



## RESEARCH PAPER

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## Effect of cadmium and chromium on fast growing pulp wood Tree species

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### Abstract

The present study investigated the effects of Cadmium (Cd) and Chromium (Cr III & Cr VI) on dry matter production, enzyme activity and metal extraction potential of three fast growing pulp wood species viz., She Oak (*Casuarina junghuhniana* Miq.), The Forest Red Gum (*Eucalyptus tereticornis* Sm.) and The White Lead tree (*Leucaena leucocephala* Lam. de Wit). Metal elements were applied in the form of Cd(Cl<sub>2</sub>)<sub>2</sub> and K<sub>2</sub>CrO<sub>4</sub> and Cr(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O at variegated concentrations viz., T<sub>1</sub> (Control), T<sub>2</sub> (lower level- 25 mg L<sup>-1</sup> Cd, 10 mg L<sup>-1</sup> Cr (VI) and 25 mg L<sup>-1</sup> Cr (III)), T<sub>3</sub> (Critical level - 50 mg L<sup>-1</sup> Cd, 20 mg L<sup>-1</sup> Cr (VI) and 50 mg L<sup>-1</sup> Cr (III)) and T<sub>4</sub> (higher level - 100 mg L<sup>-1</sup> Cd, 40 mg L<sup>-1</sup> Cr (VI) and 50 mg L<sup>-1</sup> Cr (III)) under controlled conditions of light and temperature for 3 weeks. The biometrical parameters like, shoot length, root length, shoot dry weight, root dry weight and total dry matter production were recorded at 21 Days After Sowing (DAS). Similarly, activities of plant enzymes (catalase and peroxidase) and uptake of trace metals (Cd, Cr (VI) and Cr (III)) by the above said three species were also analyzed at 21 DAS. Decline in total dry matter production and enzyme activities were founded in all the three species after 25 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> and 25 mg L<sup>-1</sup> for Cd, Cr (VI) and Cr (III) application respectively. *Leucaena leucocephala* was most affected followed by *Eucalyptus tereticornis*. But, *Casuarina junghuhniana* showed tolerance towards trace metal toxicity. Regarding uptake of trace metal, *Eucalyptus tereticornis* accumulated more Cd, Cr (VI) and Cr (III) compared to other species. In this study, Cr (VI) appeared to be more toxic to pulp wood tree species as compared to Cr (III) and Cd at germination stage (21 DAS) and Cd got accumulated more in plants than Cr (III) and Cr (VI).

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## Introduction

The increasing influx of heavy metals into water bodies from industrial, agricultural, and domestic activities is of global concern because of their well documented negative effects on human and ecosystem (Mataka *et al.*, 2006). The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.*, 2008). Among heavy metals Cd and Cr are considered potentially important environmental pollutants. Cadmium is a heavy metal with high toxicity and has an elimination half-life of 10-30 years (Jan *et al.*, 1999). People are exposed to cadmium by intake of contaminated food or by inhalation of tobacco smoke or polluted air (Järup *et al.*, 1998). High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso, 2008). Influence of cadmium toxicity on germination and growth of some common trees were investigated by Iqbal and Mehmood, (1991). At low concentration, cadmium is not toxic to plant but retards root growth (Zou, *et al.*, 2008) and cell division (Liu *et al.*, 1992). At higher concentration inhibits chlorophyll biosynthesis and decreases total chlorophyll content and chlorophyll a/b ratios (Stobart *et al.*, 1985). Similarly, chromium damages roots (Zou *et al.*, 2006) and membrane, induces chlorosis, necrosis and retardation of growth in plants (Sharma *et al.*, 2003).

Various processes including isolation, mechanical separation, chemical treatment and soil flushing are effective to clean the heavy metal contaminated soils (Mulligan *et al.*, 2001). Since these processes are costly, labour intensive, time consuming and require special equipment for the purpose, research efforts have been shifted to develop cost effective technology involving microorganisms, biomass and living plants in cleaning polluted sites (Wasay *et al.*, 1998). Phyto-extraction, a plant based technology for the removal of contaminants and heavy metals from polluted waters and soils, is evolving rapidly.

A critical parameter to evaluate phyto-extraction efficiency is the total metal uptake per hectare. It is calculated from the concentration in the harvested parts and the biomass production (Ebbs *et al.*, 1997). Plants that employ other mechanisms than hyper accumulation generally have lower shoot concentrations of metals but larger biomass (McGrath, 1998). Tobacco (*Nicotiana tabacum*), sunflower (*Helianthus annuus*), Indian mustard (*Brassica juncea*) and in-bred lines of corn (*Zea mays*) produce high yields and are known to accumulate heavy metals but they have been investigated mainly in conjunction with additives (Kayser, 2000; Wenzel *et al.*, 2003). Indian mustard has been so far the most studied of those plants because of its positive response to heavy metals and additives like EDTA (Begonia *et al.*, 1998). Nevertheless tree species, especially pioneer plants (Willow and Poplar) seem also promising for phytoextraction (Pulford and Watson, 2003). Alternatively, these tolerant plants can be used on contaminated sites to phytostabilize contamination by reducing Cd transfer through leaching or land erosion (Vangronsveld and Cunningham, 1998; Rosselli *et al.*, 2003).

Many researchers (Sanita di Toppi and Gabbrielli, 1999; Rout *et al.*, 2000; Reeves and Baker, 2000) have investigated plant species capable of accumulating unwanted metal elements and compiled a list of plant species that hyper-accumulate Cd, Cr, Ni, Pb, Se and Zn. The metal-accumulating plants identified so far are slow growing, small, and/or weedy plants that produce low biomass and have undefined growth requirements and characteristics. Many researchers documented the toxic effect of heavy metals on few tree species viz., *Prosopis juliflora* swartz (Jamal *et al.*, 2006), *Dalbergia sissoo* Roxb (Shah *et al.*, 2008) and *Albizia Lebbeck* L. Benth (Farooqi *et al.*, 2009). At present no valid research is available for the toxic effects on pulp wood tree species and their potential to uptake heavy metals. Therefore, an effort has been made to investigate the effects of Cd and Cr on fast growing

pulp wood tree species and their metal accumulating potential at seedling stage under controlled conditions of light and temperature. The species are selected because they are fast growing, high biomass producing and has wide adaptability to climatic and edaphic conditions. *Casuarina junghuhniiana* has spread in the shape of large scale energy plantations and wasteland afforestation, with potential use in pulp and paper industry. *Eucalyptus tereticornis* has emerged as a primary pulp wood species mostly grown as rain fed plantations in Tamil Nadu, India. It also has strong, hard and durable heartwood which is used for construction in heavy engineering, such as for railway sleepers and also in posts, drawers and particle boards. The leaves are used in the production of cineole based eucalyptus oil. *Leucaena leucocephala* is efficient in nitrogen fixation at more than 500 kg ha<sup>-1</sup>year<sup>-1</sup>. Hence, it was promoted as a "miracle tree" for its multiple uses such as firewood, fiber and livestock fodder. Moreover, it has been grouped under secondary pulp wood species.

## Materials and Methods

### Cultural Conditions

The seeds were collected from well grown, disease free and middle aged trees of *Casuarina junghuhniiana*, *Eucalyptus tereticornis* and *Lucaena leucocephala* found in Forest College and Research Institute, Mettupalayam, Tamil Nadu, India. The mature cones and pods were collected, shade dried properly and the seeds were extracted carefully. The seeds were graded; Small sized, immature and malformed seeds were discarded. The seeds were immersed in 3% formaldehyde solution for five minutes to avoid fungal contamination and were sown (25 seeds per cup) in plastic cups containing 250 g of sterilized sand and specified concentration of heavy metals. The ambient temperature of the chamber was maintained at 22 ± 2 °C.

### Heavy Metal Application

Cadmium and chromium (Cr VI and III) in the form of Cd (SO<sub>4</sub>)<sub>2</sub>, K<sub>2</sub>CrO<sub>4</sub> and Cr (NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O were applied at variegated concentrations viz., T<sub>1</sub> (Control), T<sub>2</sub> (lower level - 25 mg L<sup>-1</sup> Cd, 10 mg L<sup>-1</sup> Cr (VI) and

25 mg L<sup>-1</sup> Cr (III)), T<sub>3</sub> (Critical level - 50 mg L<sup>-1</sup> Cd, 20 mg L<sup>-1</sup> Cr (VI) and 50 mg L<sup>-1</sup> Cr (III)) and T<sub>4</sub> (higher level - 100 mg L<sup>-1</sup> Cd, 40 mg L<sup>-1</sup> Cr (VI) and 50 mg L<sup>-1</sup> Cr (III)) for treatment. The metal solutions were prepared in double distilled water. Control plants received no treatment except double distilled water. Seedlings were irrigated at field capacity and each time measured amount was applied. Each treatment was replicated thrice for statistical analysis.

### Assessment of Plant Dry Weight, Enzyme Activity and Uptake of Heavy Metals

The seedlings were uprooted at the end of the experiment and thoroughly washed with double distilled water. Plants were then oven dried at 70 °C for 72 hours till constant weight obtained. Oven dried weights were recorded to measure the extent of treatment application. Catalase and peroxidase activities were assayed respectively by the method of Povolotskaya and Sadenka, (1956) and by Malik and Singh, (1980). Heavy metal content in harvested plant samples were analyzed in Atomic Absorption Spectrometer (AAS) according to the procedure given by Amore *et al.*, (2005).

### Statistical Design

The data were analyzed for the possible relationship between the different parameters and analysis of variance employing Factorial Randomized Block Design as described by Panse and Sukhatme, (1985) and data were analyzed by using AGRES software.

## Results and Discussion

### Effect of Heavy Metals on Total Dry Matter Production

Among the three metals, seedlings treated with Cr (VI) significantly reduced (P < 0.01) the total dry matter production in *Casuarina junghuhniiana*, *Leucaena leucocephala* and *Eucalyptus tereticornis* when compared to seedlings treated with Cr (III) and Cd (Table 1). Total dry matter production showed reduction after 10 mg L<sup>-1</sup> Cr (VI) and 25 mg L<sup>-1</sup> Cd and Cr (III) application. Different heavy metals affect the total dry matter production significantly (P < 0.01) in different tree species. In general, seedlings grown in

cadmium chloride (C<sub>1</sub>) recorded higher total dry matter production over seedlings grown in chromium nitrate (C<sub>3</sub>) and potassium chromate (C<sub>2</sub>). In *Casuarina junghuhniiana* the total dry matter production was in the order of C<sub>3</sub> > C<sub>1</sub> > C<sub>2</sub>. In *Leucaena leucocephala* and *Eucalyptus tereticornis* it was in the order of C<sub>1</sub> > C<sub>3</sub> > C<sub>2</sub> and C<sub>1</sub> > C<sub>3</sub> > C<sub>2</sub> respectively. A significant effect of metal toxicity on total dry matter production was also observed for the varied tree species. *Leucaena leucocephala* recorded maximum total dry weight (0.189 g plant<sup>-1</sup>) which was followed by *Casuarina junghuhniiana* (0.035 g plant<sup>-1</sup>) and *Eucalyptus tereticornis* (0.028 g plant<sup>-1</sup>). The seedlings of *Casuarina junghuhniiana*, *Leucaena leucocephala* and *Eucalyptus tereticornis* also showed a gradual decrease in total dry biomass as concentrations of cadmium and chromium increased. Similar observations in crops had been observed by

Hailing *et al.*, (1991). The toxicity of some metals may be so severe that plant growth is reduced before large quantities of the element can be translocated (Haghiri, 1973). The decrease in total dry matter production with increasing concentration of heavy metals may be due to the sensitivity of enzymes of the photosynthetic carbon reduction cycle to cadmium (De Filippis and Ziegler, 1993). The results indicate the extent of tolerance of these fast growing pulp wood species to metal elements, which are in agreement with the findings of Gardea-Torresdey *et al.*, (2004). Barcelo *et al.*, (1986) found growth reduction in *Phaseolus vulgaris* at Cr (VI) dose ranging between 25-100 mg L<sup>-1</sup> which he pointed out was due to decreases in the water potential on metal element application. Cr (VI) reduces the nitrate reductase activity, thus limiting plant growth (Vajpayee *et al.*, 1999; Calabrese and Baldwin, 2003).

**Table 1.** Effect of heavy metals on total dry matter production (g plant<sup>-1</sup>) of three pulp wood tree species at 21 DAS.

Treatment Elements	Mg L <sup>-1</sup>	<i>Casuarina junghuhniiana</i>	<i>Eucalyptus tereticornis</i>	<i>Leucaena leucocephala</i>
Cd	control	0.0377 ± 0.0037	0.0360 ± 0.0023	0.2857 ± 0.0052
	25	0.0360 ± 0.0010	0.0337 ± 0.0029	0.2257 ± 0.0033
	50	0.0320 ± 0.0006	0.0310 ± 0.0010	0.1803 ± 0.0047
	100	0.0287 ± 0.0020	0.0280 ± 0.0025	0.1437 ± 0.0037
Cr (VI)	Control	0.0377 ± 0.0013	0.0357 ± 0.0050	0.2567 ± 0.0056
	25	0.0307 ± 0.0019	0.0183 ± 0.0010	0.1497 ± 0.0050
	50	0.0290 ± 0.0012	0.0140 ± 0.0010	0.1340 ± 0.0017
	100	0.0273 ± 0.0021	0.0127 ± 0.0011	0.0803 ± 0.0020
Cr (III)	Control	0.0377 ± 0.0032	0.0357 ± 0.0040	0.2567 ± 0.0052
	10	0.0367 ± 0.0022	0.0323 ± 0.0016	0.2233 ± 0.0062
	20	0.0337 ± 0.0010	0.0297 ± 0.0014	0.1897 ± 0.0043
	40	0.0300 ± 0.0020	0.0253 ± 0.0016	0.1417 ± 0.0038
ANOVA	T	P = 0.00132**		
	S	P = 0.00114**		
	T x S	P = 0.00229**		

Values are represented in mean ± Standard Error with three replicates, P = Correlation Coefficient, \*\*Indicates significance at 0.01%, ANOVA-Analysis of Variance, T-Treatment, S-Species.

#### Effects of Heavy Metals on Catalase Enzyme Activity

A significant effect ( $p < 0.01$ ) of metal toxicity on catalase activity was also observed for the varied tree species. *Casuarina junghuhniiana* recorded significantly maximum catalase activity of 20.29 µg of H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> min<sup>-1</sup> which was followed by *Leucaena leucocephala* with 18.45 µg of H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> min<sup>-1</sup> and *Eucalyptus tereticornis* with 15.27 µg of H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> min<sup>-1</sup> (Table 2). The result showed that cadmium,

chromium VI and chromium III at different concentrations reduced catalase activity in *Casuarina junghuhniiana*, *Leucaena leucocephala* and *Eucalyptus tereticornis* when compared to control. Highest reduction in catalase activity was observed in *Leucaena leucocephala* followed by *Eucalyptus tereticornis* and *Casuarina junghuhniiana*. This may be due to susceptibility of the species to heavy metal. Different heavy metals affect the catalase activity

significantly in different tree species. Among the three metals Cd exhibited significantly higher ( $p < 0.01$ ) catalase enzyme activity of  $20.10 \mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$  which was followed by Cr (III) ( $17.65 \mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$ ) and Cr (VI) ( $16.26 \mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$ ). In case of levels of heavy metals, the plant catalase activity showed a decreasing trend with increasing metal concentration. In all the three pulp wood tree species, potassium chromate significantly affects catalase activity than chromium nitrate and cadmium chloride. This was in agreement with the findings that the catalase levels increased in both roots and leaves of sorghum treated with either 50 AM Cr (VI) or 100 AM Cr (III) (Sen *et al.*, 1994). In case of levels of heavy metal, all species sown at different concentration of heavy metals recorded significantly decreased

catalase activity over controlled plants. Induction and activation of antioxidant catalase is one of the major metal detoxification mechanisms in plants (Prasad, 1998; Shanker *et al.*, 2003a). In *Echinochloa colona* L. plants supplemented with Cr at  $1.5 \text{ mg L}^{-1}$  the activity of catalase was higher in tolerant plants than in non-tolerant ones (Samantaray *et al.*, 2001). Gwozdz *et al.*, (1997) found that at lower heavy metal concentrations the activity of antioxidant enzymes increased whereas at higher concentrations catalase activity decreased. A decline in the specific activity of catalase with increase in Cr concentration from 20 to 80 ppm was observed (Jain *et al.*, 2000). Excess of Cr ( $0.5 \mu\text{M}$ ) restricted the activity of catalase in leaves of cauliflower (Chatterjee and Chatterjee, 2000).

**Table 2.** Effect of heavy metals on catalase activity ( $\mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$ ) of three pulp wood tree species at 21 DAS.

Treatment Elements	Mg L <sup>-1</sup>	<i>Casuarina junghuhniana</i>	<i>Eucalyptus tereticornis</i>	<i>Leucaena leucocephala</i>
Cd	control	26.00 ± 0.62	22.80 ± 0.07	30.69 ± 0.08
	25	21.82 ± 0.18	19.35 ± 0.23	22.29 ± 0.04
	50	17.53 ± 0.65	18.05 ± 0.21	17.56 ± 0.14
	100	15.18 ± 0.36	16.36 ± 0.17	11.59 ± 0.06
Cr (VI)	Control	26.00 ± 0.62	22.80 ± 0.07	30.69 ± 0.08
	25	21.67 ± 0.14	14.68 ± 0.26	15.50 ± 0.18
	50	19.57 ± 0.14	08.36 ± 0.11	08.80 ± 0.10
	100	17.97 ± 0.49	05.13 ± 0.06	03.90 ± 0.10
Cr (III)	Control	26.00 ± 0.62	22.80 ± 0.07	30.69 ± 0.08
	10	19.33 ± 0.17	17.76 ± 0.19	26.48 ± 0.16
	20	16.65 ± 0.11	09.40 ± 0.18	13.51 ± 0.03
	40	15.63 ± 0.13	05.17 ± 0.12	07.69 ± 0.14
ANOVA	T	P = 0.3286**		
	S	P = 0.2846**		
	T x S	P = 0.5692**		

Values are represented in mean ± Standard Error with three replicates, P = Correlation Coefficient, \*\*Indicates significance at 0.01%, ANOVA-Analysis of Variance, T-Treatment, S-Species.

#### Effects of heavy metals on peroxidase enzyme activity

A significant effect of metal toxicity on peroxidase activity was also observed for the varied tree species (Table 3). *Leucaena leucocephala* (S<sub>2</sub>) recorded maximum peroxidase activity of  $0.47 \text{ g}^{-1} \text{ h}^{-1}$  which was followed by *Casuarina junghuhniana* ( $0.32 \text{ g}^{-1} \text{ h}^{-1}$ ) and *Eucalyptus tereticornis* and *Casuarina junghuhniana* when compared *Leucaena leucocephala*. This may be due to higher uptake of metals by these tree seedlings

compared to *Leucaena leucocephala*. In general, peroxidase activity was significantly affected by different heavy metals. Among the three metals Cr (III) exhibited higher peroxidase activity of  $0.38 \text{ g}^{-1} \text{ h}^{-1}$  which was followed by Cr (VI) ( $0.34 \text{ g}^{-1} \text{ h}^{-1}$ ) and Cd ( $0.30 \text{ g}^{-1} \text{ h}^{-1}$ ). In the case of levels of heavy metals, the peroxidase activity showed a decreasing trend at lower concentration then it was increased over control at higher metal concentration. Different heavy metals affect the peroxidase enzyme significantly in different tree species. In general, seedling grown in potassium chromate recorded higher peroxidase

activity over seedling grown in chromium nitrate and cadmium chloride in *Casuarina junghuhniana*, *Leucaena leucocephala* and *Eucalyptus tereticornis*. This is may be due to the reason that chromium is more toxic than cadmium. In case of levels of heavy metal, all species sown at different concentration of heavy metals recorded decreased peroxidase enzyme activity over controlled plants. However, the rate of decrease was less at lower concentration compared to control. In *E. colona* plants supplemented with Cr at 1.5 mg L<sup>-1</sup>, activity of peroxidase was higher in tolerant calluses than in non-tolerant ones (Samantaray *et al.*, 2001). The peroxidase is an antioxidative enzyme which is an important compound in preventing the oxidative stress in plants as is based on the fact that the activity of these

enzymes is generally increased in plants when exposed to stressful conditions (Allen, 1995). In this study, peroxidase activity increased with increased concentration of Cd, Cr (VI) and Cr (III). Antioxidative enzymes are considered to be an important defense system of plants against oxidative stress caused by plants (Weckx and Clijsters, 1996). Since the peroxidase enzymes are related to free radical formation, it is evident that cadmium and chromium induce the development of free radical reactions. The relationship between metal sensitivity and lipid peroxidation was clearly illustrated in response to cadmium and chromium, indicates that these metal toxicity resulted in increased peroxidase. Similar findings were reported by Malekzadeh *et al.*, (2007).

**Table 3.** Effect of heavy metals on peroxidase activity (g<sup>-1</sup>h<sup>-1</sup>) of three pulp wood tree species at 21 DAS.

Treatment Elements	Mg L <sup>-1</sup>	<i>Casuarina junghuhniana</i>	<i>Eucalyptus tereticornis</i>	<i>Leucaena leucocephala</i>
Cd	control	0.34 ± 0.015	0.25 ± 0.017	0.65 ± 0.003
	25	0.13 ± 0.006	0.06 ± 0.009	0.35 ± 0.006
	50	0.25 ± 0.012	0.13 ± 0.009	0.44 ± 0.010
	100	0.30 ± 0.012	0.18 ± 0.012	0.52 ± 0.003
Cr (VI)	Control	0.34 ± 0.015	0.25 ± 0.003	0.62 ± 0.003
	25	0.34 ± 0.015	0.19 ± 0.012	0.16 ± 0.009
	50	0.44 ± 0.007	0.22 ± 0.020	0.34 ± 0.006
	100	0.49 ± 0.009	0.28 ± 0.006	0.55 ± 0.007
Cr (III)	Control	0.34 ± 0.015	0.25 ± 0.017	0.65 ± 0.003
	10	0.23 ± 0.009	0.11 ± 0.015	0.43 ± 0.003
	20	0.29 ± 0.006	0.16 ± 0.006	0.48 ± 0.003
	40	0.35 ± 0.009	0.79 ± 0.003	0.47 ± 0.006
ANOVA	T	P = 0.1264**		
	S	P = 0.1095**		
	T x S	P = 0.2189**		

Values are represented in mean ± Standard Error with three replicates, P = Correlation Coefficient, \*\* Indicates significance at 0.01%, ANOVA-Analysis of Variance, T-Treatment, S-Species.

**Table 4.** Phyto-extraction of heavy metals (mg kg<sup>-1</sup>) in three pulp wood tree species at 21 DAS.

Treatment Elements	Mg L <sup>-1</sup>	<i>Casuarina junghuhniana</i>	<i>Eucalyptus tereticornis</i>	<i>Leucaena leucocephala</i>
Cd	control	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	25	18.42 ± 0.58	20.31 ± 0.16	08.21 ± 0.10
	50	31.15 ± 0.41	39.24 ± 1.00	14.28 ± 0.21
	100	35.25 ± 0.14	45.22 ± 0.54	22.17 ± 0.30
Cr (VI)	Control	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	25	07.26 ± 0.15	08.21 ± 0.13	03.22 ± 0.16
	50	14.29 ± 0.18	17.17 ± 0.04	05.39 ± 0.26
	100	18.09 ± 0.58	23.18 ± 0.61	09.40 ± 0.53
Cr (III)	Control	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	10	15.15 ± 0.60	17.29 ± 1.02	07.25 ± 0.15
	20	25.21 ± 0.45	32.15 ± 0.06	13.36 ± 0.11
	40	31.22 ± 0.12	11.37 ± 0.25	18.40 ± 0.54
ANOVA	T	P = 2.3680**		
	S	P = 2.0508**		
	T x S	P = 4.5015**		

Values are represented in mean  $\pm$  Standard Error with three replicates, P = Correlation Coefficient,  
\*\* Indicates significance at 0.01%, ANOVA-Analysis of Variance, T-Treatment, S-Species.

### Heavy metal accumulation in different pulp wood species

Among the three tree species, *Eucalyptus tereticornis* accumulated significantly more heavy metals (Cd, Cr (VI) and Cr (III)) with the recorded value of 17.85 mg kg<sup>-1</sup> which was followed by *Casuarina junghuhniana* (16.33 mg kg<sup>-1</sup>) and *Leucaena leucocephala* (8.47 mg kg<sup>-1</sup>). It is important to note that, *Eucalyptus tereticornis* and *Casuarina junghuhniana* which are non leguminous species; uptake significantly higher ( $p < 0.01$ ) metals as compared to *Leucaena leucocephala*, a leguminous species, and uptakes less metal from soil (Table 4). Many researchers have investigated plant species capable of accumulating unwanted metal elements (Sanita di Toppi and Gabbriellini, 1999; Rout *et al.*, 2000). Reeves and Baker (2000) compiled a list of plant species that hyper-accumulate Cd, Cr, Ni, Pb, Se and Zn. The metal-accumulating plants identified so far are slow growing, small, and/or weedy plants that produce low biomass and have undefined growth requirements and characteristics. The two most important characters include the ability to accumulate large quantities in biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar *et al.*, 1995; Blaylock *et al.*, 1997; McGrath, 1998). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. Considering the type of heavy metals, Cd recorded significantly higher ( $p < 0.01$ ) accumulation in seedlings with 19.52 mg kg<sup>-1</sup> compared to Cr (III) (14.28 mg kg<sup>-1</sup>) and Cr (VI) (8.85 mg kg<sup>-1</sup>). This is because cadmium is a very mobile and bio-available metal which may accumulate in crops (Alloway, 1995). The poor translocation of Cr from roots to shoots is a major hurdle in using plants and trees for phyto-remediation. Pulford *et al.*, (2001) in a study with temperate trees confirmed that Cr was poorly taken up into the aerial tissues but was held predominantly in the root. These findings mean that the prospects for using trees as phyto-remediators on Cr-contaminated sites are low, their main value being to stabilize and monitor a site (Shanker *et al.*, 2003b).

### Conclusions

Increased concentration of heavy metals above the critical level severely reduced the total dry matter production and affects the activities of plant enzymes like catalase and peroxidase. Results indicated that these metal elements are toxic to *Casuarina junghuhniana*, *Eucalyptus tereticornis* and *Leucaena leucocephala* at seedling stage if applied at higher concentrations. The study also suggested that Cr (VI) is more toxic to these seedlings as compared to Cr (III) and Cd. However, *Eucalyptus tereticornis* is more tolerant to heavy metal toxicity than *Leucaena leucocephala* and *Casuarina junghuhniana*.

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