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Impact of cadmium stress on early growth stage of different multipurpose tree species: Insights into sensitivity and tolerance

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Abstract

Cadmium (Cd) contamination is a major problem for plant growth and ecosystem stability. The tolerance of different tree species to Cd is quite different. The present study investigated the reaction of selected tree species (Ziziphus mauritiana, Terminalia arjuna, Melia azedarach, Eucalyptus camaldulensis, Leucaena leucocephala, Tamarix aphylla, Salix tetrasperma, Populus deltoids, Ficus infectoria, Dalbergia sissoo) to various levels of Cd stress (0 mg kg^{-1}, 25 mg kg^{-1}, 50 mg kg^{-1}, and 100 mg kg^{-1}) in terms of growth performance, photosynthetic activity and stomatal conductance to determine their appropriateness for phytoremediation. Most species showed growth inhibition and reduced photosynthetic rate and stomatal conductance due to Cd exposure, although diverse tolerance patterns emerged. Germination and sprouting percentage of E. camaldulensis and S. tetrasperma exhibited a non significant decline till 50 mg kg^{-1} Cd stress but significantly decreased by 67.3% and 51.2% at 100 mg kg^{-1}, respectively. E. camaldulensis and P. deltoides showed relatively high tolerance, while S. tetrasperma showed higher photosynthetic activity (18 µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (0.45 mol H_2O m^{-2} s^{-1}) at 25 mg kg^{-1} Cd stress, respectively. L. leucocephala and F. infectoria, on the other hand, showed high sensitivity to Cd stress, indicating low phytoremediation potential. Findings of the present study suggest the importance of selecting suitable species for use in Cd-contaminated sites and the relevance of using P. deltoides and E. camaldulensis as possible phytoextractors. These findings underscore the need for further molecular and physiological studies to understand reduced Cd resistance in recalcitrant species, thereby facilitating the development of effective phytoremediation strategies.

Keywords: Antioxidant activities; Cd accumulation; multipurpose tree species; phytoremediation; growth response

Introduction

Cadmium (Cd) is one of the heavy metals that are not required for plant growth and development; nevertheless, its presence can lead to toxic effects that are likely to occur even at extremely low concentrations. This is released into the environment mainly through human activities, including emissions from industries, mining activities and the application of phosphate fertilizers saturated with Cd. After entering the soil, Cd readily binds to plant roots due to its high solubility, which affects various physical and chemical functions (Shen *et al.*, 2023). Due to the bioaccumulation of Cd in plants, they experience reduced yield and productivity, affecting agriculture, and it can also pose a potential threat to ecosystems or humans when it moves up the food chain, since it accumulates in the edible parts of plants. Cd has toxic effects on plants in several ways, including altering plant cellular processes and inhibiting essential physiological activities, such as photosynthesis (Kamili, 2018; Li *et al.*, 2023).

Cd toxicity in plants results in the overproduction of ROS (reactive oxygen species), which are highly reactive and cause oxidative damage to various structures and organelles. As a rule, plants have an optimal level of ROS production that serves to execute various signaling processes. The regular cellular life process is impaired in this regard by elevated levels of ROS oxidative stress, due to a considerably high exposure to Cd (Sandalio *et al.*, 2009; Huang *et al.*, 2017). Increased ROS levels contribute more to altered lipid, protein and nucleic acid profiles. This process disrupts cell membranes, leading to the release of cellular components and eventually inducing cell death (Anjum *et al.*, 2015; Juan *et al.*, 2021). Cd-induced oxidative damage has been reported in various crops. Tran and Popova (2013) reported that Cd-treated pea plants exhibited the production of plasma membrane-bound NADPH oxidase in peroxisomes, resulting

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in ROS generation. Similar findings were also reported in Scots pine (*Pinus sylvestris* L.) and alfalfa under 50 mM Cd stress (Schutzendubel *et al.*, 2001; Hattab *et al.*, 2014). Similar changes regarding oxidative damage have also been recorded in soybean, wheat, and pea (Ziaf *et al.*, 2023). Furthermore, changes in the expression of superoxide dismutase, peroxidase, and catalase have been reported in trees, including *Moringa oleifera* (Schutzendubel *et al.*, 2001; Khalofah *et al.*, 2020). The researchers reported a significant decrease in Cd-stressed moringa plants when treated with 5 mM Cd. Keeping in view this scenario there is a dire need to identify the suitable tree species for phytoremediation which can withstand and grow best under Cd contaminated conditions provided with strong antioxidant system. Therefore, this screening study was conducted to evaluate the effect of Cd stress on growth performance of different tree species by examining their morphological and physiological parameters.

Materials and Methods

Experimental layout and settings

This study was conducted under controlled conditions in the wire house of the Experimental Area of the Department of Forestry and Range Management at the University of Agriculture, Faisalabad (UAF), Pakistan. In this experiment, ten multipurpose tree species with good economic returns were selected, including Beri (*Z. mauritiana*), Arjun (*T. arjuna*), Bakain (*M. azedarach*), Sufaida (*E. camaldulensis*), Ipil ipil (*L. leucocephala*), Farash (*T. aphylla*), Willow (*S. tetrasperma*), Poplar (*P. deltoides*), Pilkan (*F. infectoria*), and Sheesham (*D. sissoo*).

The samples of sandy loam textured soil were collected from the nursery site of the Department of Forestry and Range Management at UAF. Samples were dried at room temperature, crushed to a 2 mm mesh size using a wooden roller, sieved, and stored in plastic containers for further analysis. The samples were first analyzed for physicochemical characteristics to establish their initial state before contamination. Three concentrations of Cd solution (25, 50, and 100 mg kg^{-1} as total concentration) were prepared from $CdSO_4 \cdot 8/3H_2O$, and a 0 mg kg^{-1} solution was used as the control. The prepared, sieved soil was irrigated with these Cd solutions and stored for two months. This process helped to achieve homogeneity of Cd throughout the sample matrix, following the protocol devised by Gul *et al.* (2019). This contaminated soil, weighing 600 g, was placed in 22.86 cm × 10 cm plastic bags. The planting material, seeds and cuttings of healthy trees used in the experiments were obtained from the Punjab Forestry Research Institute, Faisalabad and the experimental area of the Department of Forestry, UAF. Depending upon the propagation technique, seeds and cuttings were grown in polythene bags for six months (Febuaury to August). During this experiment, the seedlings were irrigated with pumped groundwater once a day to maintain soil moisture. To control the variability of the independent variables, the experiment was conducted using a completely randomized design (CRD), with both independent variables as factors and three replicates for statistical reliability.

Morphological parameters

Different morphological parameters, such as root length, shoot length, and the fresh and dry weights of the seedlings, were measured at the time of harvest following standard procedures. For calculating the dry biomass, the plant material of each sample was subjected to shade-drying immediately after harvest, followed by forced-air drying in an oven at 70±2 ºC for 72 hours, and the weight of the dry samples was recorded.

Physiological parameters and antioxidant activities

For recording the plant physiological parameters such as photosynthetic rate and stomatal conductance, IRGA – LC4, manufactured by Analytical Development Company of Hudson, UK, was employed to record photosynthetic rate (Bashir *et al.*, 2018) and stomatal conductance by following the protocol described by Long and Bernacchi (2003). Three to four fully expanded leaves per plant were selected and all the measurements were taken between 9 a.m. and 12 a.m. on a clear day.

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were estimated following the protocol devised by Giannopolitis and Ries (1977), based on the enzymes' capacity to suppress NBT photoreduction. SOD activity was determined by the quantity of SOD required for 50% inhibition of NBT reduction of the colored product, and absorbance was recorded at 560 nm using a UVspectrophotometer (UV-4000, O.R.I., Germany).

Catalase (CAT) activity was determined following Chance and Maehly (1955) by measuring the degradation of hydrogen peroxide (H_2O_2) at 240 nm with a UV spectrophotometer (UV-4000, O.R.I., Germany).

Peroxidase (POD) activity was determined by Chance and Maehly (1955). The absorbance was read at 470 nm at 20-second intervals up to 5 minutes in a UV spectrophotometer (UV-4000, O.R.I.). Germany).

For calculating Cd concentration in different plant parts, the samples of dry leaves, shoots, and roots were oven-dried at 60 ºC to a constant weight and ground to pass through a 2 mm sieve. The ground samples (5 g) were processed through a wet digestion procedure that included 10 mL concentrated nitric acid and 5 mL perchloric acid, using the protocol described by Rashid (1986). The presence of Cd (Cd) contents was recorded through an atomic absorption spectrophotometer (Model: Z-8200, Hitachi, Japan).

Statistical analysis

All the data were statistically analyzed using STATISTIX software, version 8.1, USA. Various data related to morphological, growth, physiological parameters, and antioxidant activities were analyzed using two-way analysis of variance. The level of significance was determined at $p < 0.05$. The difference among the mean values was determined by using Tukey's HSD test at $p < 0.05$

Results

Germination percentage

The present study found that *Z. mauritiana* showed a consistent decline in germination as Cd concentration increased, with the percentage dropping from 59.0% at 0 mg kg^{-1} to 35.6% at 100 mg kg^{-1}. Similarly, *M. azedarach* exhibited a gradual decline in germination, decreasing from 60.2% at 0 mg kg^{-1} to 50.5% at 100 mg kg^{-1}. Interestingly, *E. camaldulensis* showed a consistent germination percentage up to 50 mg kg^{-1} Cd stress, which decreased to 67.3% at 100 mg kg^{-1}. *L. leucocephala* followed a declining trend from 80.3% at 0 mg kg^{-1} Cd to 66.1% at 100 mg kg^{-1} Cd (Figure 1a). *T. arjuna* exhibited a marked decrease in germination percentage, from 54.7% at 0 mg kg^{-1} to 42.0% at 100 mg kg^{-1}, showing high sensitivity to Cd exposure.

Sprouting percentage

The results further indicated that sprouting percentage varied significantly among the species (Figure 1b). *S. tetrasperma* displayed a non-significant decline in sprouting percentage till 50 mg kg^{-1} Cd stress, which significantly decreased up to 51.2% when cuttings were subjected to 100 mg kg^{-1} Cd stress. *P. deltoides* experienced a gradual decline in sprouting, from 74.3% at 0 mg kg^{-1} to 53.6% at 100 mg kg^{-1}. At the same time, *F. infectoria* maintained stable sprouting percentages across the Cd concentrations, with only a slight reduction from 56.0% at 0 mg kg^{-1} to 51.7% at 50 mg kg^{-1}, but a significant decline to 44.8% at 100 mg kg^{-1} Cd stress, indicating moderate tolerance to the metal (Figure 1b). *T. aphylla* also showed a steady decline in sprouting, falling from 62.3% at 0 mg kg^{-1} to 51.3% at 100 mg kg^{-1}, indicating its susceptibility to Cd toxicity.

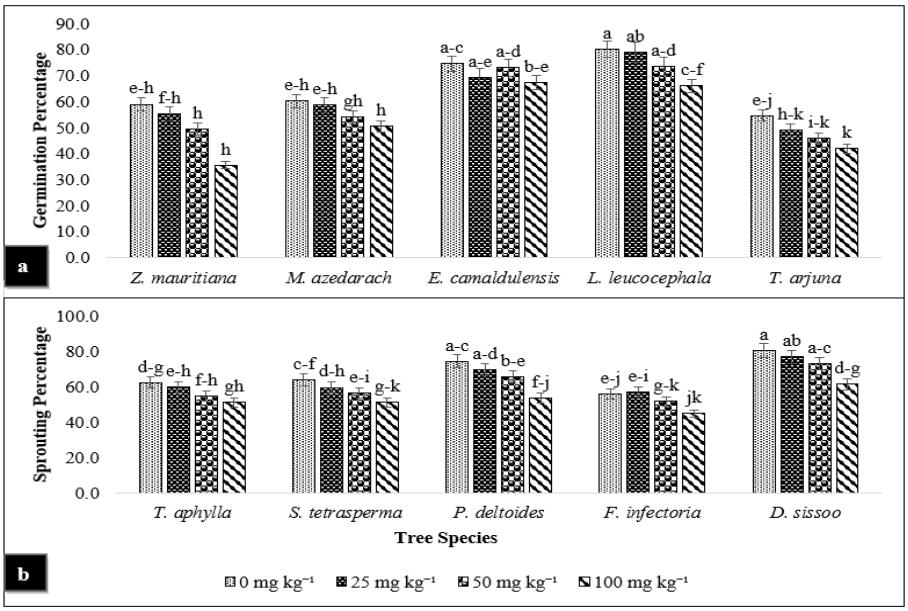

Figure 1: Germination and Sprouting percentage of selective tree species under different concentrations of Cd stress

Growth parameters

Shoot and root growth

In the present study, shoot length showed a significant decreasing trend, with some variation across all species (Figure 2a). *L. leucocephala* showed 201.3 cm shoot length when grown under 0 mg kg^{-1} Cd stress (normal conditions), with a decline up to 116.0 cm under 100 mg kg^{-1} Cd stress. Moreover, *D. sissoo* and *F. infectoria* showed comparatively less decline in exhibiting shoot length, i.e., from 172.0 cm to 137.3 cm and 154.7 cm to 119.0 cm (*D. sissoo* and *F. infectoria*, respectively). However, *P. deltoides* showed an increasing trend in shoot length, indicating higher tolerance to Cd stress. Its shoot length increased from 280.7 cm (at 0

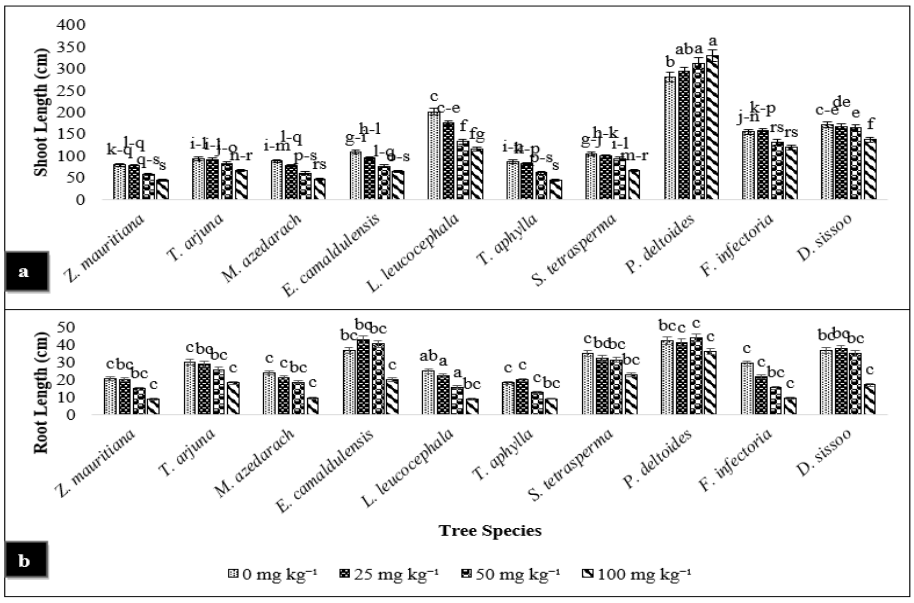

Figure 2: Shoot and Root length of selective tree species under different concentrations of Cd stress

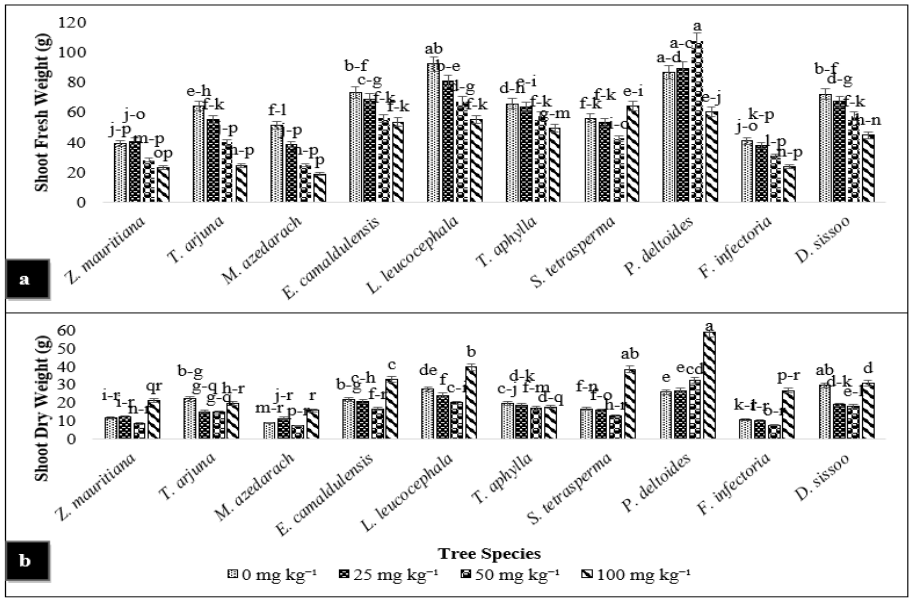

Figure 3: Shoot fresh and dry weight of selective tree species under different concentrations of Cd stress

mg kg⁻¹) to 329.0 cm (at 100 mg kg⁻¹) (Figure 2a). *Z. mauritiana* exhibited a sharp decline in its root length from 20.6 cm to 9.0 cm, *M. azedarach* showed a decline from 23.9 cm to 9.7 cm, *L. leucocephala*'s root length decreased from 24.8 cm to 9.1 cm and *F. infectoria* demonstrated a decreasing trend from 29.3 cm to 9.5 cm in its root length. Moreover, *P. deltoides* exhibited a comparatively higher tolerance, showing a decline from 42.0 cm to 36.0 cm, a 14% decrease (Figure 2b).

Shoot fresh and dry weight

Regarding shoot fresh weight (SFW), *T. arjuna* and *M. azedarach* showed a reduction in SFW, with values of 24.7 g and 18.8 g, respectively, at 100 mg kg⁻¹. However, *E. camaldulensis* and *L. leucocephala* showed relatively higher SFWs at 100 mg kg⁻¹, at 53.6 g and 55.3 g, respectively. Notably, *P. deltoides* grown under moderate Cd stress (50 mg kg⁻¹) scored the highest fresh weight of shoots, at 107.7 g and declined to 60.2 g at 100 mg kg⁻¹ (Figure 3a). Moreover, the maximum SDW among the selected tree species under Cd stress was observed for *E. camaldulensis* (33.0 g), *L. leucocephala* (39.6 g), and *P. deltoides* (59.1 g) at 100 mg kg⁻¹, indicating an adaptive response to Cd stress (Figure 3b).

Root fresh and dry weight

Furthermore, it was found that the root fresh weight (RFW) of *Z. mauritiana* increased from 16.2 g at 0 mg kg⁻¹ to 21.2 g at 100 mg kg⁻¹, while the maximum RFW (23.2 g) was noted at 50 mg kg⁻¹. However, at a concentration of 100 mg kg⁻¹, the RFW slightly declined to 21.2 g, as stated earlier.

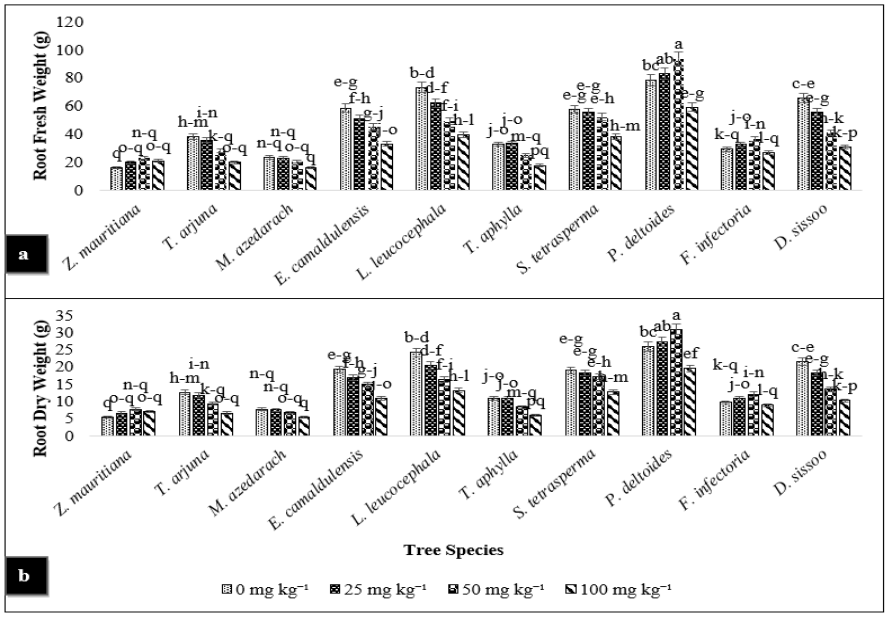

Figure 4: Root fresh and Dry weight of selective tree species under different concentrations of Cd stress

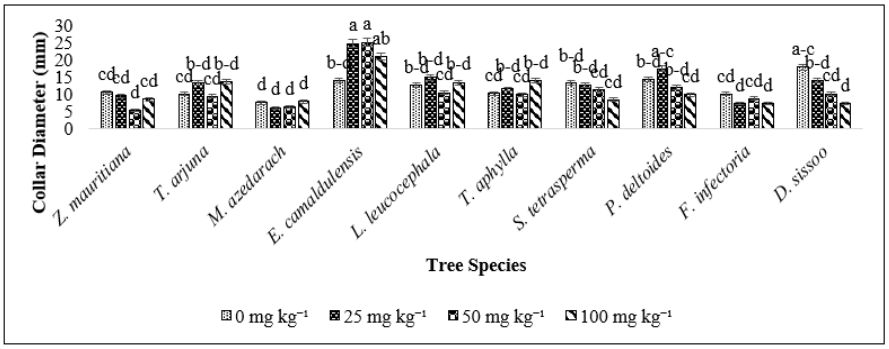

Figure 5: Collar diameter of selective tree species under different concentrations of Cd stress

It was further noted that RFW of *E. camaldulensis* declined from 58.6 g to 33.0 g while *P. deltoides* showed a different trend in exhibiting its RFW. It increased from 78.4 g to 93.6 g at 50 mg kg^{-1} Cd stress, while decreasing to 59.1 g at 100 mg kg^{-1} Cd stress, suggesting a degree of adaptability to moderate Cd exposure (Figure 4a). Root dry weight (RDW) of *T. arjuna* decreased from 12.6 g at 0 mg kg^{-1} to 6.6 g at 100 mg kg^{-1} of Cd. Likewise, RDW of *E. camaldulensis* reduced from 19.3 g to 10.9 g and *L. leucocephala* from 24.2 g to 13.1 g when Cd concentrations were increased. On the other side, *P. deltoides* showed increased RDW from 25.9 g at 0 mg kg^{-1} to 30.9 g at 50 mg kg^{-1}, then dropped to 19.5 g at 100 mg kg^{-1} (Figure 4b). Likewise, a similar trend was noted in the RDW of *F. infectoria*, which increased to 12.1 g at 50 mg kg^{-1} from 9.7 g at 0 mg kg^{-1}, then decreased to 8.9 g at 100 mg kg^{-1} (Figure 4b).

Collar diamter

In terms of collar diameter, *Z. mauritiana* showed a decreasing trend from 10.6 mm at 0 mg kg^{-1} to 9.7 mm at 25 mg kg^{-1} and 5.5 mm at 50 mg kg^{-1}, then increasing to 8.6 mm at 100 mg kg^{-1}. However, *E. camaldulensis* showed the greatest increase in collar diameter at 50 mg kg^{-1}, from 14.0 mm to 25.1 mm, followed by a reduction to 20.9 mm at 100 mg kg^{-1}. Likewise, *S. tetrasperma* and *P. deltoides* showed a decreasing trend where collar diameter decreased from 13.5 mm and 14.4 mm to 8.5 mm and 9.9 mm, respectively, with increasing Cd stress (Figure 5).

Physiological parameters

Photosynthetic rate

E. camaldulensis demonstrated the highest initial photosynthetic rate of 15.9 µmol CO_2 m^{-2} s^{-1} under 0 mg kg^{-1} Cd stress, but this rate declined to 7.7 µmol CO_2 m^{-2} s^{-1} with 100 mg kg^{-1} Cd application, resulting in a 51.6% reduction. Under Cd concentrations increasing from 0 mg kg^{-1} to 100 mg kg^{-1}, *P. deltoides* and *S. tetrasperma* experienced moderate photosynthetic reduction to 56% and 57.67% from initial values, respectively. *L. leucocephala* demonstrated the greatest photosynthesis impairment because its measurement decreased dramatically by 63% from 0 mg kg^{-1} to 100 mg kg^{-1} (Figure 6a).

Stomatal conductance

The highest stomatal conductance value among the tested plants at 0 mg kg^{-1} Cd was observed in *P. deltoides* at 0.50 mol H_2O m^{-2} s^{-1} while *E. camaldulensis* and *S. tetrasperma* had conductance values of 0.31 mol H_2O m^{-2} s^{-1} and 0.53 mol H_2O m^{-2} s^{-1} respectively. The stomatal conductance of *P. deltoides* and *E. camaldulensis* decreased significantly from their original values at 100 mg kg^{-1} Cd,

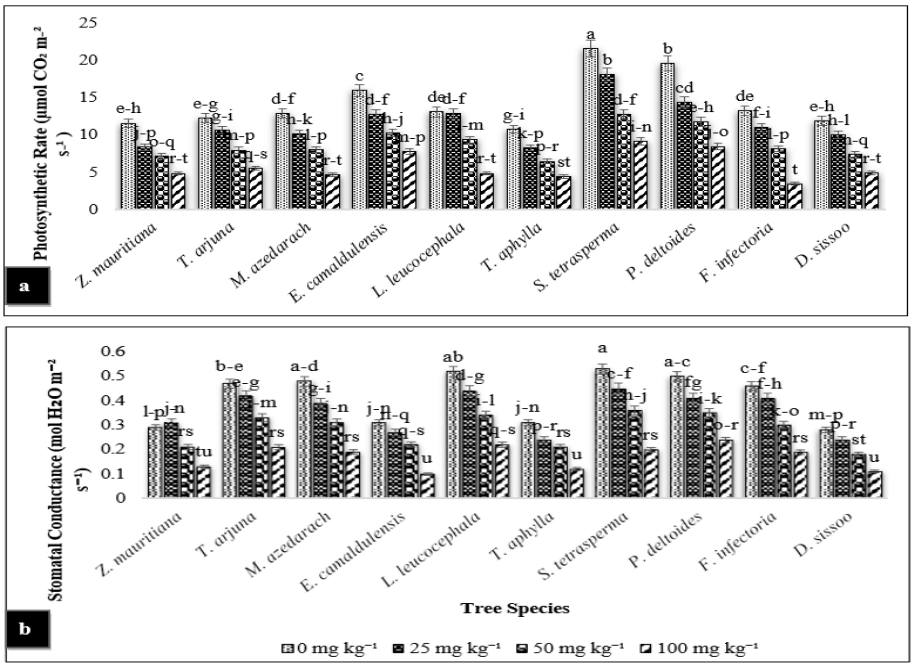

Figure 6: Photosynthetic rate and Stomatal conductance of selective tree species under different concentrations of Cd stress

reaching 0.24 mol H_2O m^{-2} s^{-1} and 0.10 mol H_2O m^{-2} s^{-1}, respectively, resulting in an overall 52–68% reduction. The stomatal conductance value of *F. infectoria* declined severely, resulting in 58% reduction at 100 mg kg^{-1}, indicating a complete stomatal closure that blocked CO_2 access and inhibited photosynthetic activities (Figure 6b).

Cd accumulation in plant parts

Overall, Cd accumulation increased with increasing Cd stress from 25 mg kg^{-1} to 100 mg kg^{-1}, though species-specific variation exists (Figure 7a, 7b). For instance, maximum Cd accumulation (38.84 mg kg^{-1}) was recorded in *P. deltoides* under 100 mg kg^{-1} Cd stress, compared with only

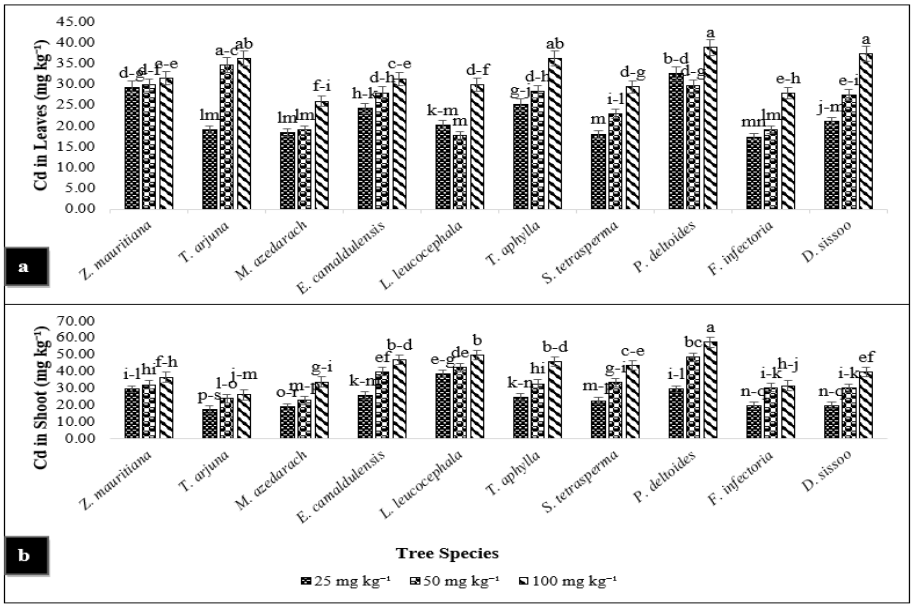

Figure 7: Cd contents (mg kg^{-1}) in the Leaves and Shoot of selective tree species under different concentrations of Cd stress

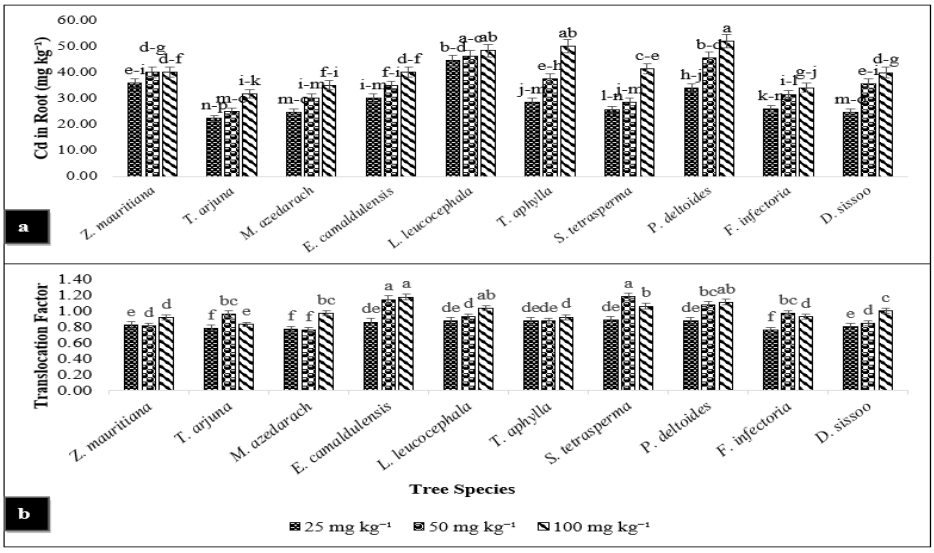

Figure 8: Cd contents (mg kg^{-1}) in the Root and Translocation factor of selective tree species under different concentrations of Cd stress

32.50 mg kg^{-1} under 25 mg kg^{-1} Cd stress. *D. sissoo* was also not an exception. Its leaves accumulated 37.33 mg kg^{-1} Cd, compared with only 21.0 mg kg^{-1} under 25 mg kg^{-1} Cd stress. Moreover, *Z. mauritiana* also exhibited a steady increase in Cd accumulation from 29.20 mg kg^{-1} at 25 mg kg^{-1} exposure to 31.43 mg kg^{-1} at 100 mg kg^{-1} Cd stress (Figure 7a).

It was further recorded that the maximum Cd accumulation (57.41 mg kg^{-1}) was observed in *P. deltoides* shoot under 100 mg kg^{-1} Cd stress, followed by *L. leucocephala* (49.76 mg kg^{-1}) and *E. camaldulensis* (46.87 mg kg^{-1}). The lowest values were recorded in *T. arjuna* and *F. infectoria*, at 26.10 mg kg^{-1} and 31.47 mg kg^{-1}, respectively, under the same Cd stress level (100 mg kg^{-1}), as shown in Figure 7b. It was also observed that *L. leucocephala* and *P. deltoides* contained the most Cd in their roots when exposed to 100 mg kg^{-1} Cd and *P. deltoides* reached 51.87 mg kg^{-1} (Figure 8a).

Translocation factor

A species' Cd translocation factor indicates its capability to transfer Cd from root zones to aboveground plant sections (shoots and leaves). *E. camaldulensis* proved to be the most effective species in translocating Cd from roots to shoots, as its translocation factor reached 1.17 at 100 mg kg^{-1}. The Cd translocation capability of *P. deltoides* and *S. tetrasperma* was strongly based on their TF values being higher than 1 at 50 and 100 mg kg^{-1} concentrations. *F. infectoria* and *T. arjuna* preserved Cd mostly within their roots since these species maintained TF values below 1 (Figure 8b).

Enzymatic antioxidant activities

Superoxide dismutase (SOD)

SOD activity in *P. deltoides* increased with increasing Cd stress up to 50 mg kg^{-1}, from 125.3 to 310.2 unit/mg Protein, but declined slightly at 100 mg kg^{-1} to 247.6 unit/mg Protein. A similar trend was observed in *S. tetrasperma*, for which SOD activity reached a maximum of 264.0 unit/mg Protein at 50 mg kg^{-1}, whereas at 100 mg kg^{-1} the value was 211.8 unit/mg Protein. In case of *D. sissoo* SOD activity increased by 42.2% at 100 mg kg^{-1} compared to control (0mg kg^{-1}). *Z. mauritiana* and *T. arjuna* demonstrated the least SOD activity (Table 1).

Table 1: Enzymatic activity of selective tree species under three levels of Cd stress

Antioxidant Enzymatic activity (unit/mg Protein)												
Tree species	**SOD**				**POD**				**CAT**			
	0 mg kg^{-1}	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}	0 mg kg^{-1}	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}	0 mg kg^{-1}	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}
Z. mauritiana	12 v	26.9 t-v	56.9 s-u	24.3 t-v	424.1 j-m	314.4 no	538.8 f-h	607.3 d-f	13.8 r	41.2 n-p	34.4 pq	43.1 n-p
T. arjuna	17.2 uv	32.1 s-v	62.1 q-s	49.5 r-t	571.4 e-g	375.6 l-n	392.8 k-n	405.3 j-m	22 qr	59.7 j-m	48.6 m-p	38.4 op
M. azedarach	79.4 p-r	111.3 m-o	164.3 h-j	101.7 n-p	455.9 h-l	425.5 j-l	656.9 d	434.8 i-l	78.8 d-h	49 m-o	44.1 n-p	47.3 m-p
E. camaldulensis	128 l-n	160.8 h-k	212.9 d-f	228.5 c-e	389.8 k-n	643.6 de	907.9 a	916.9 a	87.4 a-e	91.6 a-d	54.7 l-n	71.9 f-k
L. leucocephala	88.7 o-q	113.6 m-o	173.6 g-i	151 i-l	141.6 rs	180 p-r	156.1 q-s	54.3 tu	87.4 a-e	101.5 a	74.1 e-j	38.6 op
T. aphylla	103 n-p	137.9 j-m	187.9 f-h	220.5 c-e	587 d-g	423.8 j-m	648.6 de	755.9 c	54.3 l-n	87.4 a-e	80.3 c-h	81.4 b-g
S. tetrasperma	149.1 i-l	174 g-i	264 b	211.8 d-f	90.3 s-u	93.1 s-u	228.3 pq	479.9 h-j	41.3 n-p	75.1 e-i	80.3 c-h	81.4 f-k
P. deltoides	125.3 l-n	170.2 g-i	310.2 a	247.6 bc	434.7 i-l	246.9 op	239.9 o-q	510.7 g-i	60.7 j-m	58.8 k-m	63.8 i-l	67.1 g-l
F. infectoria	50.1 r-t	85 o-q	135 j-m	92.4 o-q	129.1 r-t	103.3 r-u	91.8 s-u	44.8 u	82.1 b-f	66.7 h-l	47.4 m-p	83.5 b-f
D. sissoo	132.3 k-n	197.2 e-g	217.2 c-f	229.8 cd	340.8 mn	466.8 h-k	879.2 ab	797.5 bc	59.2 k-m	95.4 ab	59.8 j-m	94.8 a-c

Peroxidase (POD)

In present investigation, findings indicated that POD activity rose in most of the species where it reached a peak in *E. camaldulensis* from 389.8 unit/mg Protein at 0 mg kg^{-1} to 907.9 unit/mg Protein at 50 mg kg^{-1} which was statistically at par with 100 mg kg^{-1} showing 916.9 unit/mg Protein POD activity. *D. sissoo* also showed a similar trend regarding the expression of POD activities. *P. deltoides* showed a different pattern. At 0 mg kg^{-1} of Cd stress, this activity was recorded at 434.7 unit/mg protein, which reduced to 239.9 unit/mg Protein when the plants were exposed to 50 mg kg^{-1} Cd, while an increase (510.7 unit/mg Protein) was observed when the plants were grown under 100 mg kg^{-1} Cd stress (Table 1).

Catalase (CAT)

Catalase activity was maximally recorded in *D. sissoo, which* was 94.8 unit/mg Protein at 100 mg kg^{-1} Cd stress, followed by *F. infectoria* (83.5 unit/mg Protein), while *M. azedarach* and *L. leucocephala* showed a decreasing pattern in exhibiting CAT activity, which was only 47.3 unit/mg Protein and 38.6 unit/mg Protein, respectively. In the case of *P. deltoides,* a stability in CAT activity was observed. It ranged from 60.7 unit/mg Protein to 67.1 unit/mg Protein (Table 1).

Discussion

The results of the present study revealed a diverse range of responses to Cd stress, including differences in germination and sprouting. *L. leucocephala* and *S. tetrasperma* demonstrated a high degree of tolerance, with their germination and sprouting percentages either increasing or remaining stable under Cd exposure. Conversely, species such as *T. arjuna* and *Z. mauritiana* exhibited a significant decline in both parameters, indicating their sensitivity to Cd. Moreover, growth inhibition was observed at relatively higher levels of Cd stress (100 mg kg^{-1}), whereas at 0 mg kg^{-1} stress all plants grew best; root length of these species reduced dramatically at 100 mg kg^{-1}, especially in *T. arjuna* and *M. azedarach*. This small reduction in root elongation and proliferation reflected their potential to be highly sensitive to Cd toxicity. This pattern was also observed in *E. camaldulensis* and *L. leucocephala*, in which average height growth significantly decreased as Cd stress increased. These findings are in line with those of Nouman *et al.* (2020), who also reported an inhibitory effect of Cd stress on root development. This agreement between current and previous study indicated that cadmium toxicity adversely affects plant growth and development by inhibiting root elongation and proliferation.

Cd stress caused a significant decrease in root and shoot length. These findings are in line with those of Zhang *et al.* (2020), who reported a reduction in root elongation in plants under Cd stress. Likewise, *Z. mauritiana* and *F. infectoria* showed clear reductions in root length. The findings of Nouman *et al.* (2020) supported the observation of *M. oleifera* shoot decrease because they reported that Cd stress negatively affected shoot growth of plant species by impeding cytokinin biosynthesis. The reduction in shoot growth and development might be attributed to suppressing cytokinin biosynthesis. This suggests that cadmium stress might induce inhibitory effect on hormonal regulation showing a key mechanism underlying the decrease in plant growth and development (Prasad, 2022).

The response of *P. deltoides* was distinct because shoot lengths expanded as Cd stress increased. An unusual growth pattern that contradicted traditional Cd-related growth inhibition was observed as *Populus* species demonstrated tolerance to heavy metal stress through multiple factors, including elevated cellular antioxidants and increased metal storage in vacuoles (Lu *et al*. 2021; Pramanik *et al*. 2021; Yi *et al*. 2022). Studies on *Populus* species have shown that these trees are effective for phytoremediation (Chen *et al*., 2016; Liu *et al*., 2020). Thus, *P. deltoides'* ability to tolerate Cd stress suggests its potential as a good candidate for phytoremediation, especially in regions where contamination has occurred and growth can be used to reclaim soil and repair damaged environments.

Higher Cd concentrations led to increased shoot dry weight in *E. camaldulensis*, *S. tetrasperma*, and *P. deltoides,* which can be attributed to enhanced antioxidant enzyme activity similar to results reported by Tőzsér *et al.* (2023) for Cd-tolerant poplar clones. The research conducted by Shah *et al.* (2008) demonstrated that *D. sissoo* biomass decreased due to Cd-induced oxidative stress and photochemical breakdown. The plant species *P. deltoides* and *S. tetrasperma* showed increased biomass allocation to roots and shoots at mid-range of Cd levels, indicating their successful adaptation to the stress conditions. Cd interacts with important elements such as calcium, magnesium, and zinc at the anion site and directly affects the uptake and absorption of these micronutrients, as well as disrupting the body's ionic balance. This, together with the oxidative stress caused by Cd, led to a significant reduction in root and shoot biomass in all species, as observed in several other sensitive species.

Previous studies have reported a decrease in collar diameter in Cd-stressed plant species. Liu *et al.* (2020) reported that higher Cd concentrations in Populus species led to blocked cambial function, resulting in restricted stem expansion. *D. sissoo* also suffered comparatively more than *P. deltoides* when exposed to Cd stress, mainly because of hormonal imbalances combined with lowered photosynthetic capacity,

according to Yi *et al.* (2022). The collar diameters of *E. camaldulensis* and *T. arjuna* increased slightly when exposed to moderate Cd levels, likely through an adaptive cellular division mechanism, according to Feng *et al.* (2021).

According to the research findings, Cd was accumulated mostly in plant roots, as previously established scientific studies demonstrated that roots act as the main heavy metal storage zone. The research by Yi *et al.* (2022) reported that *P. deltoides* and *L. leucocephala* roots accumulated more Cd than shoots and leaves, findings supported by this study. The ability of *P. deltoides*, *L. leucocephala*, and *T. aphylla* to accumulate Cd in roots and shoots suggested their potential for phytostabilization and phytoextraction applications. Similar findings were reported by Yang *et al.* (2024), who reported that *E. camaldulensis* effectively restricted Cd translocation to aerial parts, thereby reducing its toxic impact on photosynthesis and overall growth. Such regulation might include Cd binding and sequestration in root cells to decrease its activity, as well as sequestration in xylem leaf vacuoles to shield metabolic processes (Al-Khayri *et al.* 2023).

The research results supported previous studies documenting the toxic impact of Cd stress, which impairs both photosynthesis and stomatal conductance. Plants contaminated with Cd exhibit reduced photosynthetic efficiency because Cd disrupts multiple physiological processes, including chlorophyll formation, stomatal function, and carbon fixation enzyme activity (Liu *et al.* 2018). All tested tree species showed a decrease in photosynthetic rate as Cd stress increased, consistent with previous research. The photosynthetic inhibition caused by Cd exposure results primarily from chlorophyll breakdown, disrupted electron transport, and altered carbon assimilation pathways (Hasanuzzaman *et al.*, 2017). The concentrations of Cd in *E. camaldulensis* decreased by 51.6%, and L. leucocephala experienced a severe 63.1% decline, confirming previous research by Yang *et al.* (2024) and Zhang *et al.* (2020) on Cd-exposed *Populus* and *Eucalyptus* species.

Very few stomatal openings were detected in F. infectoria, *M. azedarach*, and T. aphylla because Cd concentrations reached 100 mg kg^{-1} and caused severe stress reactions due to hormonal imbalance mediated by disrupted abscisic acid (ABA) signaling, according to Zhang *et al.* (2019). The significant reduction in *P. deltoides* and *E. camaldulensis* suggests that, while these species exhibited some tolerance to Cd, their gas exchange capacity remained compromised at higher Cd levels.

The consequences of Cd stress on antioxidant activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were examined in different tree species. The data showed differential Cd stress, demonstrating differences in species' sensitivity to oxidative stress induced by Cd toxicity. Oxidative stress from Cd exposure becomes less severe because antioxidant enzymes use reactive oxygen species (ROS) for scavenging purposes. The moderate Cd exposure level increased SOD, POD, and CAT enzyme activities in plant tissues, as reported by Hasanuzzaman *et al.* (2017) in their study of wheat and rice plants under Cd stress. The strong initial ROS defense mechanisms of *Z. mauritiana* and *T. arjuna* are evident from their enhanced SOD activity, as this defense strategy is essential for mitigating oxidative cell damage (Shahid *et al.*, 2014). Enzyme activity decreased at 100 mg kg^{-1} Cd in *P. deltoides*, but not in *T. arjuna* and *Z. mauritiana*, indicating that the defensive capabilities could no longer protect against oxidative stress. Studies in *L. leucocephala*, along with other tree species, confirm that Cd stress induces the breakdown of protein synthesis and the inactivation of enzymes (Abbas *et al.*, 2019; Zhao *et al.*, 2020). Literature review shows that higher CAT activity is a typical homeostatic mechanism used by tolerant species to combat oxidative stress (Nouman *et al.,* 2020). However, reduced CAT activity in sensitive species like *M. azedarach* suggests that, beyond certain concentrations, their antioxidant system is highly stressed, leading to cellular dysfunction.

According to the findings presented in this research, various tree species used different mechanisms to counter Cd stress. *P. deltoides* with greater shoot Cd content is suitable for phytoextraction, since mobilizing metals in root zones is crucial for averting the dissemination of metallic elements into the biosphere. On the other hand, *S. tetrasperma and F. infectoria* showed higher Cd accumulation in all tissues, suggesting that these plants are good candidates for a phytoextraction site. The moderate accumulation and balanced translocation strategy observed in *E. camaldulensis* make it a versatile option for applications that require a balance between stabilization and extraction. These findings emphasize the importance of selecting species based on specific remediation goals, as their physiological responses to Cd stress can significantly influence their effectiveness in restoring contaminated environments.

Conclusion

Cd contamination significantly affected the growth and physiology of tree species, with considerable differences between species in tolerance levels. This research suggested that proper species selection is essential to the success of phytoremediation in-Cd contaminated areas. *E. camaldulensis*, *P. deltoides* and *S. tetrasperma* were found to be relatively more suitable species for remediation approaches owing to their greater tolerance to Cd stress. The

phytostabilization technique could use *F. infectoria* and *T. arjuna* plants as they showed efficient root-based cadmium retention for soil-heavy metal control. Moreover, *E. camaldulensis* and *P. deltoides* exhibited both effective metal uptake and strong translocation factors indicating their suitability for phytoextraction purpose. The research also highlighted the need for holistic considerations of species-specific physiological traits, metal uptake mechanisms and ecological suitability when developing phytoremediation approaches for practical applications. It is not enough for remedial species to have good growth, they must also be able to sustain functional stability under contaminant stress and support ecosystem restoration. In conclusion, this study highlights the need for species selection and promotes the development of sustainable phytoremediation programs, providing a platform for future research into maximizing remediation potential through physiological, molecular and soil treatment strategies.

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