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RESPONSE OF *EUCALYPTUS CAMALDULENSIS* TO EXOGENOUS APPLICATION OF CADMIUM AND CHROMIUM

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Abstract

A pot experiment was conducted to evaluate the effect of synthetic wastewater containing various concentrations of cadmium and chromium on *Eucalyptus camaldulensis*. Six month-old seedlings of *E. camaldulensis* were treated with Cd (II) and Cr (VI) for 18 months. Treatments were; T_0: (Tap water, control), T_1: 0.05 +1.0 mg L^{-1}, T_2: 0.10+2.0 mg L^{-1}, T_3: 0.20+4.0 mg L^{-1} and T_4: 0.40+8.0 mg L^{-1} of Cd and Cr respectively. Maximum growth in terms of shoot length (248.33 cm), collar diameter (1.78 cm), number of branches (20), number of leaves (504), root diameter (1.3 cm), leaf fresh weight (166.33 g), stem fresh weight (353.33 g), root fresh weight (201.33 g), leaf oven dry weight (73.67 g), stem oven dry weight (166 g) root oven dry weight (100 g) and root/shoot ratio (0.3) was recorded at T_1 treated plants beyond that a gradual decline in growth was observed with a maximum reduction of 10.5, 18.7, 31.7, 53.4, 21.8, 18, 57.6, 35.8, 20, 58, 34, 27 and 0.07% respectively in T_4 treatment compared to control (T_0). Cd and Cr accumulation in tissues increased (roots>shoot>leaves) as external metal concentration increased, while nutrients accumulation (K, P, Mg, Fe) and chlorophyll contents declined. The results indicated that Cd and Cr are toxic to *E. camaldulensis*.

Introduction

The discharge of metals into wastewater is a serious environmental problem, mainly in developing countries (Nouairi *et al*., 2006). In several countries raw wastewater with excess of heavy metals is used for agricultural production. This practice has raised the concentration of toxic elements to dangerous levels (Kisku *et al*., 2000). Among these elements Cr and Cd are of special concern because of their toxicity to plant kingdom, even at low concentrations (Shukla *et al*., 2007). Cr toxicity reduces yield affecting leaf and root growth and altering biochemical processes (Zou *et al*., 2006). Similarly, Cd has proven to be highly toxic to plants (Pinto *et al*., 2004). The interactions of Cd and metal nutrients can change nutrient concentration and composition in some plant species (Peralta-Videa *et al*., 2002). Higher contents of Cd in soil retard plant growth, reduce biomass production (Rai *et al*., 2005; Zou *et al*., 2008), cause changes in various physiological and biochemical characteristics and inhibits photosynthesis and mineral assimilation in plants (Scebba *et al*., 2006). The accumulation of these metals in plants may also pose a serious health hazard to human beings through food chain (Wagner, 1993).

The Hudiara drain (31 32′ 59″ N, 74 20′ 37″ E) carrying raw wastewater of over 120 different industrial units (textile, dying, tanneries, pharmaceutics and others) and municipal wastewater (Rashid & Majeed, 2002). The drain constitutes main tributary of the Ravi River with a total length of 98.6 Km; 44.2 km of which is in India and 54.4 Km in Pakistan. The discharge of the Hudiara drain at its source in Pakistan R.D. (Reduced distance) 138 is 73.3 m^3s^{-1} and at outfall R.D. (308) is 141.2 m^3s^{-1}. The overall characterization of the Hudiara drain revealed that the concentration of Cd and Cr were 0.11 mg L^{-1} and 2.6 mg L^{-1} respectively; almost several times higher than the permissible limits for irrigation water (Yasir *et al*., 2005). Currently, more than 120 pumps are installed along the drain in Pakistani territory for irrigation of agricultural land by local farmers, particularly to raise vegetables, fodder and fruit. Population in the vicinity of the drain are suffering from serious health hazards due to drinking drain contaminated shallow aquifer groundwater (Afzal *et al*., 2000).

This situation demands that wastewater be treated for safe agricultural use. Various techniques such as isolation, containment, mechanical separation, chemical treatment or soil flushing can effectively be employed to clean soils contaminated with metals (Mulligan *et al*., 2001). However, these techniques are not commonly used due to their inherent disadvantages (costly, labor intensive, requiring special equipments, causing soil disturbance and being effective for small areas). Phytoremediation, on the other hand, has attracted the researchers because of its cost-effectiveness, environment friendly nature, equal applicability for the removal of both organic and inorganic pollutants present in the soil, water and air (Yu *et al*., 2007). However, phytoremediation is effective only for shallow soil pollution and requires longer time period (Mulligan *et al*., 2001). Phytoremediation appears as a technically viable option for large-scale applications. However, plants that can guarantee high biomass yield as well as high heavy metal accumulation in their aerial parts are needed. Trees could be good choice for phytoremediation (Pulford *et al*., 2002).

The present study was designed to investigate the effects of two metals (Cd and Cr) on *Eucalyptus camaldulensis,* a moderately fast growing tree with high biomass production and wide adaptability to climatic and edaphic conditions and falls at the bottom of food chain. The species is best suited for plantation of degraded lands (waterlogged and saline soils) and disposal of untreated industrial wastewater safely and economically to tree plantation which helps to recapitulate the soil status and quality (Bhati & Singh, 2003) Six month old plants were treated for 18 months with various concentrations of Cd and Cr before harvesting. The effects of the treatments were evaluated through plant growth, metal uptake and chlorophyll production using different spectroscopic techniques.

Materials and Methods

Six month-old *Eucalyptus camaldulensis* seedlings were raised in plastic pots (diameter 35 cm and height 40 cm) containing thoroughly mixed loamy soil at the Botanical Garden of University of the Punjab, Lahore, Pakistan. Seedlings were irrigated with tap water for two months for proper establishment in the pots prior to treatment application. Stock solutions of Cd and Cr were prepared using salts of Cd $(SO_4)_2$ and K_2CrO_4 (Merck, Germany). Seedlings were treated with various concentrations of Cd and Cr for 18 months (February 2006 to October 2007). Seedlings were irrigated at field capacity by applying six liter of water per pot contaminated with metal elements as tap water-control (To), 0.05+1.0 mg L^{-1} of Cd+Cr (T_1), 0.10+2.0 mg L^{-1} of Cd+Cr (T_2), 0.20+4.0 mg L^{-1} of Cd+Cr (T_3) and 0.40+8.0 mg L^{-1} of Cd+Cr (T_4). These concentrations were chosen because overall content of Cd and Cr in the Hudiara drain water used for crop irrigation were 0.11 mg L^{-1} and 2.6 mg L^{-1} respectively; almost several times higher than the permissible limits for irrigation water (Yasir *et al*., 2005). For the purpose of statistical analysis, each treatment was replicated three times in a completely randomized design.

At the end of the experiment, the number and weight of leaves was recorded, plants were uprooted, thoroughly washed with running tap water and rinsed with distilled water for subsequent analysis. Shoots were removed at the collar and collar diameter was measured using a vernier caliper and plant height was measured with a measuring tape. Shoot fresh weight, number of roots, root length and roots fresh weight were determined. Roots, shoots and leaves of all plants were cut into small pieces and dried in an oven (MMM, Medcenter, Einrichtungen GmbH, Gräfelfing, Germany) at 70 ºC until constant weight for dry biomass calculation and elemental analysis. Oven dried plant samples were ground in a Willey mill (Plant grinder 2000 SM, Retsch, Germany), passed through 2 mm sieve and kept in a desiccator before weighing for analysis of metals. Homogenous tissue samples were digested in 3:1 HNO_3:$HCLO_4$ (v/v) solution on a hot plate at 150-175ºC for about two h until clear liquid was obtained. Metal elements such as Fe, Mg, Cd and Cr were determined using flame atomic absorption spectrophotometer (Spectra 250 Plus, Varian, Mulgrave, Victoria, Australia). The concentration of phosphorous (P) was measured by using UV/Vis Spectrophotometer (Lambda 25, Perkin Elmer, Shelton, CT, USA) at 430 nm wavelength and sodium (Na) and potassium (K) contents were determined by flame photometer (PFP-7, Essex, England) after Jones *et al*., (1991). Control standard solutions were run at the start, during and at the end of sample analyses for continuous accuracy of the results and calibration of the instruments.

Chlorophyll a, chlorophyll b and total chlorophyll contents of plants were measured by extracting a one gram sample of fresh and fully expanded leaves sample in 80% acetone and measuring the color intensity of the extract at 470, 644.3 and 661.6 nm wavelength using UV/Vis Spectroscopy. Chlorophyll contents were computed by using the formula described by Shah *et al*., (2008). Data were analyzed using one way analysis of variance (ANOVA) after Steel & Torrie (1981) by using statistical package SPSS 11.0. (SPSS, Chicago, IL, USA). Treatment means were compared using Duncan Multiple Range Test ($p \leq 0.05$.

Results and Discussion

Effects of combined application of various concentrations of Cd and Cr on biomass production of *Eucalyptus* seedlings are presented in Table 1. Maximum seedling growth in terms of plant height (248.33 cm), collar diameter (1.78 cm), number of branches (20), number of leaves (504), root diameter (1.30 cm), root length (75 cm), leaf fresh (166.33 g) and dry weight (73.67 g), stem fresh (353.33 g) and dry weight (166 g), root fresh (201.33 g) and dry weight (100 g), root/shoot length ratio (0.30) and total seedling length (323.33 cm) was observed at T_1 treatment with a significant increase ($p<0.05$) of 12, 7.2, 41.4, 46.7, 9.2, 19, 51.2, 13.4, 12.7, 26.3, 11.9, 15.4, 7.1 and 13.6% respectively compared to control. Beyond T_1 treatment, a gradual reduction in growth parameters was observed. The increase in growth parameters at low concentration of Cd and Cr could be due to the presence of the phenomenon of hormesis, a dose dependent response of the seedlings where the low dose stimulates the growth while high dose suppresses the growth (Peralta-Videa *et al*., 2001; Calabrese, 2002; Shah *et al*., 2008).The results correspond to the findings of Dong *et al*., (2005), where an increase in the height of two cultivars of tomato was observed at low concentration of Cd (0.1 µmol L^{-1}).

Table 1. Effect of combined application of Cd and Cr on biomass production of *Eucalyptus camaldulensis*.

Treatment	Stem height (cm)	Diameter (cm)	No. of branches	No. of leaves	Root diameter (cm)	Root length (cm)	Fresh weight (g)			Oven dry weight (g)			Root/shoot ratio
							Leaf	Stem	Root	Leaf	Stem	Root	
T_1	248.33	1.78	20.0	504.0	1.30	75.0	166.33	353.33	201.33	73.67	166.0	100.0	0.30
	$(1.67)^{a}$	$(.10)^{a}$	$(2.31)^{a}$	$(32.08)^{a}$	$(.10)^{a}$	$(3.2)^{a}$	$(29.04)^{a}$	$(8.99)^{a}$	$(6.33)^{a}$	$(11.67)^{a}$	$(7.81)^{a}$	$(8.66)^{a}$	$(.11)^{a}$
T_2	212	1.53	15.67	316.0	1.11	60.33	101.67	298.67	173.33	51.0	129.0	80.0	0.28
	$(3.71)^{bc}$	$(.04)^{bc}$	$(1.45)^{ab}$	$(6.81)^{b}$	$(.03)^{bc}$	$(2.4)^{bc}$	$(6.36)^{b}$	$(20.69)^{b}$	$(8.35)^{b}$	$(4.62)^{ab}$	$(13.65)^{bc}$	$(5.77)^{abc}$	$(.01)^{a}$
T_3	211.33	1.45	11.33	190.0	1.01	53.33	65.0	230.0	158.33	32.0	106.67	75.0	0.26
	$(2.84)^{bc}$	$(.05)^{cd}$	$(1.45)^{bc}$	$(31.75)^{c}$	$(.04)^{bc}$	$(2.91)^{bc}$	$(9.87)^{bc}$	$(20.21)^{c}$	$(11.67)^{bc}$	$(6.24)^{bc}$	$(11.67)^{cd}$	$(8.66)^{bc}$	$(.01)^{a}$
T_4	198.33	1.35	9.33	160.0	.93	51.67	46.67	200.0	143.0	24.67	97.0	63.0	0.26
	$(3.61)^{c}$	$(.02)^{d}$	$(1.45)^{c}$	$(14.77)^{c}$	$(.03)^{c}$	$(3.33)^{c}$	$(4.18)^{c}$	$(10.41)^{c}$	$(3.21)^{c}$	$(2.23)^{c}$	$(4.04)^{d}$	$(3.75)^{c}$	$(.02)^{a}$

T_0= Tap water (control); T_1= 0.05+1.0 mg L^{-1}; T_2= 0.10+2.0 mg L^{-1}; T_3= 0.20+4.0 mg L^{-1}; T_4= 0.40+8.0 mg L^{-1}, (n=3; values in parenthesis ± SE m, values with different letters are significantly different from each other at $p \leq 0.05$).

Roots being the first plant organ in contact with the metal element(s) appeared to be heavily affected by their toxicity. In this study, a maximum reduction in root length (17.9%) and root diameter (21.8%) was observed at T_4 treatment which was greater than the reduction in shoot length (10%) and shoot diameter (18.67%) at the same treatment. The reduction in root growth may be due to the hampering of nutrient uptake by plants because of high solute potential in the rhizosphere leading to a decrease of mitotic division of meristematic cells (Odjegba & Fasidi, 2004). Similar growth inhibition in *Lolium perenne* L., treated with chromium was observed by Vernay *et al.,* (2007). There was a gradual reduction in seedling length with an increase in the concentration of metals (Cd and Cr) with a maximum reduction of 12.3% at T_4 compared to control (Table 1). The direct influence of metals on the cellular metabolism of shoots may lead to the reduction of seedling length in metal stressed seedlings (Shanker *et al*., 2005). Leaf growth and total number of leaves are considered a suitable bioindicators of metal toxicity. It is apparent from the results that Cd and Cr application severely affected the plant growth in terms of leaf biomass production. The increasing concentration of metal dose reduced the number of leaves significantly ($p<0.05$), and maximum reduction in number of leaves (53%) was observed at T_4 treatment. Similar reduction in leaf weight (fresh and oven) was recorded with a maximum reduction of 58% at T_4 compared to control. There was a significant ($p<0.05$) reduction in biomass of seedlings under increasing concentration of metal elements (Table 1) which is in agreement with the findings of Shukla *et al*., (2007) where combined application of Cd and Cr showed an inhibitory effect on the growth of *Bacopa monnieri* L., and gradual reduction in its biomass with increasing Cd and Cr contents in nutrient medium. Reduction in biomass production under the influence of Cd and Cr may be due to impairment of uptake and translation of nutrients and water in aerial plant parts (Svetková & Fargašová, 2007).

The highest values of chlorophyll a (6.69 mg g^{-1}), chlorophyll b (5.43 mg g^{-1}) and total chlorophyll (12.1 mg g^{-1}) contents were recorded at T_1 with a significant increase of 27.4, 9.5 and 18.5% compared to control (Table 2). Beyond that a significant ($p<0.05$) reduction in chlorophyll contents with increasing concentration of Cd and Cr compared to control was recorded. However, values for chlorophyll a/b ratio with treatment application were not significantly changed ($p<0.05$). A gradual reduction in chlorophyll a (27%), chlorophyll b (32%) and total chlorophyll (29%) with increasing dose of metal elements (Cd and Cr) was recorded with a maximum reduction at T_4. This reduction may be due to chromium toxicity affecting chlorophyll synthesizing system and chlorophyllase activity (Van Assche & Clijsters, 1990). Decrease in chlorophyll contents of *Datura innoxia* treated with Cr has been reported by Vernay *et al*., (2008). Similarly, Cd may cause disorder in chlorophyll biosynthesis, PS II reaction centre is affected by Cd impairing enzyme activity and protein structure by interacting with functional SH- group of protein (Šimonová *et al*., 2007). A reduction of 20% and 50% in chlorophyll contents of maize plants at 15 and 25 mM Cd treatments, respectively was observed by Kranteva *et al*., (2008). An increase in chlorophyll a/b ratio at T_1 compared to control plants (T_0) is recorded which is mainly due to the fact that chlorophyll b contents are affected more by the metal toxicity than chlorophyll a. Roots accumulated maximum K (3.56%), P (0.08%), Mg (1024.3 mg kg^{-1}) and Fe (824.5 mg kg^{-1}) at T_1 beyond that nutrient accumulation gradually declined (Table 3). However, Na, Cd and Cr showed a reverse trend and a gradual increase in the accumulation of these elements was evident with increasing concentration of metal elements in irrigation water. Cd and Cr are non essential and toxic for plant growth hence plants have no specific mechanism for uptake and translocation in plant system. These metal elements (Cd and Cr) effectively compete with the essential nutrients (P, Fe, K, Mg) for rapid entry into the plant system resulting in the reduction in uptake and translocation of essential nutrients in plant parts (Shanker *et al*., 2005).

Table 2. Variation in chlorophyll contents in response to industrial effluent application.

Treatment	Effect of industrial effluents on chlorophyll content (mg g^{-1})			Chhlorophyll a/b
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	
T_0	5.25(067) ^{ab}	4.96(0.55) ^{a}	10.21(1.22) ^{ab}	1.05(0.02) ^{a}
T_1	6.69(069)^{a}	5.43(0.52)^{a}	12.1(1.11)^{a}	1.24(0.11)^{a}
T_2	5.09(052)^{ab}	4.59(0.27) ^{a}	9.67(0.79) ^{abc}	1.1(0.05) ^{a}
T_3	4.69(0.19) ^{b}	4.27(0.14) ^{ab}	8.94(0.33) ^{bc}	1.09(0.01) ^{a}
T_4	3.83(0.08) ^{b}	3.37(0.03) ^{b}	7.21(0.11) ^{c}	1.14(0.17) ^{a}

T_0= Tap water (control); T_1= 0.05+1.0 mg L^{-1}; T_2= 0.10+2.0 mg L^{-1}; T_3= 0.20+4.0 mg L^{-1}; T_4= 0.40+8.0 mg L^{-1}; (n=3; values in parenthesis ± SE m, values with different letters are significantly different from each other at $p \leq 0.05$).

Table 3. Accumulation of mineral nutrients in various plant parts of *Eucaltptus camaldulensis.*

Treatment	K	Na	P	Mg	Fe	Cd	Cr
	(%)			(mg kg^{-1})			
				Roots			
T_0	3.83 (0.15) ^{a}	3.69 (0.07) ^{bc}	0.067 (0.008) ^{ab}	935.5 (22.06) ^{ab}	587.47 (56.39) ^{b}	1.67 (.33) ^{c}	136.83 (12.7) ^{d}
T_1	3.56 (0.32) ^{a}	3.11 (0.54) ^{c}	0.08 (0.016) ^{a}	1024.3 (21.42) ^{a}	824.5 (78.5) ^{a}	5.83 (1.7) ^{bc}	283.17 (64.14) ^{cd}
T_2	3.49 (0.27) ^{a}	3.61 (0.11) ^{bc}	0.057 (0.003) ^{abc}	896.17 (16.64) ^{ab}	545.23 (50.89) ^{b}	8.3 (1.92) ^{ab}	424.17 (80.76) ^{c}
T_3	3.2 (0.12) ^{ab}	4.32 (0.52) ^{ab}	0.039 (0.002) ^{bc}	824.5 (106.56 ^{b}	491.93 (55.7) ^{b}	11.17 (3.18) ^{ab}	875 (42.14) ^{b}
T_4	2.68 (0.06) ^{b}	4.87 (0.15) ^{a}	0.031 (0.01) ^{c}	786.5 (13.77) ^{b}	439.03 (43.43) ^{b}	12.83 (1.01) ^{a}	1199.8 (123.17)^{a}
				Shoots			
T_0	3.45 (0.32) ^{ab}	1.35 (0.11) ^{c}	0.09 (0.006) ^{ab}	672.33 (47.48) ^{a}	134.23 (8.74) ^{b}	2.13 (0.20) ^{b}	307.83 (18.46) ^{c}
T_1	3.85 (0.34) ^{a}	1.35 (0.04) ^{c}	0.12 (0.02) ^{a}	719 (27.25) ^{a}	161.5 (14.5) ^{a}	2.63 (0.23) ^{ab}	318.33 (35.48) ^{c}
T_2	3.28 (0.13) ^{ab}	1.65 (0.17) ^{bc}	0.09 (0.001) ^{ab}	631.5 (45.18) ^{a}	132.06 (5.9) ^{b}	2.67 (0.19) ^{ab}	369.33 (5.55) ^{bc}
T_3	3.02 (0.09) ^{b}	1.91 (0.05) ^{ab}	0.08 (0.01) ^{b}	626.5 (13.1) ^{a}	112.9 (5.16) ^{bc}	2.92 (0.04) ^{ab}	408.17 (18.18) ^{ab}
T_4	2.8 (0.08) ^{b}	2.17 (0.09) ^{a}	0.06 (0.005) ^{b}	487.7 (16.5) ^{b}	98.67 (3.17) ^{c}	3.17 (0.49) ^{a}	447.33 (16.66) ^{a}
				Leaves			
T_0	5.51 (0.21) ^{b}	0.85 (0.04) ^{b}	0.11 (0.001) ^{ab}	1139.7 (3.94) ^{ab}	457.67 (13.84)^{ab}	0.86 (0.05) ^{b}	388.83 (30.46) ^{c}
T_1	6.92 (0.62) ^{a}	0.89 (0.04) ^{b}	0.13 (0.01) ^{a}	1165 (12.97) ^{a}	536.93 (25.17) ^{a}	1 (0.28) ^{b}	416.67 (22.83) ^{bc}
T_2	5.88 (0.38) ^{ab}	1.0 (0.05) ^{b}	0.11 (0.01) ^{ab}	1114.8 (9.27) ^{ab}	313.23 (67.17) ^{c}	1.17 (0.17) ^{ab}	422.33 (16.17) ^{bc}
T_3	5.31 (0.42) ^{b}	1.27 (0.25) ^{ab}	0.10 (0.001) ^{b}	1066.5 (38.9) ^{b}	445.67 (36.13)^{ab}	1.33 (0.33) ^{ab}	477.33 (24.4) ^{ab}
T_4	4.9 (0.21) ^{b}	1.6 (0.25) ^{a}	0.09 (0.001) ^{ab}	912.3 (40.21) ^{b}	375.67 (16.5) ^{bc}	1.83 (0.17) ^{a}	512.5 (20.5) ^{a}

T_0= Tap water (control); T_1= 0.05+1.0 mg L^{-1}; T_2= 0.10+2.0 mg L^{-1}; T_3= 0.20+4.0 mg L^{-1}; T_4= 0.40+8.0 mg L^{-1}, (n=3; values in parenthesis ± SE m, values with different letters are significantly different from each other at $p \leq 0.05$).

Roots usually accumulate significantly more metal than above ground plant parts might be due to the sequestration in the vacuoles of the root cells, which has been identified as a detoxification mechanism (Shanker *et al*., 2005). In the present study metal accumulation in plant tissues was in order of roots>shoot>leaves and the increase was proportional to the external application of metal elements (Nyquist *et al*., 2007).

Above ground plant parts (shoot and leaves) followed the similar trend as far the accumulation of nutrients and metal elements is concerned and highest values for K (3.85%), P (0.12%), Mg (719 mg kg^{-1}) and Fe (161.5 mg kg^{-1}) were recorded at T_1 beyond that they significantly ($p<0.05$) declined. However, with an increase in external Cd and Cr, the accumulation of these elements significantly ($p<0.05$) increased and highest values for Na (2.17%), Cd (3.17 mg kg^{-1}) and Cr (447.33 mg kg^{-1}) were recorded at T_4. Metal toxicity hampered the uptake and accumulation of essential nutrient in the above ground parts adversely affecting their growth and functioning.

The increase in the concentration of Cd and Cr in the irrigation water resulted in reduced uptake of nutrients essential for plant growth (Table 3). It may also be due to the inhibition of superoxide dismutase (SOD) and catalase (CAT) enzyme activity caused by heavy metal stress, which might have weakened the superoxide and H_2O_2 scavenging system ultimately reducing plant growth (Dey *et al.,* 2007). The data showed a gradual reduction in growth and biomass production of *Eucalyptus* seedlings under the influence of external application of Cd and Cr in the growth medium. Metal adversely affected the physiological functioning through reduction in chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll). This decline in chlorophyll concentrations might be caused by a reduction in the synthesis of chlorophyll by possibly increasing chlorophyllase activity, disorderness of chloroplast membrane, and inactivation of electron transport of photosystem II. Similarly, metals disturbed the nutritional balance of the seedlings through competitive uptake at the binding sites resulting in the reduction of plant growth. The results demonstrate a gradual reduction in growth and biomass production of *Eucalyptus* seedlings with increasing exogenous application of Cd and Cr in irrigation water.

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