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Original Article

Metal Accumulation Ability of Different Eucalyptus Species at the Early Stage

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ABSTRACT

Eucalyptus species is crucial in the phytoremediation of hazardous metals such as lead and cadmium. This study involved the experimentation of our different *Eucalyptus* species to determine the best accumulator among them. The experimentation took place in Tamil Nadu Agricultural University, Coimbatore, using sand as the growing medium. The two toxic metals cadmium and lead were used at various levels on *Eucalyptus camaldulensis*, *Eucalyptus globulus*, *Eucalyptus citriodora*, and *Eucalyptus tereticornis* in the investigation, using analytical reagents grade salt of $CdCl_2$ and $Pb(NO_3)_2$. In comparison to the other *E. species*, *E. citriodora* had the highest cadmium (17.09 mg kg^{-1}) and lead (31.68 mg kg^{-1}) accumulation in the root. In shoots of *Eucalyptus* species, peroxidase activity varied from 1.31 to 0.38 g^{-1} h^{-1}, with 1.35–0.38 g^{-1} h^{-1} being the highest and 0.38 g^{-1} h^{-1} being the lowest, and 1.35–0.38 g^{-1} h^{-1} being the lowest. The enzymes like peroxidase and catalase played important role in the phytoremediation of toxic metals due to their antioxidant nature. With a higher concentration of heavy metals, peroxidase activity was reduced.

Keywords: Cadmium, catalase, lead, peroxidase, phytoremediation

Introduction

Heavy metal contamination is currently causing a lot of health concern in humans. The current degree of industrialization and some kinds of detrimental anthropogenic activities are responsible for release of large quantity of heavy metals in the environment causing disturbance in its normal functioning (Manisalidis et al., 2020). Cadmium and lead are assimilated into food chain due to the use of phosphorus fertilizers, sewage, sludge, and air pollution caused by various industrial activities. Among all elements discovered so far, 53 elements have been identified as heavy metals. However, the majority of them do not contribute anything to plant metabolism (Hassan et al., 2020). Heavy metals have been linked to an increased risk of cancer, kidney problems, and hypertension in humans (Lin et al., 2019; Satarug et al., 2011; Sidhu et al., 2020). It also causes membrane and DNA damage, perturb protein function, and enzyme activity (Witkowska et al., 2021).

The use of biological materials to clean up heavy metal contaminated soils has been recommended as a cost-effective and efficient bioremediation approach. Phytoremediation, a process that uses plants to help with reclamation, is presently being investigated in current biotechnology researches. Phytoremediation is the practice of using plants to remove the environmental contaminants from soil by absorbing them throughout their life cycle (Harvey et al., 2002; Muthusaravanan et al., 2018; Parmar & Singh, 2015). The following mechanisms or processes are thought to play an important role in the phytoremediation of contaminated soils: phytoextraction, phytostabilization, phytodegradation, phytovolatilization, rhizofiltration, and rhizodegradation (Greipsson, 2011; Shah & Daverey, 2020). Phytoremediation is a green technique for cleaning contaminated areas (Melinda et al., 2013) in which many hyper-accumulating species are native to metal-rich substrates but have a restricted distribution which is seen as a rising problem in current world scenario.

These species are characterized by their tolerance to toxic amounts of metals such as cobalt, copper, zinc, manganese, lead, selenium, nickel, and cadmium (Briffa et al., 2020). To remove such toxic metals from the soil, the phytoremediation approach is the best alternative technique at present that is a cost-effective and eco-friendly (Steliga & Kluk, 2020). The Eucalyptus species is known to be a fast-growing, short-rotation species, according to trial experimental findings. A preliminary experiment was carried out before the main experiment to ensure

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that the effects of some similar tree species were subjected to heavy metals accumulation. Eucalyptus species are some of the plant species that have been harmed in the experiment. The purpose of the experiment was to investigate the best hyperaccumulator Eucalyptus species over other species of Eucalyptus that really can assist to minimize the degree of heavy metal contamination in polluted soil and water sources as a solution.

Materials and Methods

Four Eucalyptus species, namely *Eucalyptus tereticornis*, *Eucalyptus camaldulensis*, *Eucalyptus globulus*, and *Eucalyptus citriodora*, were selected for this study. The two toxic metals cadmium and lead were used at various levels in the investigation, using analytical reagent grade salt of $CdCl_2$ and $Pb(NO_3)_2$ also acting as cadmium and lead source, respectively (Patil & Uma, 2014).

Experiment with Metal Toxicity

The sand was used as a growing medium for screening Eucalyptus species in order to find hyperaccumulators. Seed germination and screening were performed using sterilized sand. The samples of sand were sterilized by autoclaving. Table 1 displays the different concentrations of Cd (M1) and Pb (M2) imposed on sterilized sand. The Cd and Pb levels were established depending on the outcomes of the test. According to Chen & Lee (1997), the levels of Cd and Pb were adjusted based on critical limits (Patil & Umadevi, 2014). Throughout the experiments, various levels of Cd and Pb dissolved in distilled water were applied to plants. The experiment involved 25 seeds each repetition.

Screening of Suitable Eucalyptus Species for Heavy Metal Uptake and Accumulation

The seeds were sown in plastic bowls containing 250 gm of sterilized sand and a specific concentration of heavy metals, using 25 seeds per plastic bowl. The following are the details of the treatment:

Total Metal Content Analysis of Harvested Plant Samples

Plant samples were collected at the end of the experiment's 21 days after sowing (DAS) period and dried in a hot air oven at 65°C to remove all moisture. Whole plant samples of known weight were taken and digested with the tri-acid mixture (9:2:1 of HNO_3:H_2SO_4:$HClO_4$). The digested materials were filtered through what man No. 42, yielding in a volume of 25 mL (Lindsay & Norwell, 1978). The samples were then examined in an atomic absorption spectrophotometer for heavy metals (Perkin-Elmer, Analyst 800 AAS).

Performance of the Peroxidase Enzyme (g^{-1} h^{-1})

In 5 mL of 0.25 M Tris–HCl buffer solution 250 mg leaf samples were uniformed and centrifuged it at 5000 rpm for 10 minutes (pH 6.0). Pyrogallol 0.5 mL 0.5% aqueous solution, 0.4 and 0.5 mL 1% H_2O_2 was added to the mixture of plant enzyme extract and incubated at 25°C for 10 minutes. In the solution by adding 0.5 mL of 5% (v/v) H_2SO_4, the reaction was finished. In a UV spectrophotometer at 420 nm, the OD values at zero time and then after 10 minutes were measured 11, 12 (Umadevi et al., 2014). The mean value of peroxidase activity was computed using the following formula, and the difference in OD has been used to express the mean value g^{-1} h^{-1}.

$$\text{Peroxidase activity} = \frac{X \times 60 \times 10 \times 1000}{1 \times 30 \times 500} \text{microgram of } H_2O_2 \text{ g}^{-1}\text{h}^{-1} \quad (1)$$

Performance of the Catalase Enzyme (g of H_2O_2 g^{-1} min^{-1})

About 250 mg of leaf samples were homogenized in 0.066 M phosphate buffer solution after centrifuging at 2000 rpm for 10 minutes (pH 6.8). In 0.2 mL of extracted plant enzyme, 5 mL of triprotic acid with pH 6.8 and 4 mL of 0.3 N H_2O_2 (substrate) were added, and after 15 minutes of incubation, 10 mL of 2 N H_2SO_4 were added to stop this reaction. With the addition of 2 N H_2SO_4 and 0.2 mL of distilled water, the blank was maintained for each set. The sample were titrated using 0.1 N $KMnO_4$ and titrate reading was recorded based on the occurrence of pink color (Umadevi et al., 2014). The volume of permanganate corresponding to enzyme activity was calculated using the difference in titrate values.

$$\text{Catalase activity} = \frac{X}{1 \times 0.5} \times 10 \times 1 \times 0.85 \text{ microgram of } H_2O_2 \text{ g}^{-1}\text{min}^{-1} \text{ v} \quad (2)$$

Statistical Analyses

The study was set up using a factorial CRD design. The experiment's data were computed and tabulated for statistical analysis applying standard statistics/package MS office Excel and MS-DOS AGDATA; AGRES was used for all mathematical and statistical calculations.

Results

The aim of the research was to identify the best accumulator of *Eucalyptus species* in a controlled environment. The effects of heavy metals, including lead and cadmium, on the four *Eucalyptus species,* were examined 21 DAS. A total of seven treatments including control were applied with three replications to each treatment (Table 2).

Plant Roots Accumulate Heavy Metals

The study's findings on the accumulation of heavy metals in the roots of different Eucalyptus species differed significantly in terms of heavy

Table 1.
Levels of Metals

S. no.	Heavy metals	Level of metals (mg kg^{-1})
1	Lead [$Pb(NO_3)_2$]	100, 125, 250
2	Cadmium ($CdCl_2$)	25, 50, 100

Note: 1. Cd (M1)—0, 25 (less than critical point), 50 (critical point), 100 (higher than critical point) (mg kg^{-1}).
2. Pb (M2)—0, 100 (less than critical point), 125 (critical point), 250 (higher than critical point) (mg kg^{-1}).

Table 2.
Treatment Combinations

Plant species	E1—*Eucalyptus tereticornis* E2—*E. camaldulensis* E3—*E globulus* E4—*E. citriodora*
Treatments	T0—Control T1 M1—Cadmium 25 mg kg^{-1} T2 M1—Cadmium 50 mg kg^{-1} T3 M1—Cadmium 100 mg kg^{-1} T1 M2—Lead 100 mg kg^{-1} T2 M2—Lead 125 mg kg^{-1} T3 M2—Lead 250 mg kg^{-1}
Replications	3
Design	FCRD (Patil & Uma, 2014)

Note: FCRD = Factorial completely randomized design.

Table 3.
Phytoextraction Concentration (ppm) in the Root of Four Eucalyptus Species at 21 DAS

		Phytoextraction root (mg kg^{-1})			
Metal	Treatments	E1	E2	E3	E4
Cadmium	T1	6.64 ± 0.012	4.37 ± 0.067	6.35 ± 0.090	10.39 ± 0.073
(M1)	T2	8.59 ± 0.045	5.33 ± 0.009	7.7 ± 0.084	12.62 ± 0.129
	T3	10.56 ± 0.006	6.71 ± 0.113	8.72 ± 0.064	17.09 ± 0.134
Lead	T1	17.19 ± 0.262	9.22 ± 0.006	14.33 ± 0.13	19.59 ± 0.038
(M2)	T2	18.5 ± 0.023	10.31 ± 0.058	16.21 ± 0.33	21.88 ± 0.216
	T3	26.33 ± 0.215	13.49 ± 0.023	21.05 ± 0.003	31.68 ± 0.191
ANOVA test of significance at 0.05	$E = 0.202^{**}$	$T = 0.175^{**}$	$M = 0.143^{**}$	$E \times M \times T = 0.495^{**}$	

Note: DAS, days after sowing; E1—*Eucalyptus tereticornis*, E2—*E. camaldulensis*, E3—*E globulus*, E4—*E. citriodora*; M1T1—cadmium 25 ppm, M1T2—cadmium 50 ppm, M1T3—cadmium 100 ppm, M2T1—lead 100 ppm, M2T2—lead 125 ppm, M2T3—lead 250 ppm.

metal concentrations (Table 3). Among the two heavy metals, lead accumulation was higher in the root part of all four Eucalyptus species, ranging from 9.22 ± 0.006 to 31.68 ± 0.191 mg kg^{-1}, whereas cadmium accumulation was lower, ranging from 4.37 ± 0.067 to 17.09 ± 0.134 mg kg^{-1}. The research revealed that when heavy metal concentrations increased, so did the accumulation. *Eucalyptus citriodora* had the highest metal accumulation in its root, at 31.68 ± 0.191 mg kg^{-1}, while *E. camaldulensis* had the lowest at 13.49 ± 0.023 mg kg^{-1}. The root of different *Eucalyptus species* considerably differed in terms of distinct heavy metal concentrations (Table 3).

Plant Shoots Accumulate Heavy Metals

Table 4 presents data on heavy metal accumulation in four Eucalyptus species' shoots, including cadmium and lead. The findings revealed that the quantity of metal accumulated in the shoots of different Eucalyptus species varied significantly depending on the concentration. Lead deposition was higher in the shooting parts of four Eucalyptus species, ranging from 7.72 ± 0.007 to 24.59 ± 0.728 mg kg^{-1}, among the two heavy metals examined. Cadmium levels were significantly lower, ranging from 2.17 ± 0.037 to 12.53 ± 0.382 mg kg^{-1}

The Elements Translocation Factor

The metal transfer ratio from root to shoot that has been calculated is the translocation factor (TF). Lead metals had a higher TF than cadmium metals at all stages, indicating stronger biomagnification of metals (Table 5). Metal movement from the lower section (root) to the upper part (shoot) of the plant were estimated applying TFs.

Peroxidase Activities in Shoots and Roots

Tables 6 and 7 show the effect of lead and cadmium on peroxidase enzyme activity in the root of four distinct Eucalyptus species. When compared to the untreated plant, substantial results were observed in the roots of Eucalyptus species related to peroxidase enzymatic activity due to heavy metals. In the roots of the four Eucalyptus species, lead and cadmium showed reduced peroxidase activity. With increasing heavy metal concentrations, peroxidase activity in the roots of all four Eucalyptus species decreased. The root of *E. citriodora* had the highest peroxidase activity (1.14 ± 0.02 g^{-1} h^{-1}) of the four Eucalyptus species studied, whereas *E. camaldulensis* seemed to have the lowest lower peroxidase activity (0.41 ± 0.01 g^{-1} h^{-1}).

In the shoots of Eucalyptus species, the activity of the peroxidase plant enzyme was altered by lead and cadmium, as shown in Table 7. In the shoot, a similar pattern to that of the root was found. With increasing heavy metal concentrations, peroxidase activity in the shoots of all four Eucalyptus species reduced. The shoot of *E. citriodora* had the highest peroxidase activity (1.10 ± 0.16 g^{-1}h^{-1}) and *E. camaldulensis* had the lowest peroxidase activity (0.38 ± 0.05 g^{-1} h^{-1}).

Table 4.
Phytoextraction Concentration (ppm) in the Shoot of Four Eucalyptus Species at 21 DAS

		Phytoextraction shoot (mg kg^{-1})			
Metal	Treatments	E1	E2	E3	E4
Cadmium	T1	4.45 ± 0.126	2.17 ± 0.037	4.46 ± 0.135	6.25 ± 0.028
(M1)	T2	6.19 ± 0.075	4.2 ± 0.003	5.74 ± 0.075	9.88 ± 0.337
	T3	7.52 ± 0.009	5.6 ± 0.116	6.83 ± 0.009	12.53 ± 0.382
Lead	T1	12.47 ± 0.034	7.72 ± 0.007	12.36 ± 0.068	15.67 ± 0.052
(M2)	T2	12.84 ± 0.012	8.32 ± 0.012	12.64 ± 0.155	16.65 ± 0.098
	T3	20.44 ± 0.251	10.47 ± 0.033	17.35 ± 0.088	24.59 ± 0.728
ANOVA test of significance at 0.05	$E = 0.207^{**}$	$T = 0.179^{**}$	$M = 0.148^{**}$	$E \times M \times T = 0.508^{**}$	

Note: DAS, days after sowing.

Table 5.
Translocation Factor of Four Eucalyptus species at 21 DAS

		Translocation factor			
Metal	Treatments	E1	E2	E3	E4
Cadmium	T1	0.971 ± 0.0174	0.956 ± 0.0227	0.859 ± 0.0177	0.794 ± 0.0028
(M1)	T2	0.991 ± 0.0033	0.975 ± 0.0019	0.875 ± 0.0194	0.915 ± 0.0355
	T3	0.996 ± 0.0012	0.983 ± 0.0012	0.898 ± 0.0068	0.947 ± 0.0187
Lead	T1	0.958 ± 0.0168	0.945 ± 0.0003	0.931 ± 0.0098	0.946 ± 0.0109
(M2)	T2	0.946 ± 0.0020	0.926 ± 0.0320	0.964 ± 0.0227	0.944 ± 0.0102
	T3	0.966 ± 0.0049	0.923 ± 0.0038	0.982 ± 0.0192	0.986 ± 0.0232
ANOVA test of significance at 0.05	$E = 0.025^{**}$	$T = 0.022^{**}$	$M = 0.018^{**}$	$E \times M \times T = 0.062^{**}$	
Note: DAS, days after sowing.					

Table 6.
Effect of Cadmium and Lead on Peroxidase Activity of Root (g^{-1} h^{-1}) of Eucalyptus Species at 21 DAS

		Peroxidase root (g^{-1} h^{-1})			
Metal	Treatments	E1	E2	E3	E4
	T0	1.35 ± 0.02	1.29 ± 0.01	1.30 ± 0.01	1.30 ± 0.18
Cadmium (M1)	T1	1.12 ± 0.03	0.56 ± 0.03	1.00 ± 0.15	1.14 ± 0.02
	T2	0.76 ± 0.02	0.47 ± 0.03	0.73 ± 0.04	1.00 ± 0.08
	T3	0.75 ± 0.03	0.41 ± 0.01	0.67 ± 0.02	0.88 ± 0.06
Lead	T1	1.25 ± 0.02	0.86 ± 0.03	1.22 ± 0.17	1.27 ± 0.14
(M2)	T2	0.97 ± 0.07	0.53 ± 0.03	1.12 ± 0.14	1.17 ± 0.07
	T3	0.85 ± 0.04	0.65 ± 0.04	0.87 ± 0.05	1.07 ± 0.10
ANOVA test of significance at 0.05	$E = 0.011$	$T = 0.009$	$M = 0.008$	$E \times M \times T = 0.027$	
Note: DAS, days after sowing.					

Table 7.
Effect of Cadmium and Lead on Peroxidase Activity of Shoot (g^{-1} h^{-1}) of Eucalyptus Species *at 21 DAS*

		Peroxidase shoot (g^{-1} h^{-1})			
Metal	Treatments	E1	E2	E3	E4
	T0	1.31 ± 0.06	1.24 ± 0.13	1.26 ± 0.12	1.28 ± 0.04
Cadmium (M1)	T1	1.08 ± 0.07	0.52 ± 0.06	0.98 ± 0.06	1.10 ± 0.16
	T2	0.71 ± 0.05	0.43 ± 0.04	0.73 ± 0.10	0.94 ± 0.07
	T3	0.67 ± 0.01	0.38 ± 0.05	0.63 ± 0.09	0.76 ± 0.09
Lead (M2)	T1	1.20 ± 0.20	0.82 ± 0.12	1.2 ± 0.15	1.21 ± 0.12
	T2	0.91 ± 0.06	0.43 ± 0.05	1.02 ± 0.05	1.13 ± 0.20
	T3	0.82 ± 0.04	0.62 ± 0.11	0.84 ± 0.08	1.05 ± 0.03
ANOVA test of significance at 0.05	$E = 0.012$	$T = 0.010$	$M = 0.008$	$E \times M \times T = 0.028$	
Note: DAS, days after sowing.					

Shoot and Root Catalase Activity

Table 8 depicts the effect of heavy metals, lead, and cadmium on catalase activity in the roots of four Eucalyptus species. The activity of the catalase plant enzyme was found to differ significantly across Eucalyptus species' roots. Higher cadmium and lead concentrations increased the catalase enzyme's specific activity. In the roots of the four Eucalyptus species, both metals exhibited higher catalase activity. Cadmium concentrations ranged from 30.22 ± 0.57 µg of H_2O_2 g^{-1} $min^{-1}$to 23.14 ± 0.01 µg of H_2O_2 g^{-1} min^{-1}, whereas lead concentrations ranged from 22.160.11 g of H_2O_2 g^{-1} min^{-1} to 26.87 ± 0.58 µg of H_2O_2 g^{-1}

Table 8.
Effect of Cadmium and Lead on Catalase Activity of Root (µg of H_2O_2 g^{-1} min^{-1}) of Eucalyptus Species at 21 DAS

		Catalase root (µg of H_2O_2 g^{-1} min^{-1})			
Metal	Treatments	E1	E2	E3	E4
	T0	21.54 ± 0.32	21.15 ± 0.03	21.87 ± 0.09	21.16 ± 0.05
Cadmium (M1)	T1	25.38 ± 0.58	23.14 ± 0.01	24.47 ± 0.01	27.18 ± 0.59
	T2	26.46 ± 0.10	24.15 ± 0.02	25.20 ± 0.06	28.18 ± 1.15
	T3	28.44 ± 0.31	25.11 ± 0.05	26.27 ± 0.08	30.22 ± 0.57
Lead (M2)	T1	22.46 ± 0.11	22.16 ± 0.11	22.55 ± 0.23	24.50 ± 0.09
	T2	23.57 ± 0.32	23.16 ± 0.01	22.16 ± 0.02	25.34 ± 0.02
	T3	24.34 ± 0.01	23.78 ± 0.10	22.88 ± 0.07	26.87 ± 0.58
ANOVA test of significance at 0.05	$E = 0.069$	$T = 0.060$	$M = 0.049$	$E \times M \times T = 0.169$	
Note: DAS, days after sowing.					

Table 9.
Effect of Cadmium and Lead on Catalase Activity of Shoot (µg of H_2O_2 g^{-1} min^{-1}) of Eucalyptus Species at 21 DAS

		Catalase shoot (µg of H_2O_2 g^{-1} min^{-1})			
Metal	Treatments	E1	E2	E3	E4
	T0	21.12 ± 054	21.09 ± 0.57	21.35 ± 0.99	21.12 ± 1.02
Cadmium (M1)	T1	23.18 ± 0.01	22.44 ± 0.88	22.91 ± 0.01	25.37 ± 0.14
	T2	24.54 ± 0.15	22.86 ± 0.05	23.44 ± 1.21	26.51 ± 0.03
	T3	25.32 ± 0.50	23.29 ± 0.09	24.77 ± 0.57	28.22 ± 0.58
Lead	T1	21.85 ± 0.01	21.45 ± 0.14	21.56 ± 0.10	22.68 ± 0.01
(M2)	T2	22.16 ± 0.07	22.33 ± 0.21	21.92 ± 0.14	23.30 ± 0.08
	T3	22.88 ± 0.12	22.87 ± 0.06	22.36 ± 0.06	24.15 ± 0.02
ANOVA test of significance at 0.05	$E = 0.057$	$M = 0.040$	$T = 0.049$	$E \times M \times T = 0.140$	
Note: DAS, days after sowing.					

min^{-1}. Catalase activity significantly greater in the root of *E. citriodora* recorded 30.22 ± 0.57 µg of H_2O_2 g^{-1} min^{-1} while lowest 22.16 ± 0.11 µg of H_2O_2 g^{-1} min^{-1} were observed in *E. camaldulensis*.

Table 9 illustrates the catalase enzyme activity in the shoots of four Eucalyptus species. Catalase activity in the shoots of *Eucalyptus species* showed a significant change owing to heavy metals. In the shoots of the four *Eucalyptus species*, lead and cadmium showed higher catalase activity. Cadmium concentration measurements from 22.44 ± 0.88 µg of H_2O_2 $g^{-1}min^{-1}$ to 28.22 ± 0.58 µg of H_2O_2 g^{-1} min^{-1}, whereas lead concentrations varied from 21.45 ± 0.14 µg of H_2O_2 $g^{-1}min^{-1}$ and 24.15 ± 0.02 µg of H_2O_2 g^{-1} min^{-1}. *Eucalyptus citriodora* had the highest catalase activity (28.220.58 g of H_2O_2 g^{-1} min^{-1}) and *E. camaldulensis* had the lowest catalase activity (21.45 ± 0.14 µg of H_2O_2 g^{-1} min^{-1}).

Discussion

Heavy Metal Concentration in Eucalyptus Species' Shoots and Roots

Eucalyptus citriodora accumulated one of most metals inside its roots (31.68 ± 0.191 mg kg^{-1}), while Eucalyptus camaldulensis accumulated the least (13.49 ± 0.023 mg kg^{-1}). It has been suggested that the roots of actively growing plants operate as a barrier, preventing metal from moving to the above-ground plant parts; as a consequence, metal accumulation in the roots is more severe than in the above-ground plant parts (Labidi et al., 2021; Wierzbicka, 1987). The Cruciferae family includes *Thlaspi caerulescens*, *Brassica nigra*, and *Brassica juncea*, all of which accumulate a lot of metals. More biomass production, higher metal accumulation, and phytoextraction of heavy metal from root to shoot from the soil (Xiongz, 1998) all were qualities of hyperaccumulator plant species (Turan & Angin, 2013; Xiongz, 1998). The research demonstrated that as the concentration of heavy metals increased, so did the accumulation. The present investigation found that cadmium and lead accumulation was higher at higher doses, as reported by Aria et al. (2017). In comparison to cadmium, the harmful metal lead accumulated more and suitable species can be helpful for bioremediation of metals (Altaf et al., 2021). A higher concentration of lead had no deleterious influence on the growth characteristics of any Eucalyptus species in our investigation (Pb). Cadmium has its own toxic effects on a plant's metabolic and physiological activities, varying in severity and plant resistance to cadmium. Lower concentrations of cadmium damages the chloroplast structure, resulting for the chlorosis in leaves,

necrotic lesions, water strain, reticence of root elongation, reduces gas exchange, induce wilting, and impair macro and micronutrients absorption. (Lasat et al., 2000; Subašić et al., 2022).

The addition of Zn significantly reduced Cd toxicity, resulting in a smaller loss in growth and suppression of Cd concentration (Garg & Kaur, 2012; Ismael et al., 2019). Other findings suggest that lead ions were sequestered and safely fixed into part of the cell wall or vacuole by thiol peptides and that this sequestration does not disrupt other key physiological activities (Sharma & Dubey, 2005). The plants have an exclusion mechanism that allows them to absorb metals through their roots rather than their shoots, allowing them to accumulate more in the root than in the shoot (Kastori et al., 1998) also supported this assertion. A good heavy metal hyperaccumulator plant must have metal tolerance capacity, according to (Amin et al., 2021). According to another study, the concentrations of arsenic and cadmium in tea plants were shown to be in the following order: feeding roots > stems > old leaves > young shoots. In comparison to plants growing in polluted soil, their roots may have worked as a barrier, preventing contamination from reaching above-ground sections (Haider et al., 2021).

The current investigation discovered that *E. citriodora* has the ability to remove toxic metals from the soil at greater levels of hazardous metal concentration. *Eucalyptus citriodora* grew faster than the other species due to a genetic trait that allows it to tolerate higher levels of toxicity, similar to *Arabidopsis species* (Bechsgaard et al., 2006). The direct interaction of Cd ions with the guard cells caused due to closing of stomata or may be initial impact of Cd accumulation in root and shoot (Haider et al., 2021). At initial stage of *Salix species* acted as a good phytoremediator due to its high heavy metal accumulation (particularly Zn and Cd) and transportation ability, as well as high biomass production (Greger, 1999; Kabata & Pendis, 1993). Nonetheless, vegetation cover plays an important role in the restoration of polluted sites because it stabilizes and dries them out, initiates biological processes, and provides a protective barrier for the surrounding areas (Turan & Angin, 2013). As a result, a majority of plant species are excluded and accumulate heavy metals in their subterranean organs (Lal, 2010). In this situation, limiting metal uptake in the shoots will be dependent on cadmium and lead transport and distribution in plant tissues. Metal tolerance and accumulation may also be influenced by metal binding to the plant cell wall or apoplast fixation (Krämer et al., 2000).

Metal tolerance is characterized by a detoxifying mechanism that is dependent on the distribution pattern of metals in plant tissues. Because metal is transported between the cytosol and vacuole in cells, as well as between apoplast and symplasm tissues, transmembranes play a key role in metal transportation for hyperaccumulation. This is in line with the findings of Smith et al. (1993), who found that the behavior of phytoextraction of water, nutrients, and heavy metals differs by species, and that plants that are able to reap the benefits of increased phosphorus, water, and mineral nutrients create higher growth. Root tissue sequesters a higher quantity of lead and other metals than shoot tissue (Dong et al., 2019; Sharma & Dubey, 2005).

With increasing treatments concentration with long exposure of cadmium, deposition in different areas of the plant *H. odorata* and *I. palembanica* increased at the time of exposition (Moreno-Caselles et al., 2000) reported a similar outcome. Phytoextraction efficiency is measured by metal concentration in plants and dry matter production. As a result, the plant species chosen for phytoremediation should have a high producing ability, which may aid in metal tolerance and increased metal accumulation (Marlborough, 2016). *Acacia mangium* and *H. odorata* primary roots and stems did not acquire large amounts of lead. Some plants' roots secrete a range of organic chemicals that regulate metal solubility in the rhizosphere. The root exudates complex metal ions, which the roots can absorb (Ang et al., 2010; Sharma & Dubey, 2005). Although several of the data demonstrated good phytoextraction capabilities, no plant possesses all of the necessary characteristics. Some hyperaccumulator plants required ongoing plant breeding and genetic engineering research on their characteristics (Marlborough, 2016).

The Factor of Translocation

Any plant species' metal TF aids in determining the metal distribution pattern in various plant parts (Xiongz, 1998). Metal buildup in the above-ground vegetative plant portion is influenced by a variety of physiological, morphological, and biochemical factors (Singh et al., 2010). *Eucalyptus citriodora* had the highest metal accumulation in the shoot (14.26 mg kg^{-1}) and *E. camaldulensis* had the lowest accumulation (6.41 mg kg^{-1}) among the four Eucalyptus species used in this study, indicating that plants with a low TF are better for phytostabilization (Yan et al., 2020).

Table 5 shows the data on cadmium and lead TFs by shoot and root of different Eucalyptus species. Overall, the larger TF for lead was reported, while the lower TF for cadmium was recorded. The TF was found to be highest in *E. tereticornis* (0.991 ± 0.0033), followed by *E. citriodora* (0.794 ± 0.0028) among the four *Eucalyptus species* used in the study. The ATP-dependent proton pumps which catalyze H^+ extrusion across the cell wall membrane of root cells is responsible for overall metal uptake and transport. The shuttling ability of hazardous cations across plant membranes is mediated by the plant transporter (Singh et al., 2010). According to dozens of studies, the metal tolerant mechanism is based on metal ion compartmentalization, which is restored in the vacuolar compartment. These compartments keep them out of cellular regions where functions like cell division and respiration take place, proving to be a useful safeguard (Chaney et al., 1997; Singh et al., 2010). Metal cations such as Cd, Cu, and Zn bind better to cysteine-rich proteins (metallothionein) (Dong et al., 2019; Singh et al., 2010). The key ways for Pb tolerance in geranium plant roots are cell wall lignification and the creation of metal lignin complexes. Because the translocation in this study is higher (i.e., ratio >1), the species might be classified as an accumulator species (Dan et al., 2000; Singh et al., 2010).

Peroxidase Activity in Shoots and Roots

The shoot of *E. citriodora* seemed to have the highest peroxidase activity (1.10 ± 0.16 $g^{-1}h^{-1}$) and *E. camaldulensis* was the lowest peroxidase activity (0.38 ± 0.05 g^{-1} h^{-1}). As protection against stress in the plant, the peroxides enzyme eliminated excessive levels of hydrogen peroxide (Aria et al., 2017). A natural defense system is present in plants as enzymatic and nonenzymatic antioxidants activities protects against the oxidative damage caused by environmental stresses (Hassan et al., 2020). In both the root and the shoot, peroxidase activity was higher in the control than in the other treatments. At 21 DAS, the peroxidase activity declines as the metal concentration rises. The findings showed that the increased toxicity of lead and cadmium lowers peroxidase activity in seedling roots and shoots. The antioxidant enzyme's activity was lowered as a result of Cd-induced oxidative pressure (Garg & Kaur, 2012).

By increasing the number of free radicals, oxidative stress increased the activity of stress-related enzymes such as catalase, peroxidase, and superoxide dismutase (Ashraf et al., 2010). The basic function of peroxidase is to convert hydrogen peroxide (H_2O_2) into water (H_2O) and

oxygen (Lin et al., 2015). Peroxidase acts to combat the stress caused by heavy metals. Concentrations of Cd and plant species reduce or encourage the activity of various antioxidative enzymes before any observed symptoms of toxicity (Martínez et al., 2020).

Cadmium causes oxidative stress by interfering with antioxidative resistance, disrupting the electron transport chain, or causing lipid peroxidation. Cadmium exposure can sometimes activate lipoxygenase, an enzyme that promotes lipid peroxidation (Bhaduri & Fulekar, 2012). These systems can inhibit oxidative chain reactions by slowing or even stopping the oxidation of biomolecules. Because peroxidase uses phenolic forms that are cell wall bond soluble, it participates in the oxidation of lignin monomers, which aids in lignin synthesis and other physiological processes. Peroxidase aids in the formation of lignin, which acts as a physical barrier against heavy metal poisoning such as cadmium toxicity, as well as any pathogen reaction (Janusz et al., 2017).

The activity of peroxidase is proportional to the amount of cadmium in the body. The peroxidize activity rises in tandem with the cadmium concentration.

The peroxidase activity increased as the cadmium treatments increased. The results showed that as the cadmium concentration was increased, peroxidase specific activity went up as well, peaking at the highest concentration. Environmental stress changes the peroxidize enzyme, and these enzymes are utilized as nonspecific biomarkers in the research of environmental contamination (Kachout et al., 2009).

Root and Shoot Catalase Activity

With increasing heavy metal concentrations, catalase activity in the roots of all four Eucalyptus species increased. Catalase catalyzes the removal of hydrogen peroxide from chloroplasts. Catalase's roles include stress-resistance signaling in plants, apoptotic factor modulation, and maintaining the oxidation-reduction dynamic equilibrium in plant cells (Lin et al., 2015). Roots have lost the ability to absorb nutrients and develop the capability to support plant development as a result of the defence response caused by cadmium (Schützendübel & Polle, 2002).

Cd^{2+} and Pb^{2+} frequently limit enzyme activity when they interact with them. Several enzyme activities have been observed to be enhanced by Cd^{2+} and Pb^{2+}. As a result, both cadmium and lead have been found to encourage the development of active oxygen forms in cells. Activity of antioxidant enzyme decrease in against to oxidative stress, neutralizing free radicals and peroxides. Plant cells have developed defense mechanisms activation of antioxidant enzymes (Dutta et al., 2018). The antioxidant enzyme activity, on the other hand, are dependent on the stage of plant development and can be lowered even after a brief exposure to these metals (Shaw, 1995). Cadmium and lead do not exert a particular inhibition; other cations with similar affinity for protein functional groups can also cause inhibition. This theory is supported by a number of inhibitory effects of Cd^{2+} and Pb^{2+} on enzymes, as well as the solubility of the related sulfides.

Metals have been shown to boost enzyme activity in some studies. However, there is no direct evidence that cadmium and lead stimulate catalase, peroxidase, or superoxide dismutase when immobilized enzymes are treated (e.g., horseradish peroxidase) (Lal, 2010). Furthermore, because all of these enzymes are metalloenzymes, their activity may be reduced if the necessary metal is replaced with cadmium or lead. The plant's tolerance to heavy metals may be attributed to the activation of the enzyme system during the cell's metabolic process, which is triggered by heavy metal stress (Ashraf et al., 2010).

At all phases, *E. citriodora* had the highest catalase defense activity, followed by *E. tereticornis*, *E. globulus*, and *E. camaldulensis*. Some plant species have antioxidant defense mechanisms that scavenge reactive oxygen species after plant enzyme catalases, peroxidases, and superoxide dismutases are triggered in response to oxidative stress (Liu et al., 2007). The greater concentration of heavy metals also impacts physiological activity, according to Schützendübel and Polle (2002). The accumulation of cadmium in spruce needles was controlled by the solubility of peroxidase isoenzyme pattern generated by increased cadmium concentrations (Alberto & Sigua, 2013). With increasing heavy metal concentrations, catalase activity in the shoots of all *Eucalyptus species* increased. Heavy metal stress causes changes in the membrane's lipid composition, which alters the function of enzymes linked to membranes. To protect against oxidative stress, the plant produces antioxidant enzymes such as catalase, superoxidase dismutase, peroxidize, glutathione reductase, and S-transferase (Kachout et al., 2009; Lal, 2010).

The findings demonstrated that the harmful effects of cadmium and lead vary depending on the stage of plant development. In the early stages of *Cajanus cajan*, forced cadmium reduced photosynthesis and enzymatic activity by around 50%, whereas later stages were less affected (Sheoran et al., 1990). Cadmium poisoning may increase the catalase enzyme's ability to scavenge H_2O_2 (Brunetti et al., 2009). Every plant species has a unique strategy for resisting the same enzyme. As a result, most enzyme activity reduced when cadmium and lead are present. Metal toxicity's multidirectional effects differed depending on cell metabolic activity (Lal, 2010). Due to the participation of the catalase enzyme during photo-respiration, scavenging H_2O_2 is released (Bhaduri & Fulekar, 2012). It served as the plant's defense system against harmful metals. As a result, higher activity of these enzymes could be interpreted as evidence of increased generation of reactive oxygen radicals such dO_2, dOH, and H_2O_2.

In the case of metals, cadmium treatment seemed to have the highest catalase activity at all stages as compared to lead. At 21 DAS, the antioxidant enzyme catalase activity in the root was 26.18 g H_2O_2 g^{-1} due to cadmium treatment. Long-term exposure to a higher quantity of cadmium stimulates oxidative metabolism in plants, according to a study conducted on spruce needles. Due to increasing cadmium concentrations, the buildup of cadmium in spruce needles and the solubility of catalase isoenzyme pattern changes (Alberto & Sigua, 2013). The seedlings antioxidant defense system was observed. Long-term treatment results revealed that increasing cadmium concentration increased the capacity of the seedlings antioxidative defense system, reducing the damaging effects of metal ions (Jaishankar et al., 2014). At greater cadmium concentrations, oxidative metabolism increases, resulting in more free radicals and reactive oxygen species. Due to chelating proteins, cadmium complexes can be discovered in many trees (Alberto & Sigua, 2013). Some metal-bearing plants, on the other hand, have not accumulated phytochelatins as a result of this. Because of the energy necessary for sulfate reduction to promote phytochelatin synthesis, the massive creation of phytochelatin was an unrelated mechanism for metal tolerance (Alberto & Sigua, 2013; Lal, 2010). Due to cadmium serving as an inducer of oxidative stress in cells, various antioxidative enzymes activities were enhanced before obvious indications of toxicity effects of cadmium (El-Beltagi et al., 2010).

In *Thlaspi caerulescens*, catalase appears to be the most susceptible enzyme to heavy metal stress. The greatest root catalase activity in *E. citriodora* was 27.04 g H_2O_2 g^{-1} min^{-1}at 21 DAS, compared to 25.04 g H_2O_2 g^{-1} min^{-1} in the shoot. *E. citriodora* and *E. tereticornis* were found to have a higher capacity to resist stress than *E. globulus* and

E. camaldulensis, a more rapidly growing and antioxidative defensive system. The increased activity of the catalase enzyme and the cadmium and lead-induced antioxidant defense system in the plant with greater catalase response were shown by the higher metal concentrations (Malecka et al., 2001; Brunetti et al., 2009). A higher cadmium content raises the catalase level, which lowers the rate of respiration and lowers the CO_2 refraction point (Aria et al., 2017).

Conclusion

Eucalyptus citriodora accumulated the most harmful elements in both its root and shoot parts of the four Eucalyptus species studied. Among all the species, *E. camaldulensis* had the lowest accumulation rate. Toxic metal buildup was found to be higher in the roots of all species than in the shoots. In comparison to cadmium, the harmful metal lead accumulated more in the current study. Cadmium was shown to be more hazardous than lead. The heavy metal application had a considerable effect on the peroxidase and catalase activities in the shoot and root of all Eucalyptus species. Higher amounts of heavy metals reduced peroxidase activity in both the roots and the shoots of *Eucalyptus* species. In the shoots and roots of the four *Eucalyptus* species, lead and cadmium had increased catalase activity. With increasing heavy metal concentrations, catalase activity in the shoot and root of all *Eucalyptus* species increased.

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