical conductivity (EC) measurement. Soil organic matter (OM) content was determined by the method of Walkley–Black (Jackson, 1962), and lime ($CaCO_3$) contents were determined as described by Manousaki and Kalogerakis (2009). Soluble cations and anions were measured by titration (Richards, 1954) and used for residual sodium carbonate (RSC) and sodium adsorption ratio (SAR) calculations. Canal water (Table 1) was used to irrigate the plants.

Table1. The characteristics of soil and water used in the experiment

Characteristic	Unit	Value
Soil analysis		
Texture	-	Sandy loam
OM	%	0.36±0.08
Available P	mg kg^{-1}	5.5±0.52
pHs	-	8.11±1.00
EC	dS m^{-1}	1.30±0.06
TSS	$mmol_c$ L^{-1}	13.0±1.50
SAR	$(mmol\ L^{-1})^{1/2}$	4.10±0.45
Cd	mg kg^{-1}	0.30±0.07
Water analysis		
EC	dS m^{-1}	0.21±0.02
TSS	$mmol_c$ L^{-1}	2.10±0.41
SAR	$(mmol\ L^{-1})^{1/2}$	1.91±0.33
RSC	mmol L^{-1}	Nil
Cd	mg kg^{-1}	Not detected

Where OM= organic matter, pHs = pH of saturated soil paste, EC= electrical conductivity, TSS= total soluble salts, SAR= sodium adsorption ratio, RSC= residual sodium carbonate, and Cd= cadmium concentration.

***Experimental set-up and treatments*:** Soil was spiked with different Cd concentrations (0, 5, 10 and 15 mg kg^{-1} soil) using $CdCl_2$ salt and was equilibrated at 70% water holding capacity for four weeks as described by Niazi *et al.* (2011). Soil water holding capacity was measured using SM150T soil moisture sensor. The available Cd concentrations after spiking were determined by DTPA-extractable method (Lindsay and Norvell, 1978). These concentrations were 4.20, 8.50 and 13.41 for 5, 10 and 15 mg kg^{-1} applied soil Cd levels, respectively. The soil was filled in pots at the rate of seven kg per pot. All the pots were arranged in a completely randomized design and each treatment was replicated three

times. Two-month-old uniform plants of *E. camaldulensis* and *C. erectus* were transferred to pots keeping one plant of each species in each pot. All the cultural practices such as irrigation, weeding and hoeing were carried out uniformly in all the pots.

***Plant harvesting and growth measurements*:** The plants were harvested after six months of growth in pots. After harvesting of shoots, the roots were carefully collected from each pot. Adhering soil was removed by thorough washing of roots in tap water. The roots were rinsed in 0.01 M H_2SO_4 for 30 seconds, and thereafter washed with distilled water to remove surface adsorbed Cd. Plant height and root length were measured using a scale. Shoot and root samples were air dried, and then oven dried at 65 °C for 48 hours to record their dry weights. Plant height stress tolerance index (PHSTI), root length stress tolerance index (RLSTI), shoot dry matter stress tolerance index (SDMSTI) and root dry matter stress tolerance index (RDMSTI), were calculated using the following equations as described by Kausar *et al.* (2012).

PHSTI = plant height of stressed plant / plant height of control plant × 100

RLSTI= root length of stressed plant/ root length of control plant× 100

SDMSTI = shoot dry matter of stressed plant / shoot dry matter of control plant × 100

RDMSTI = root dry matter of stressed plant / root dry matter of control plant × 100

***Chlorophyll and leaf water contents*:** At harvest, fully mature leaf samples (0.4 g) were randomly collected from upper part of each plant. Following washing in distilled water, the samples were homogenized in 80% acetone solution using a porcelain mortar and pestle. The extract was centrifuged at 3000 rpm for 10 minutes, and the absorbance of the extract was noted at 663.2, 646.8 and 470 nm against an extraction buffer control using a UV–Vis spectrophotometer (UV-visible Spectrometer Lambda 25/35/45, PerkinElmer, Inc. USA).

The pigment contents (chlorophyll a, b and total chlorophylls a + b) were calculated using the equations described by Lichtenthaler (1987).

Chlorophyll-a = 12.25* Abs 663.2 – 2.798* Abs 646.8

Chlorophyll-b = 21.5* Abs 646.8 – 5.1* Abs 663.2

Total chlorophyll = 7.15* Abs 663.2 + 18.71* Abs 646.8

Fresh weight (FW) and dry weight (DW) of leaves were used for calculating leaf water contents (WC) as given below.

WC= (FW-DW)/ (FW) × 100

***Wet digestion and cadmium determination*:** Root and shoot samples were ground using a grinding machine. One-gram ground plant sample was taken in conical flask and digested following the protocols as described by AOAC (1990) using HNO_3 and $HClO_4$ in 2:1 ratio. Cadmium concentrations in aliquot were determined by atomic absorption spectrophotometer, Perkin Elmer Model: PinAAcle 900F, Inc. USA). Internal standards, reagent blanks and certified reference materials were used for quality assurance and accuracy in Cd determination. Shoot and root Cd uptake was calculated by multiplying the Cd concentration of each part with dry weight of that part.

***Bioconcentration and translocation factors*:** Bioconcentration factor (BCF) and translocation factor (TF) were calculated as described by Marrugo-Negrete *et al.* (2015). The BCF was calculated as the ratio of Cd concentrations in the roots to that in soil. The TF was expressed as the ratio Cd concentration in the shoots to that in roots.

***Statistical analysis*:** A completely randomized design (CRD) with three replicates was used for the analysis of data. Two-way analysis of variance (ANOVA) of the data was carried out (Steel *et al.*, 1997) using statistical software package ''Statistix 8.1''. Comparison of treatments was done by standard error of means and least significant difference (LSD) test at 5% significance level.

RESULTS

***Cadmium tolerance*:** Cadmium tolerance of both tree species was expressed in terms of tolerance indices for various growth attributes. Plant height stress tolerance index (PHSTI) of both tree species was decreased with increasing soil Cd levels (Fig. 1a). The comparison of both species showed that PHSTI of *C. erectus* was decreased from 98% to 83% as the soil Cd concentration was increased from 5 to 15 mg kg^{-1} soil. The respective decrease in PHSTI of *E. camaldulensis* was from 96% to 73% when soil Cd concentration was increased from 5 to 15 mg kg^{-1} soil. Root length stress tolerance index (RLSTI) was also gradually decreased in both species with increasing soil Cd contamination (Fig. 1b). It decreased from 96% to 82% in *C. erectus*, and from 94% to 71% in *E. camaldulensis*, respectively as the soil Cd concentration was increased from 5 to 15 mg kg^{-1} soil. A gradual decrease was observed in shoot dry matter stress tolerance index (SDMSTI) of both species with increasing soil Cd levels (Fig. 1c). It decreased from 95% to 82% in *C. erectus*, and from 93% to 73% in *E. camaldulensis* when soil Cd level was increased from the lower to higher level. Likewise, root dry matter stress tolerance index (RDMSTI) decreased from 96% to 82%, and from 95% to 72% in *C. erectus* and *E. camaldulensis*, respectively, with increasing soil Cd levels from 5 to 15 mg kg^{-1} soil (Fig. 1d).

***Leaf water and chlorophyll contents*:** Leaf water and chlorophyll contents of *C. erectus* were not much affected at various soil Cd levels except for 15 mg kg^{-1} soil Cd level. However, in case of *E. camaldulensis*, both 10 and 15 mg kg^{-1} soil Cd levels caused significant reduction in these attributes as compared to the control treatment. Leaf water contents were decreased by 17% and 22% in *C. erectus* and *E. camaldulensis*, respectively at 15 mg kg^{-1} soil Cd level (Fig. 2a). Chlorophyll contents (chl a, chl b and total chl) were

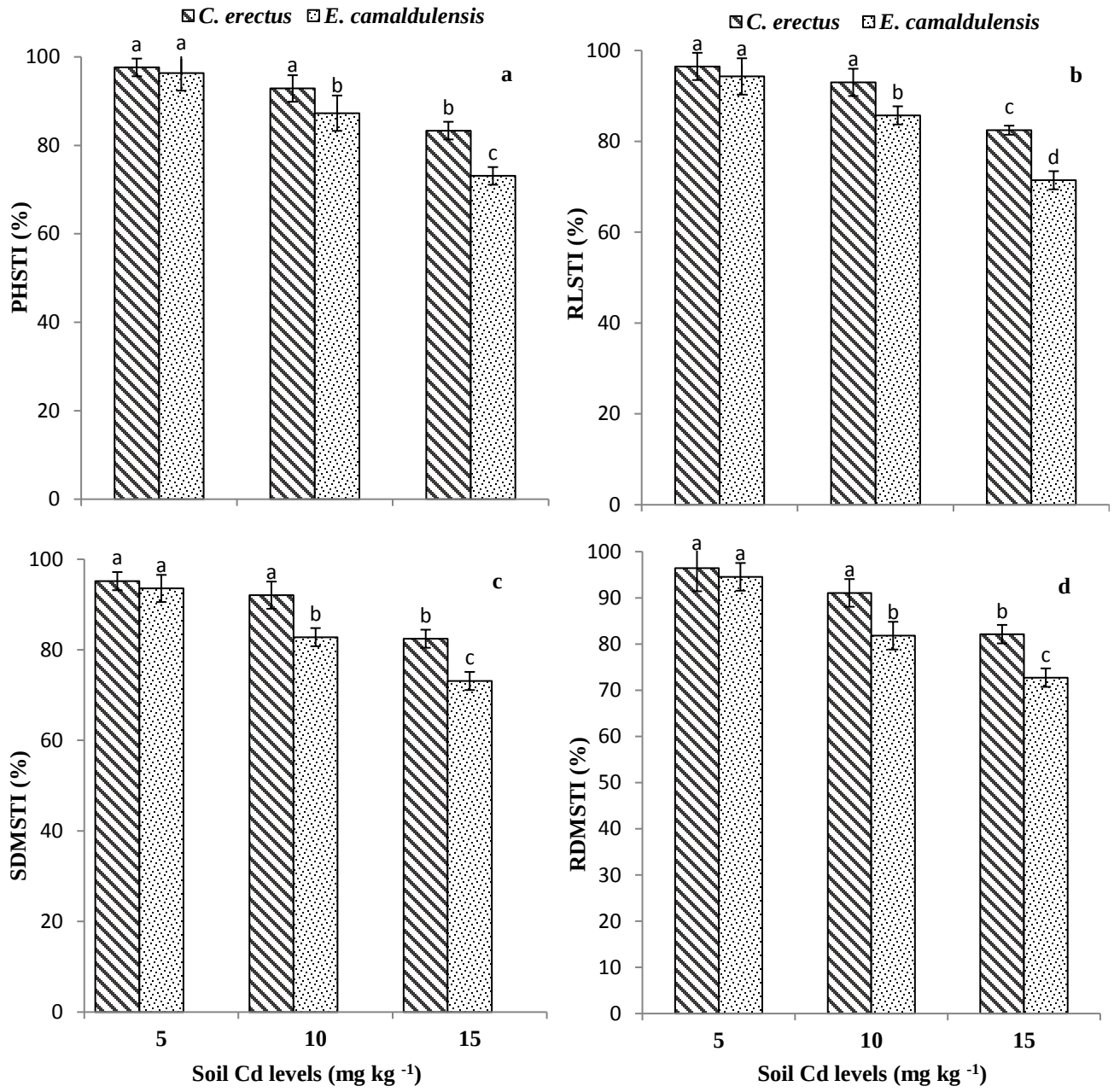

Figure 1. Effects of different soil Cd levels (mg kg^{-1}) on Cd tolerance attributes of *C. erectus* and *E. camaldulensis*. Values are the mean ± standard error of three replicates.

decreased by 16%, 15% and 16% in *C. erectus* and by 27%, 26% and 27% in *E. camaldulensis*, respectively at the highest soil Cd level (15 mg kg^{-1} soil) as compared to respective control treatment (Fig. 2b, c, d).

***Shoot and root Cd concentration and uptake*:** Data regarding Cd concentration showed that shoot and root Cd concentrations in both species were increased with increasing soil Cd levels (Fig. 3a, b). Shoot Cd concentrations were increased from 4.20 to 9.70 in *C. erectus* and from 3.30 to 8.50 mg kg^{-1} in *E. camaldulensis*, as the soil Cd concentration was increased from 5 to 15 mg kg^{-1} soil. Root Cd concentrations were increased from 8.20 to 24.0 and from 6.10 to 17.40 mg kg^{-1} in *C. erectus* and *E. camaldulensis*, respectively with increasing the soil Cd concentration from the lower level (5 mg kg^{-1} soil) to the higher level (15 mg kg^{-1} soil).

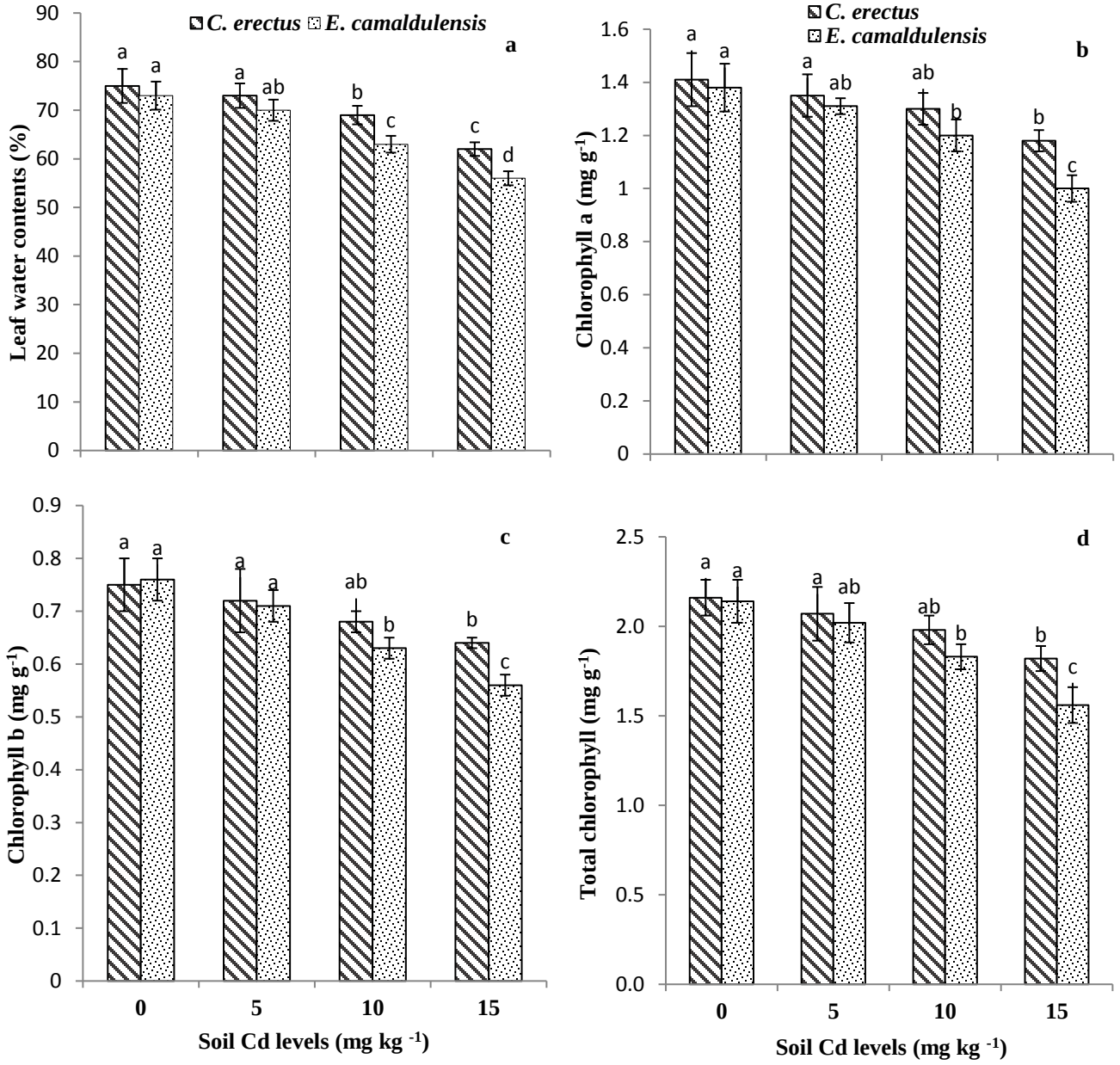

Figure 2. Effects of different soil Cd levels (mg kg^{-1}) on leaf water contents (a) chlorophyll a (b) chlorophyll b (c) and total chlorophyll contents (d) of *C. erectus* and *E. camaldulensis.* Values are the mean ± standard error of three replicates.

Shoot and root Cd uptake (concentration * dry weight) also increased in both species with increasing the soil Cd levels (Fig. 4 a, b). Shoot Cd uptake was increased from 38.6 to 77.3 µg Cd plant^{-1} in *C. erectus* and from 28.7 to 57.8 µg Cd plant^{-1} in *E. camaldulensis* with increasing soil Cd concentration from 5 to 15 mg kg^{-1} soil. Root Cd uptake was increased from 44.3 to 110.4 in *C. erectus* and from 31.7 to 69.6 µg Cd plant^{-1} in *E. camaldulensis* as the soil Cd level was increased from the lower level (5 mg kg^{-1} soil) to the higher level (15 mg kg^{-1} soil).

***Bioconcentration factor* (*BCF*) *and translocation factor* (*TF*):** The values of BCF were greater than one for both the species for all the soil Cd levels (Table 2). The comparison of both species showed that BCF was higher for *C. erectus* than *E. camaldulensis*. Data regarding root to shoot Cd translocation showed that the TF was less than one for both species for all the soil Cd levels (Table 2). Comparison of both

tree species indicated that TF was higher in *E. camaldulensis* than C. *erectus.*

Table 2. Effects of different soil Cd levels (mg kg^{-1}) on translocation factor (TF) and bioconcentration factor (BCF) of *C. erectus* and *E. camaldulensis*

Soil Cd levels	Translocation factor		Bioconcentration factor	
	C. erectus	*E. camaldulensis*	*C. erectus*	*E. camaldulensis*
0	0.42±0.05	0.33±0.04	2.37±0.11	2.67±0.10
5	0.51±0.06	0.54±0.08	1.64±0.10	1.22±0.11
10	0.48±0.04	0.56±0.06	1.56±0.15	1.10±0.10
15	0.40±0.02	0.48±0.08	1.60±0.18	1.16±0.12

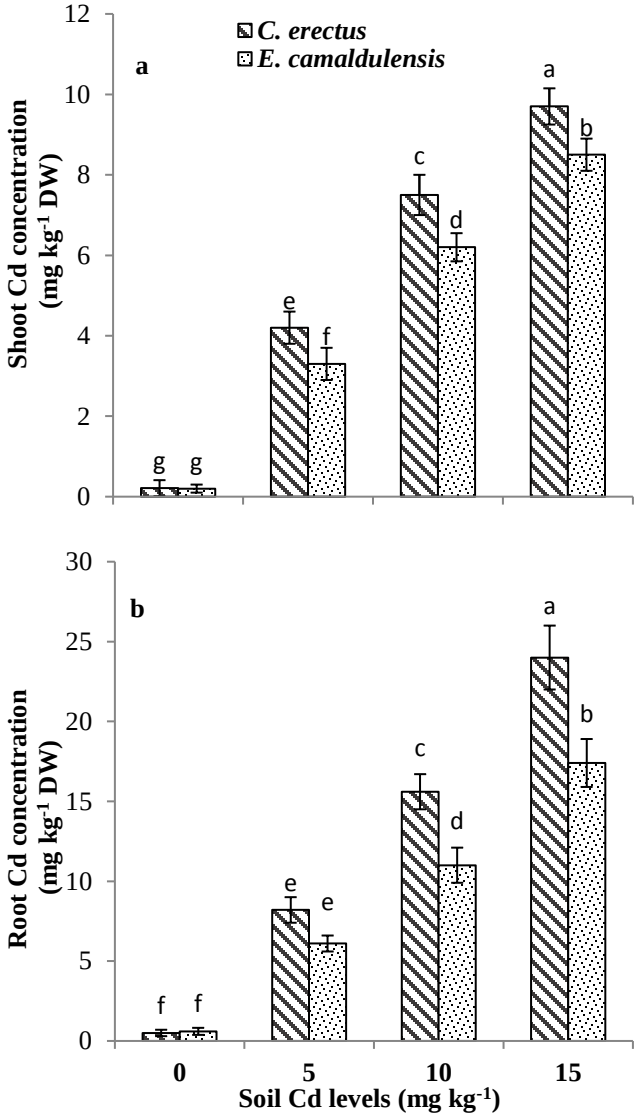

Figure 3. Effects of different soil Cd levels (mg kg^{-1}) on shoot (a) and root (b) Cd concentrations of *C. erectus* and *E. camaldulensis.* Values are the mean ± standard error of three replicates.

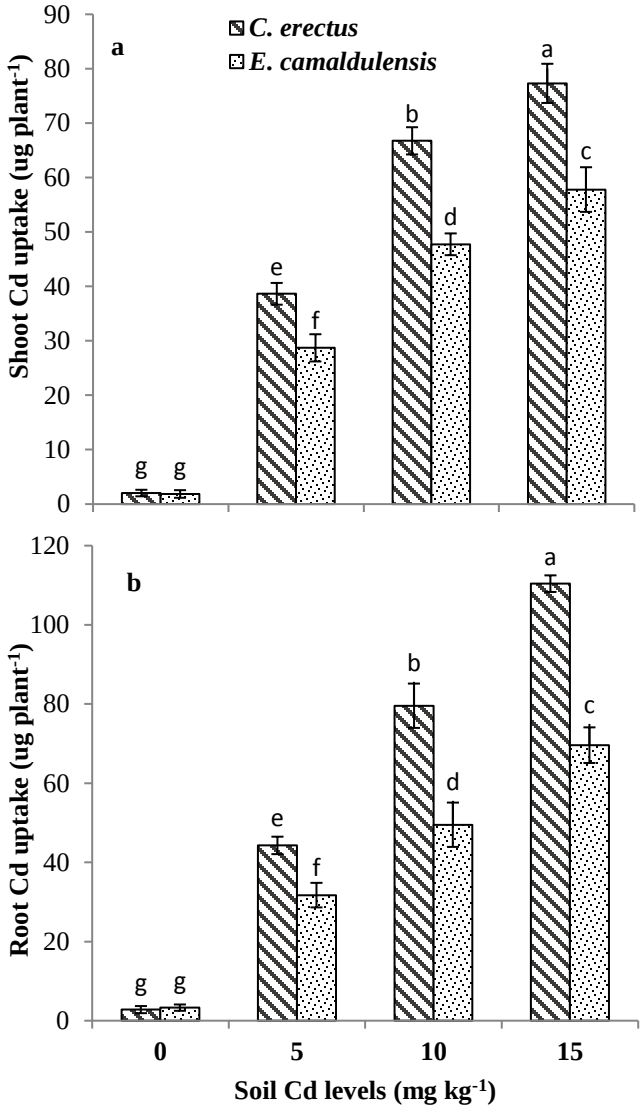

Figure 4. Effects of different soil Cd levels (mg kg^{-1}) on shoot (a) and root (b) Cd uptake of *C. erectus* and *E. camaldulensis.* Values are the mean ± standard error of three replicates.

DISCUSSION

The results of the present study demonstrated that root and shoot growth of both tree species was differently affected by various soil Cd levels. Root and shoot growth of *E. camaldulensis* was reduced at the lower (10 mg kg^{-1} soil) soil Cd level. On the other hand, only the highest soil Cd level (15 mg kg^{-1} soil) caused a noticeable reduction in root growth of *C. erectus* as compared to control treatment. Many previous studies (Wu *et al.,* 2010; Bauddh and Singh, 2012; Abbasi *et al.,* 2015; Hammami *et al.,* 2016) reported reduction in growth of various plant species in response to Cd stress. Contrary to these findings, Manousaki and Kalogerakis

(2009) did not observe any significant effect of Cd on the root growth of *Atriplex halimus* and declared it as a Cd tolerant species. Qados (2015) observed a significant reduction in shoot dry biomass of three tree species at higher soil Cd level (100 mg kg^{-1} soil) as compared to the Cd levels used in the present study. Such differences might be due to different growth conditions (both the soil and climate), and the tree species under consideration.

Growth inhibition, an index of tolerance determination, is a commonly observed response in non-hyper accumulating plants in response to Cd stress (Wu *et al.,* 2010; Pietrini *et al.,* 2015), and is related to Cd induced water and nutrient imbalance, oxidative stress, changes in enzymatic activities, injuries to the photosynthetic apparatus, and disturbance to many other metabolic activities (Gallego *et al.,* 2012; Andresen and Kupper, 2013; Shabir *et al.,* 2017).

Leaf water contents and chlorophyll contents are regarded as important components of metal tolerance of plants, and both of these attributes are generally decreased when plants are exposed to Cd contamination (Liu *et al.,* 2016; Pietrini *et al.,* 2015). We found that *C. erectus* maintained relatively higher leaf water contents than *E. camaldulensis* which is an indication of its higher tolerance than its counterpart. Similarly, Manousaki and Kalogerakis (2009) observed that leaf water contents of *Atriplex halimus* were not much affected by various soil Cd treatments showing its tolerance against Cd stress.

Cadmium is not involved in any known function in plants (Wu *et al.,* 2010). It is easily taken up by plant roots and translocated to shoots where it affects many crucial metabolic activities in different cellular compartments (Prasad, 2004; Pietrini *et al.,* 2015). Due to detrimental effects of Cd to chloroplasts, the pigment contents are reduced (Mobin and Khan, 2007; Pietrini *et al.,* 2010b). We observed that chlorophyll contents (chl a, chl b and total chl) were decreased in both the species, however, the reduction was more in the case of *E. camaldulensis* than *C. erectus* indicating relatively higher tolerance of the later species against Cd stress. Our results are in accordance with the findings of Pietrini *et al.* (2015), who observed that chlorophyll contents of two eucalyptus clones (Viglio and Velino) were reduced when the plants were grown in Cd contaminated water. The clone Velino was regarded as more tolerant than Viglio due to relatively less reduction in its chlorophyll contents.

Shoot and root Cd concentrations and uptake were increased in both the species with increasing Cd levels in the soil. Physicochemical characteristics of the soil (texture, pH, lime contents, organic matter, salinity, form and amount of metal, presence of other metals), agro-climatic conditions of the experimental site, and the plant species have great influence on the bioavailability and uptake of Cd from the soil (Naidu *et al.,* 2003; Bauddh and Singh, 2012; Shahid *et al.,* 2017; Shabir *et al.,* 2017). In our study, about 75% of the total Cd added to the soil was available for plant uptake mainly due to sandy loam texture and very low organic matter content of the soil. We found that root Cd concentration and uptake were much higher than shoot Cd concentration and uptake in both the species, and *C. erectus* showed more Cd concentration in both parts than *E. camadulensis*.

Such preferential accumulation of Cd in the roots of various eucalyptus species has also been reported by other researchers (Bauddh and Singh, 2012; Gomes *et al.,* 2012; Pietrini *et al.,* 2015). The mechanisms contributing to Cd sequestration and immobilization in roots include chelation of Cd by thiol-containing peptides such as metallothioneins and phytochelatins, Cd compartmentalization in root vacuoles, and adsorption to the root cell walls (Nocito *et al.,* 2011; Fine *et al.,* 2013). It has been reported that Cd adsorption to roots mainly contributes to Cd binding in roots (Meighan *et al.,* 2011). In our experiment, the contribution of adsorption to root uptake was eliminated by washing the roots with acid prior to drying. It is important to note that, although Cd concentration achieved in the roots of *C. erectus* was not much higher yet, due to its fast growth and high root biomass production potential, considerable quantity of Cd can be stabilized by growing this species on Cd contaminated soils.

In the present study, BCF was greater than 1.0 for both tree species (Table 2). Michałowski and Gołas (2001) described a four-degree scale for accumulation of metals in plants. According to this scale, BCF less than 0.01, indicates no accumulation, 0.01-0.1 indicates low bioaccumulation, 0.1-1.0 corresponds to medium bioaccumulation and > 1.0 represents a high bioaccumulation. Similarly, according to Negrete *et al.* (2015) and Melgar *et al.* (2009), a BCF value greater than one corresponds to metal accumulating behavior of the plants.

Based on the BCF values obtained in the present study, both tree species showed great potential for Cd accumulation in roots. On the other hand, TF was less than one for both tree species (Table 2), showing less transfer of Cd from roots to shoots. The plant should have BCF and TF greater than one for phytoextraction of metals. The higher BCF and lower TF obtained for both the species indicate their suitability to be used for phytostabilization of Cd in contaminated soils as reported by Tu *et al.* (2003), Fine *et al.* (2013) and Marrugo-Negrete *et al.* (2015). However, due to relatively greater accumulation of Cd in roots and its less transfer to shoots in the case of *C. erectus* indicates that this trees species is more suitable than *E. camadulensis* for phytostabilization of Cd in contaminated soils.

***Conclusions*:** The results of the present study suggest that the effects of Cd on growth and physiological attributes were less pronounced in *C. erectus* than *E. camadulensis.* Both tree species accumulated higher levels of Cd in roots which was indicted by their lower TF values. Less transfer of Cd from root to shoot indicated the phytostabilization behavior of both the species. Due to relatively higher Cd tolerance, and greater

capacity to retain higher concentration of Cd in roots, *C. erectus* is a better choice than *E. camadulensis* for phytostabilzation of Cd contaminated soils.

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