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THE INFLUENCE OF CADMIUM AND CHROMIUM ON THE BIOMASS PRODUCTION OF SHISHAM (*DALBERGIA SISSOO* ROXB.) SEEDLINGS

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Abstract

The present study investigated the effects of Cadmium (Cd) and Chromium (Cr) on the growth of Shisham (*Dalbergia sissoo Roxb.*) seedlings. Metal elements were applied in the form of $Cd(SO_4)_2$ and K_2CrO_4 at variegated concentrations of 0, 10, 20, 40 and 80 mg L^{-1} under controlled conditions of light and temperature for four weeks. Response of the seedlings was monitored in terms of number of leaves, shoot and root length, leaves, root and shoot weight (fresh and oven dry) and chlorophyll contents. Decline in growth was recorded after 10 mg L^{-1} and 40 mg L^{-1} for Cr and Cd application respectively. Similarly, combined application of Cd and Cr showed growth reduction beyond 20 mg L^{-1} dose. Chromium appeared to be more toxic to *Dalbergia sissoo* as compared to Cd at seedling stage.

Introduction

Industrialization and urbanization have not only adversely effected the crop production through land degradation but also contaminated the water resources (Zekri & Koo, 1994). In addition to carrying organic pollutants, the effluents are impregnated with heavy metals like Cd, Cr, Fe, Zn, Pb etc. A continuous use of such effluents for irrigation of soil may result in the accumulation of the metal elements to a level that may turn it phytotoxic (Davis & Jones, 1989). Among heavy metals Cd and Cr are considered potentially important environmental pollutants. At low concentration, cadmium is not toxic to plant but retards root growth (Zou, *et al.*, 2008) and cell division at higher concentration (Liu *et al*., 1992), 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 isoloation, 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). Phytoextraction, a plant based technology for the removal of contaminants and heavy metals from polluted waters and soils, is evolving rapidly. Plants are reported to be used to clean wastewater for decades because they serve as effective biological sieves and inhibits contamination of ground water sources through extensive root system (Karpiscak *et al*. 1996). Many researchers have investigated plant

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species capable of accumulating unwanted metal elements (Sanita´ di Toppi & Gabbrielli, 1999; Rout *et al*., 2000). Reeves & Baker (2000) compiled a list of plant species that hyperaccumulate 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. Therefore, an effort has been made to investigate the effects of Cd and Cr on biomass production of *Dalbergia sissoo* at seedling stage under controlled conditions of light and temperature. Shisham is selected because it is moderately fast growing; high biomass producing plant and has wide adaptability to climatic and edaphic conditions and comes at the bottom of food chain of human and animal

Materials and Methods

Dalbergia sissoo seeds were collected from botanical garden of the University of the Punjab, Lahore, during April, 2007. The seeds were immersed in 3% v/v formaldehyde for five minutes to avoid fungal contamination and were sown in plastic pots containing 100 g thoroughly mixed loamy soil. Saturation percentage of the soil was calculated using US Salinity Lab procedure (USDA Handbook, 1954).

The pots containing the seeds were placed in the growing chamber giving 300 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation with 16:8 hours photoperiod. The pots were rotated daily for the uniform light exposure. The ambient temperature of the chamber was maintained at 22±2 $^{\circ}$C. Cadmium and chromium in the form of $Cd(SO_4)_2$ and K_2CrO_4 were applied at 10,20,40 and 80 mg L^{-1} for treatment. Cd and Cr solutions were prepared in deionized water. Control plants received no treatment except distilled water. Treatment was started when seedlings attained two to three leaves and was applied for four weeks. Seedlings were irrigated at field capacity and each time measured amount was applied. Each treatment was replicated thrice for statistical analysis.

The seedlings were uprooted at the end of the experiment and thoroughly washed with deionized water. Root length, shoot length and number of leaves were recorded immediately. The seedlings were separated into roots, shoots and leaves for fresh weight calculation. Plant parts were then oven dried at 70 $^{\circ}$C for 72 h till constant weight. Oven dried weights were recorded to measure the extent of treatment application.

The chlorophyll contents of the plants were extracted by homogenizing 0.1 g fresh leaf material with 10 ml of 80% acetone in a mortar and the mixture was then centrifuged at 5000 rpm for ten minutes. The color intensity of the extract was measured at wave length of 445, 645 and 663nm using UV spectrophotometer and *chlorophyll a, chlorophyll b* and *total chlorophyll* were calculated using a relationship after Arnon (1949).

The data were analyzed using a one way ANOVA (Steel & Torrie, 1981) and statistical package SPSS, version 11.0. The treatment mean values were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

As shown in table 1, an increase of 22%, 4.8% and 8.2% in number of leaves as compared to control seedlings was observed at 10 mg L^{-1} dose of Cd, Cr and Cd+Cr respectively. Beyond that a significant reduction ($p<0.05$) in leaves number was recorded as the concentration of metal elements applied was increased. However, a substantive decrease of 47%, 31% and 34% in number of leaves was noted at 80 mg L^{-1} dose of Cr, Cd and combined Cd/Cr, respectively. Tripathi *et al*., (1999) reported similar results in an experiment where 200ppm of Cr (VI) severely affected the leaf area and biomass of *Albizia lebbek.*

A significant ($p<0.05$) reduction in shoot length was evident beyond 40, 20 and 20 mg L^{-1} dose of Cd, Cr and combination of Cd/Cr. At 80 mg L^{-1} dose of each treatment, heavy metals remarkably retarded the seedling growth and shoot length reduction up to 33%, 61% and 39% as compared to control was recorded for Cd, Cr and Cd/Cr mixture. Our results are in agreement with the findings of Barton *et al.*(2000) in lucerne culture.

Reduction in shoot growth could be attributed to the reduction in chlorophyll contents and activity of photosystem I induced by heavy metal stresses (Skorzynska-Polit & Baszynski, 1997). Similarly, metal elements transported to above ground plant part reduced height by disturbing the cellular metabolism of the shoots (Shanker *et al*., 2005).

The effect of treatment was more pronounced in case of root length as compared to shoot length. A significant reduction in root length was found at 20 mg L^{-1} and above doses of Cd compared to control while all the Cr applications reduced the root length significantly. However, combined application of Cd and Cr ameliorated the effect of Cr toxicity to some extent and a significant reduction in root length was observed beyond 20 mg L^{-1} dose of Cd/Cr mixture. Maximum reduction of 33%, 51% and 28% as compared to control was recorded at 80 mg L^{-1} application of Cd, Cr and Cd+Cr respectively. Roots are more prone to heavy metal toxicity relative to shoots (Oncel *et al.*, 2000) and it is evident that roots are the first victim of toxicity of metals than shoots. Cr is more toxic to *Dalbergia sissoo* at seedling stage than Cd. However, the combined application of Cd

Table 1 Metal element dose effect on the number of leaves, shoot length, root length and seedling length of *Dalbergia sisso* Roxb.

Metal	Dose mg L^{-1}	Metal element effect on various parts of seedlings			
		No. of Leaf	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)
Cd	0	13.67(0.88)^{b}	13.40(.45)^{a}	7.90(.21)^{a}	21.30(.30)^{a}
	10	16.67(0.88)^{a}	12.17(.60)^{a}	7.47(.49)^{a}	19.97(.42)^{a}
	20	13.33(0.90)^{b}	12.0(1.77)^{a}	7.2(.30)^{ab}	19.2(1.55)^{a}
	40	11.0(1.15)^{bc}	11.97(.32)^{a}	6.9(1.25)^{ab}	18.87(1.54)^{a}
	80	9.33(.85)^{c}	9.03(.02)^{b}	5.22(.06)^{b}	14.25(.05)^{b}
Cr	0	13.67(0.88)^{a}	13.40(.45)^{a}	7.90(.21)^{a}	21.30(.30)^{a}
	10	14.33(.67)^{a}	14.0(3.55)^{a}	7.33(.17)^{b}	21.67(3.71)^{a}
	20	10.67(.88)^{b}	11.50(1.44)^{ab}	6.003(.95)^{c}	17.503(2.32)^{a}
	40	9.0(.58)^{bc}	6.37(.09)^{bc}	5.0(.06)^{d}	11.37(.09)^{b}
	80	7.20(.40)^{c}	5.20(.17)^{c}	3.90(.23)^{e}	9.1(.07)^{b}
Cd+Cr	0	13.67(0.88)^{ab}	13.40(.45)^{a}	7.90(.21)^{ab}	21.30(.30)^{a}
	10	15.33(2.90)^{a}	13.63(.35)^{a}	7.87(.32)^{ab}	21.5(.66)^{a}
	20	12.67(2.33)^{ab}	11.87(.24)^{a}	8.17(.33)^{a}	20.07(.38)^{a}
	40	10.67(.88)^{ab}	9.33(1.20)^{b}	7.2(.173)^{b}	16.53(1.37)^{b}
	80	9.07(.07)	8.19(.07)^{b}	5.7(.15)^{c}	13.89(.19)^{c}

Values in parenthesis± SE Means with different letters are significantly different from each other ($p \leq 0.05$; n=3) and Cr somehow stifles the overall toxicity of Cr. Sensitivity of Alfalfa to Cr (VI) over Cd (II) and other heavy metals has been reported by Peralta-Videa *et al*. (2002). Similarly, Cr (VI) at low level reduces the chlorophyll biosynthesis and activity of various enzymes and thus becomes phytotoxic (Vajpayee, *et al*., 2000). Reduction in root length owes to the accumulation of metals in the root mass, which lowers the rate of mitosis in the meristematic zones of roots, especially blocking the metaphase in meristematic cells. Consequently, a decrease in root length. These results are in agreement with the findings of Peralta-Videa *et al*. (2000) where the 5ppm dose of Cd (II), Cr (VI), Cu(II), Ni (II) and Zn (II) promoted root and shoot growth of alfalfa compared to control.

As far as the seedling length is concerned, it followed the same trend of reduction as in the case of shoot and root length. Similar reduction in root, shoot and seedling length and seedling dry weight in *Albizia lebbeck* and *Leucaena leucocephala* due to the application of different concentrations of lead and cadmium was reported by Iqbal & Shazia (2004).

Table 2 reports the fresh and oven dry biomass production of *Dalbergia sissoo* seedlings as affected by four week application of various concentrations of metal elements. Significant ($p<0.05$) reduction in leaf fresh weight was recorded beyond 20 and10 mg L^{1}dose of Cd and Cr respectively. While an increase of 30% in leaf fresh weight over control was observed at the combined application of these metal elements and a significant ($p<0.05$) reduction was observed at 80 mg L^{-1} concentration compared to control plants. An increase of 38% and 4% over control in terms of leaf weight (oven dry) at 10 and 20 mg L^{-1} Cd was recorded beyond that reduction was significant. Similarly, all Cr treatments affected the seedling leaf weight (oven dry weight) significantly over control, while the combination of Cd/Cr enhanced the leaf oven dry weight by 73% and 12% at 10 and 20 mg L^{-1} dose respectively, beyond that significant reduction in leaf weight (oven dry) was observed. Maximum reduction of 19%, 73% and 19% was recorded at 80 mg L^{-1} Cd, Cr and Cd+Cr application, respectively. Growth reduction may be generally linked to a loss of cellular turgor resulting in either a decrease of mitotic activity and/or an inhibition of cell elongation (Gabrielli *et al*., 1990).

Shoot fresh weight was appeared to be reduced beyond 40 mg L^{-1} dose of Cd while fresh weight reduction was significant after 10 mg L^{-1} in case of Cr treatment; however, Cd/Cr combined application showed significant shoot weight (fresh) reduction at 80 mg L^{-1}. An increase of 42% and 100% over control was observed at 20 and 40 mg L^{-1} dose of Cd and Cd+Cr. In case of Cr treatment, the shoot oven dry weight showed no significant difference among treatments.

Trend of increase in root weight (fresh and oven dry) was observed and maximum increase of 39% and 51% in root weight (fresh and oven dry) over control was recorded at 20 mg L^{-1} Cd. However, 40 mg L^{-1}dose of Cd affected the root fresh and oven dry weight over control significantly. The decrease in shoot biomass 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). Root dry weight showed reduction after 10 mg L^{-1} Cr application; however Cd+Cr treatment showed maximum dry weight at 40 mg L^{-1}. The results indicate the extent of tolerance of shisham 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*

Table 2 Effect of toxic metals on biomass production of *Dalbergia sissoo* seedlings

Metal	Dose mg L^{-1}	Effect of heavy metals on biomass of seedlings					
		Fresh weight(g)	Oven dry weight(g)				
		Leaf	Shoot	Root	Leaf	Shoot	Root
Cd	0	0.077(0.001) ^{b}	0.069(0.004) ^{a}	0.019(0.001) ^{bc}	0.026(0.003) ^{ab}	0.019(0.002) ^{b}	0.008(0.001) ^{c}
	10	0.093(0.001) ^{a}	0.072(0.001) ^{a}	0.026(0.003) ^{abc}	0.036(0.006) ^{a}	0.026(0.001) ^{a}	0.015(0.001) ^{ab}
	20	0.089(0.002) ^{a}	0.07(0.004) ^{a}	0.031(0.005) ^{a}	0.027(0.001) ^{ab}	0.027(0.002) ^{a}	0.017(0.001) ^{a}
	40	0.064(0.002) ^{c}	0.066(0.003) ^{a}	0.029(0.001) ^{ab}	0.022(0.002) ^{b}	0.022(0.001) ^{b}	0.012(0.001) ^{bc}
	80	0.05(0.005) ^{d}	0.04(0.006) ^{b}	0.017(0.003) ^{c}	0.021(0.001) ^{b}	0.02(0.002) ^{b}	0.01(0.001) ^{c}
Cr	0	0.077(0.001) ^{a}	0.069(0.004) ^{a}	0.019(0.001) ^{a}	0.026(0.003) ^{a}	0.019(0.002) ^{a}	0.008(0.001) ^{a}
	10	0.087(0.03) ^{a}	0.07(0.026) ^{a}	0.02(0.01) ^{ab}	0.02(0.004) ^{ab}	0.02(0.006) ^{a}	0.008(0.002) ^{ab}
	20	0.06(0.015) ^{ab}	0.05(0.01) ^{ab}	0.01(0.005) ^{ab}	0.02(0.002) ^{ab}	0.02(0.001) ^{a}	0.007(0.001) ^{ab}
	40	0.03(0.001) ^{b}	0.03(0.001) ^{b}	0.02(0.002) ^{ab}	0.01(0.001) ^{bc}	0.015(0.063) ^{a}	0.007(0.002) ^{ab}
	80	0.02(0.005) ^{b}	0.02(0.006) ^{b}	0.009(0.001) ^{b}	0.007(0.001) ^{c}	0.01(0.007) ^{a}	0.003(0.001) ^{b}
Cd+Cr	0	0.077(0.001) ^{bc}	0.069(0.004) ^{a}	0.019(0.001) ^{b}	0.026(0.003) ^{b}	0.019(0.002) ^{b}	0.008(0.001) ^{b}
	10	0.11(0.003) ^{a}	0.07(0.001) ^{a}	0.03(0.001) ^{a}	0.045(0.006) ^{a}	0.039(0.006) ^{a}	0.009(0.0004) ^{b}
	20	0.09(0.01) ^{b}	0.07(0.01) ^{a}	0.03(0.003) ^{a}	0.029(0.005) ^{b}	0.038(0.005) ^{a}	0.014(0.003) ^{ab}
	40	0.08(0.006) ^{b}	0.07(0.003) ^{a}	0.02(0.003) ^{ab}	0.023(0.002) ^{b}	0.038(0.001) ^{a}	0.017(0.001) ^{a}
	80	0.06(0.003) ^{c}	0.04(0.003) ^{b}	0.02(0.002) ^{b}	0.021(0.002) ^{b}	0.02(0.001) ^{b}	0.009(0.003) ^{b}

Values in parenthesis± SE Means with different letters are significantly different from each other ($p \leq 0.05$; n=3)

vulgaris at Cr (VI) dose ranging between 25-100 mg L^{-1} 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).

As shown in Table 3, a significant ($p< 0.05$) increase of 11%, 21% and 15% in c*hlorophyll a*, *chlorophyll b* and total *chlorophyll* contents over control was evident at 40 mg L^{-1} dose of Cd. However, beyond that a decline in chlorophyll values can be seen. A reduction in chlorophyll owes to the cadmium toxicity as it effects stomata and mesophyll cells, which decreases their efficiency of light utilization and electron transport rates involving PS II and PS I. Consequently, there is a decline in chlorophyll contents (Elloumi *et al*., 2007). Reduction in chlorophyll values was appeared to be significant ($p<0.05$) at all Cr treatments and maximum reduction of 36%, 39% and 38% in c*hlorophyll a*, *chlorophyll b* and total *chlorophyll* contents was recorded at 80 mg L^{-1} of Cr. Toxic effects of chromium on photosynthetic pigment of crops and tree species are well documented by Vajpayee *et al*. (1999).

Combined application of these metals showed significant reduction in c*hlorophyll a*, *chlorophyll b* and total *chlorophyll* beyond 40 mg L^{-1} dose. The data correspond to the findings of Peralta-Videa *et al.* (2001). The reduction in chlorophyll contents can be explained as irrigation of seedlings of shisham with metal elements loaded water may cause stomatal closure due to elevated CO_2 contents. This phenomenon leads to the chlorophyll reduction (Vitoria *et al*., 2003; Van Assche & Clijsters, 1983).

Response of seedling to metal elements was in accordance with chemical hormesis process quite common in nature (Peralta-Videa *et. al*., 2001; Calabrese & Baldwin,

Table 3 Effects of heavy metals on chlorophyll contents of *Dalbergia sissoo* seedlings

Metal	Dose mg L^{-1}	Effect of heavy metals on chlorophyll contents of seedlings		
		Chlorophyll a **(mg g^{-1})**	***Chlorophyll b*** **(mg g^{-1})**	***Total Chlorophyll*** **(mg g^{-1})**
Cd	0	19.41(.99) ^{ab}	13.16(.92) ^{ab}	32.57(1.91) ^{abc}
	10	18.93(1.84) ^{ab}	10.96(.77) ^{b}	29.89(2.48) ^{bc}
	20	21.09(.03) ^{a}	14.25(.17) ^{ab}	35.46(.129) ^{ab}
	40	21.49(1.09) ^{a}	15.93(1.89) ^{a}	37.5(2.93) ^{a}
	80	17.24(.44) ^{b}	10.85(1.28) ^{b}	28.02(1.56) ^{c}
Cr	0	19.41(.99) ^{a}	13.16(.92) ^{a}	32.57(1.91) ^{a}
	10	17.54(.60) ^{ab}	10.98(.49) ^{ab}	28.52(1.09) ^{ab}
	20	16.96(2.76) ^{ab}	10.98(2.01) ^{ab}	24.94(5.11) ^{ab}
	40	14.76(.16) ^{bc}	9.05(.059) ^{b}	22.6(.798) ^{b}
	80	12.30(.115) ^{c}	8.00(1.15) ^{b}	20.30(.58) ^{b}
Cd+Cr	0	19.41(.99) ^{ab}	13.16(.92) ^{a}	32.57(1.91) ^{a}
	10	19.98(2.74) ^{a}	15.28(1.78) ^{a}	33.47(4.64) ^{a}
	20	21.39(1.02) ^{a}	15.29(.97) ^{a}	36.68(1.95) ^{a}
	40	20.41(.71) ^{a}	14.50(.51) ^{a}	34.92(1.22) ^{a}
	80	14.95(.90) ^{b}	9.34(.46) ^{b}	24.29(1.31) ^{b}

Values in parenthesis± SE Means with different letters are significantly different from each other ($p \leq 0.05$; n=3)

2003). Low dose of metal elements accordingly stimulate the seedling growth while gradual increase of dose inhibit the growth.

Conclusions: At low concentrations, these metals act as micronutrient causing a rapid overall growth of seedlings as compared to control. However, increasing the concentration of these metals above the critical level severely inhibits the seedling growth in terms of seedling length, root and shoot length, number of leaves, biomass and chlorophyll contents. Results indicate that these metal elements are toxic to shisham at seedling stage if applied at higher concentrations. The study suggests that Cr is more toxic to *Dalbergia sissoo* seedlings as compared to Cd.

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