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EFFECTS OF CADMIUM ON GERMINATION AND SEEDLING GROWTH OF *PROSOPIS JULIFLORA* (SWARTZ) DC. – A POTENTIAL METALLOPHYTE

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ABSTRACT

Germination and seedling growth of *Prosopis juliflora* (Swartz) DC. is investigated against cadmium toxicity. Cadmium concentration up to 400 ppm, *in vitro*, showed no detrimental effect on germination of abraded seeds, germination velocity was, of course impeded. Cotyledonary area per seedling (CAS) remained more or less unaffected (r = - 0.0025, NS) but radicle growth was inhibited drastically above 25 ppm. Compared to radicle, reduction in growth of hypocotyl was relatively of lower order. In Cd concentrations higher than 50 ppm, radicle appeared somewhat dehydrated, turned brown and almost burnt gradually in higher concentrations. TL_{50} values for Hypocotyl and radicle growth were 288 and 36 ppm of Cd, respectively. Such values calculated on the basis of regression for hypocotylar growth turned out to be 300 ppm. Radicle length related with Cd curvilinearly indicating 50 % reduction at 45.4 ppm of cadmium.

Seedling emergence and their growth, for a period of 50 days, were also evaluated in pot containing soil contaminated with 10 – 100 mg cadmium per kg soil. Soil appeared to provide protection to the seedlings against cadmium in comparison to their, *in vitro*, vulnerability to cadmium. There was delay in emergence velocity but (CAS) remained more or less unaffected up till 15th day of growth – CAS declined at later stage. Cadmium had no significant effect on hypocotylar growth. There was delay in primary leaf development. Fifty per cent reduction in growth of radicle, epicotyl, shoot (hypocotyl + epicotyl), number of leaves, number of leaflets per seedling and seedling biomass corresponded with 129.5, 136, 249.8, 169.2, 104.9, and 180 mg of cadmium per kg soil, respectively. Cadmium inhibited internodal elongation significantly. One individual each in 80 and 100 mg Cd per kg soil treatments died after 24 days of growth. The results are discussed in eco-physiological context.

Key words: *Prosopis juliflora* (Swartz) DC., Cadmium, Germination, Seedling growth.

INTRODUCTION

Industrial and agricultural activities of man result in heavy-metal pollution of soils. Their concentrations in soil may range from less than 01 mg/kg to as high as 100,000 mg/kg due to geological origin of the soil or as a result of human activity (Blaylock and Huang, 2000). Cadmium is among the top ten toxins in the environment and its toxicity is generally considered to be 2 – 20 times higher than other heavy metals (Jagodin *et al.*, 1995). It has no biological function and is highly toxic to plants and animals (Alloway, 1990). The problems associated with Cd-contaminated soils may partially be solved by phyto-remediation (Perfus-Barbeoch, *et al*, 2000; Raskin and Ensley, 2000; Prasad and Freitas, 2003). Such a strategy, however, requires understanding of Cd-toxicity at whole-plant basis as well as at cellular and molecular level.

P. juliflora is reported to accumulate a variety of elements such as Sr, B, and Ba when growing on pegmatite tailings from Nallore Mica Belt, Andhra Pradesh, India (Nagaraju and Prasad, 1998). Sr, B, Cu, Zn, and Pb were found in several times larger concentrations in leaves of *P. juliflora* than in the soil abounded by the plants in the region of Mengampeta Barytes deposit, Cuddapah Basin, Andhra Pradesh, India (Nagaraju and Rajesh, 2003). Al-Faraj and Al-Wabel (2007) have reported substantial bioaccumulation of cadmium in *Prosopis juliflora* plants growing on mining area at Mahad Ad'Dahab, Saudi Arabia. *Prosopis juliflora* is known to be sensitive to Chromium but much tolerant to Aluminum at germination and seedling growth stages (Jamal *et. al*, 2006). In view of accumulation efficiency of this species to Cd and Cu (Senthilkumar *et. al*., 2005), this paper investigates the performance of *Prosopis juliflora* against cadmium at germination and seedling growth stages, which constitute the key events for the establishment of plants under any prevailing environment (Welbaum *et. al.*, 1998). Such short-term growth/survival tests using seedlings or vegetative propagules are useful and have provided clear-cut results among species differing in their tolerance to metal toxicity (Grime, 1965; Baker, 1987).

MATERIALS AND METHODS

Germination and seedling growth in petri plates

Seeds of *Prosopis juliflora*, collected from Korangi Industrial area, Karachi, were surface sterilized in 2% sodium hypochlorite solution and slightly abraded with sand paper to overcome dormancy imposed due to hard seed coat (Khan *et al*, 1984). Twenty seeds were placed in 9 cm diameter petri plates lined with Whatman filter paper No.

1 and soaked with a series of cadmium sulphate solutions containing 25, 50, 100, 150, 200, 250, 300, 350, and 400 ppm cadmium. Controls received deionized water. The petri plates of each treatment and control were incubated in a growth chamber at 20 -22 ^{o}C. Germination counts were made daily. A seed was considered germinated if its radicle protruded out of the seed and attained a length of not less than 1.5 mm (Taylor, 1942). After six days of incubation, radicle and hypocotyl were measured.

Germination and seedling growth in pots

Ten surface sterilized and slightly scarified (for early emergence) seeds of *P. juliflora* were sown in plastic pot (with no basal perforation) containing 500g sandy garden soil of basic reaction (texture - sand: 86.5, silt: 8.2 and clay: 5.3%) treated with solution of cadmium sulphate ($(3CdSO_4.8H_2O)$; Merck, Germany; Mol. wt. 769.51) at the rate of 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mg Cd / Kg soil. In each case seeds were sown at a depth of 0.5 cm. The controls as well as treated plants were irrigated with 25 ml of de-ionized water at alternate day. The pots were kept in a propatray and light intensity atop pots was 3000 lux for 14 h day length. The experiment was continued up to 50 days of seedling growth – when cotyledons in nearly all seedlings had abscised. The emergence of seedlings was observed daily. Seedlings were carefully harvested at various point of time to measure seedling growth, which included parameters such as expansion of cotyledons, elongation of radicle, epicotyl, hypocotyl, and internodes, number of leaflets per leaf and number of leaves per seedling, etc. The one-sided area of cotyledons was measured by drawing their outlines on a graph paper. The ambient temperature during experimental period (October 2004 – December 2004) varied considerably. The mean monthly temperature which was 25.7 ^{o}C in October (lowest being 18 ^{o}C) declined to 21.8 ^{o}C in December (lowest being 12.5 ^{o}C).

Statistical analyses

Germination / emergence velocity was measured by Djavashir and Pourbeik (1976) index – GV3 = (Σ cn / d) / dm.gp) where cn is the cumulative number of seeds germinated or emerged by daily intervals; d, the serial number of the day; dm, the number of germination / emergence observations and gp, the final germination percentage. This index has been shown to be a better estimator of germination velocity (Shaukat and Gulzar, 2004). A 50% tolerance limit (TL_{50}) at which growth reduced to 50%, was computed using formula proposed by Davis *et al* (1972) - TL 50 = $C_1 + [(C_2 – C_1) (50 – P_1)] / (P2 – P_1)$ where C_1 = Highest concentration giving less than 50% growth reduction, C_2 = lowest concentration giving more than 50% growth reduction, P_1 percentage growth at C_1, and P_2 = percentage growth at C_2. Alternately, a technique of correlation and regression analysis was employed. ANOVAs were calculated wherever necessary. In case of two mortalities - one in 80 and one in 100 mg cadmium per Kg soil treatments in last days of experiment, missing values were calculated by the method described by Choudhry (1973).

$$x = rB + kT – G / (k-1) (r – 1)$$

Where r = number of replicates, k = number of treatments, B = sum of the remaining values in the block with the missing value, T = sum of the remaining values of the treatments with missing value and G = grand total of all observed values.

OBSERVATION AND RESULTS

Effect of Cadmium on Germination of Seeds and Seedling Growth in Petri-plates

Cadmium concentration up to 400 ppm showed no detrimental effect on germination of abraded seeds of *P. juliflora*. There was no germination on the first day but it was almost complete more or less within 72 h of incubation (Table 1). Seedling growth was, however, reduced significantly in cadmium environment. Cotyledonary area per seedling (CAS) remained more or less unaffected (r = - 0.0025, NS) but radicle growth was inhibited drastically in cadmium concentration above 25 ppm. Compared to radicle, reduction in growth of hypocotyl was relatively of lower order. Hypocotyl appeared somewhat thicker under influence of cadmium. In Cd concentrations higher than 50 ppm, radicle appeared somewhat dehydrated, turned brown and almost burnt gradually in higher concentrations (Table 2). Following equations were significant in accounting for the seedling growth. TL_{50} values calculated as per Davis *et al.* (1972) for Hypocotyl and radicle growth were 288 and 36 ppm of Cd, respectively. Such values calculated on the basis of equation # 1 for hypocotyl length turned out to be 300 ppm of Cd. Radicle

related with Cd curvilinearly (Fig. 1) and best fitted with equation # 2 indicating 50 % reduction in radicle length at 45.4 ppm of cadmium.

Hypocotyl Length (cm) = 1.9372 – 0.0032188 Cd (ppm) ± 0.453
t = 30.33 t = -11.26
p < 0.001 p < 0.001
r = - 0.6879; df =141; p < 0.001; F = 126.7 ……. (1)

Radicle Length (cm) = 3.1378 – 0.021369 Cd (ppm) + 0.0000395 Cd (ppm)^2 ± 0.547
t = 31.67 t = - 16.81 t = 12.49
p < 0.001 p < 0.001 p < 0.001
R^2 = 0.7692; adj. R^2 = 0.7659; df = 141; p < 0.001; F = 233.30……. (2)

Table 1. Effect of cadmium concentration on germination of *P. juliflora* seeds.

Day	Cadmium Concentration (ppm)									
	Control	25	50	100	150	200	250	300	350	400
1	0	0	0	0	0	0	0	0	0	0
2	80	80	80	80	66.6	80	80	86.6	66.6	86.6
3	86.6	100	93.3	86.6	100	80	80	93.3	80	86.6
4	100	100	93.3	100	100	100	100	93.3	86.6	100
5	100	100	100	100	100	100	100	93.3	86.6	100
6	100	100	100	100	100	100	100	93.3	86.6	100

Table 2. Effect of cadmium concentration on seedling growth of *P. juliflora*.*

Parameter	Cadmium Concentration (ppm)									
	Control	25	50	100	150	200	250	300	350	400
Radicle Length (cm)	3.93 ± 0.23	2.19± 0.15	1.69 ± 0.13	1.03 ± 0.09	0.71 ± 0.06	0.63 ± 0.06	0.55 ± 0.07	0.65 ± 0.05	0.59 ± 0.04	0.55 ± 0.02
Hypocotyl Length (cm)	1.92 ± 0.15	2.15 ± 0.13	1.96 ± 0.16	1.37 ± 0.09	1.20 ± 0.08	1.23 ± 0.07	1.11 ± 0.80	0.85 ± 0.04	0.75 ± 0.09	0.93 ± 0.12
Cotyledonary Area per Seedling (cm^2) **	1.46 ± 0.048	1.52 ± 0.058	1.46 ± 0.060	1.48 ± 0.042	1.50 ± 0.044	1.56 ± 0.046	1.46 ± 0.044	1.48 ± 0.046	1.47 ± 0.047	1.49 ± 0.048

*, The figures given in italics are significantly different from the control at least at p < 0.05 as given by t-test.
**, One-sided cotyledonary area.

Emergence of *P. juliflora* seedlings from soil added with Cadmium

Final percentage of emergence of seedlings in cadmium environment from 0 to 100 mg Cd / kg soil remained almost unaffected. Emergence velocity was definitely impeded significantly under the influence of cadmium (Fig. 2 & 3).

Seedling Growth of *P. juliflora* in soil contaminated with Cadmium

Various parameters of seedling growth were studied during 50-day period of growth of *P. juliflora* seedlings in soil added with 0 to 100 mg cadmium per kg of soil. Soil appeared to provide protection to the developing seedlings.

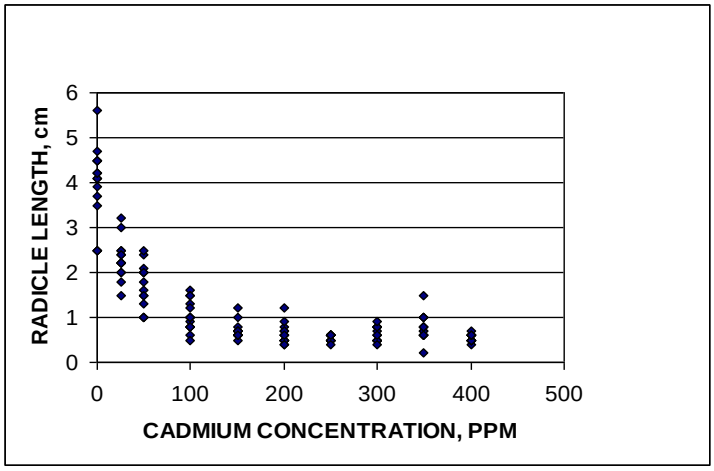

Fig. 1 . Effect of cadmium on radicle length of *P. juliflora* in petriplates.

Effect of Cadmium on Cotyledonary Expansion

The one-sided cotyledonary area per seedling (CAS) from emergence up till 20 days of growth under varying concentration of cadmium is presented in Fig.4. Area per cotyledon on second day of emergence was c 0.707 ± 0.024 cm^2. There was a gradual increase in cotyledonary area with age at least up to 15 days of growth. Compared to the controls, CAS on second and 5^{th} day of growth remained quite unaffected with the presence of Cadmium in the root environment. On 10^{th} day, Cd up to 80 mg / kg of soil appeared to generally induce promotion in cotyledonary area over control. Such a promotion, however, began to mitigate on 15^{th} day of growth. On 20^{th} day, clear cut inhibition of CAS over control took place more or less consistently with the increasing cadmium concentration.

Mortality

One seedling each in 80 and 100 mg Cd / kg soil treatments died on 24^{th} day of growth. The stems of these seedlings blackened near the soil surface, seedlings lost turgidity, bent and dehydrated.

Effect of Cadmium on Shoot and Root growth

For around 2 weeks after emergence much of the aboveground activity in shoot was in form of growth of hypocotyls and expansion of cotyledons. No appreciable development of epicotyls took place up till 20^{th} day of growth after emergence. Cadmium had no significant effect on hypocotylar length (R^2: 0.0069; F: 0.435 NS) but inhibited the growth of epicotyls significantly (Fig. 5 & 6) (F: 4.178; $p < 0.00027$). Two-Way ANOVA for the growth of epicotyl in soil containing 0 to 100 mg cadmium / kg soil for seedlings of various ages indicated significant negative effect of cadmium (F: 22.18, $p < 0.001$) and positive effect of seedling age (F: 258.21, $p < 0.001$) on epicotylar length (Fig.6). The interaction of cadmium with age was, however, statistically insignificant (F: 1.208, $p < 0.1508$).

For 50-day old seedlings, following equation (# 3) significantly related the epicotylar growth negatively with Cd concentration in the root zone. By this relationship 50% reduction in epicotylar growth corresponded with Cd concentration of 136 mg per kg of soil.

Epicotyl length (cm) = 5.8563 – 0.021476 Cd (mg / kg soil) ± 1.2045
t = 20.99 t = - 4.46
$p < 0.001$ $p < 0.001$
$r = - 0.4925$; df = 62; $p < 0.001$; F = 19.87 ……. (3)

The shoot (hypocotyl + epicotyl) as measured in 50-day old seedlings exhibited significant reduction in growth under influence of Cd (Fig. 7) and related well with the following equation (# 4). By this relationship 50% reduction in shoot growth corresponded with Cd concentration of 249.8 mg per kg of soil.

Shoot length (cm) = 9.65621 – 0.019326 Cd (mg / kg soil) ± 1.35
t = 30.69 t = - 3.56
p < 0.001 p < 0.001
r = - 0.4117; df = 62; p < 0.001; F = 12.65 …….... (4)

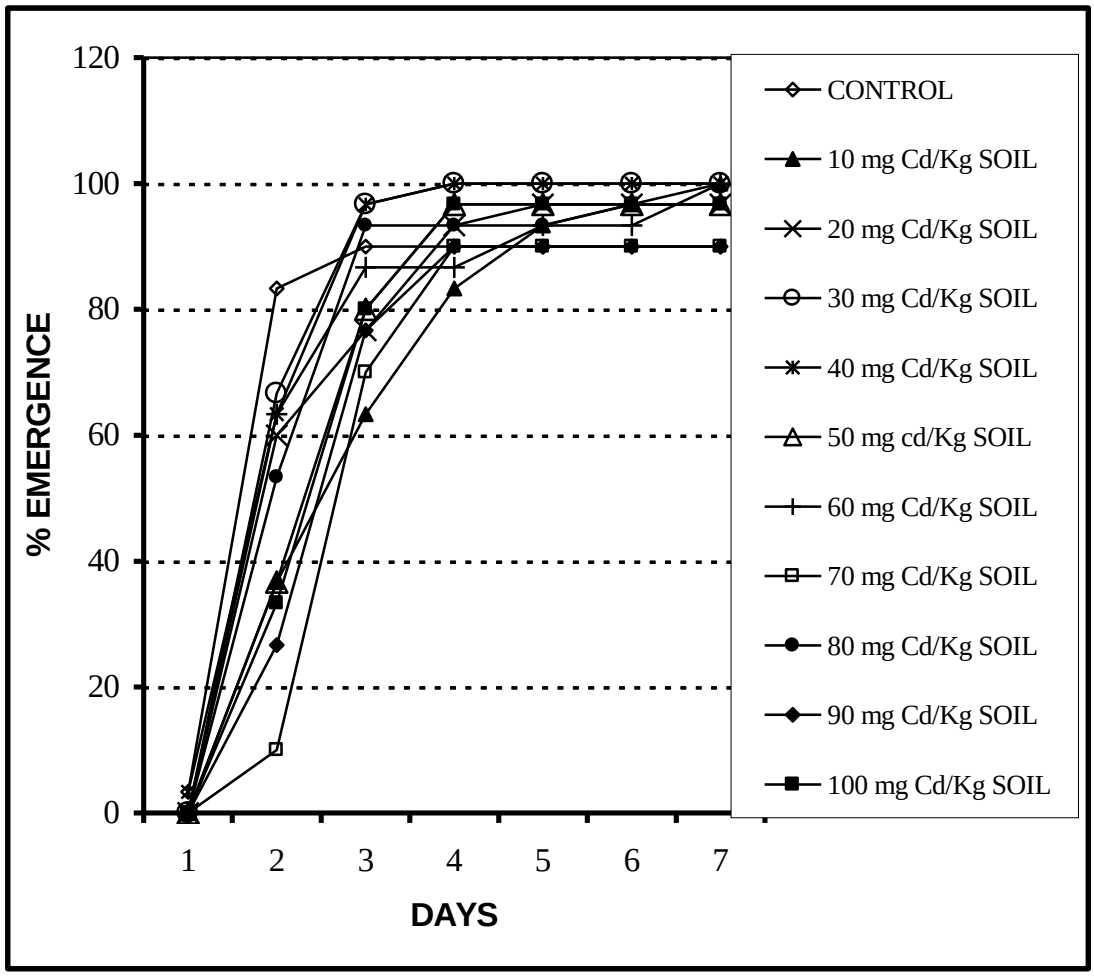

Fig. 2. Effect of cadmium on seedling emergence of *P. juliflora*.

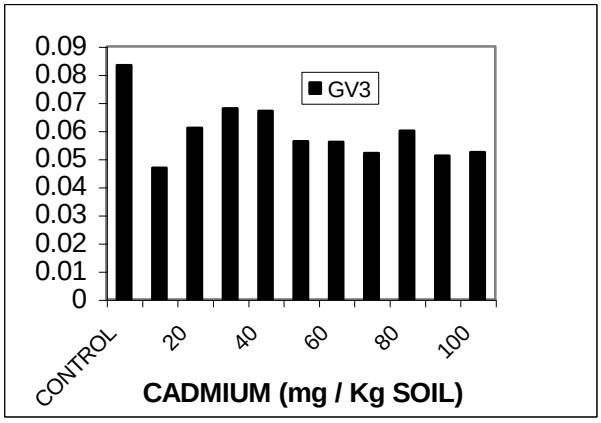

Fig. 3. Effect of cadmium on germination / emergence Velocity of *P. juliflora* as per Djavanshir and Pourbeik's (1976) index (GV3).

The root growth of *P. juliflora* was more strongly inhibited by cadmium (Fig. 7) as is evident from the following equation (# 5). By this relationship 50% reduction in root growth corresponded with Cd concentration of 129.5 mg per kg of soil

Root length (cm) = 16.25252 – 0.062744 Cd (mg / kg soil) ± 3.714
t = 18.89 t = - 4.22
p < 0.001 p < 0.001
r = - 0.4727; df = 62; p < 0.001; F = 17.84 ……. (5)

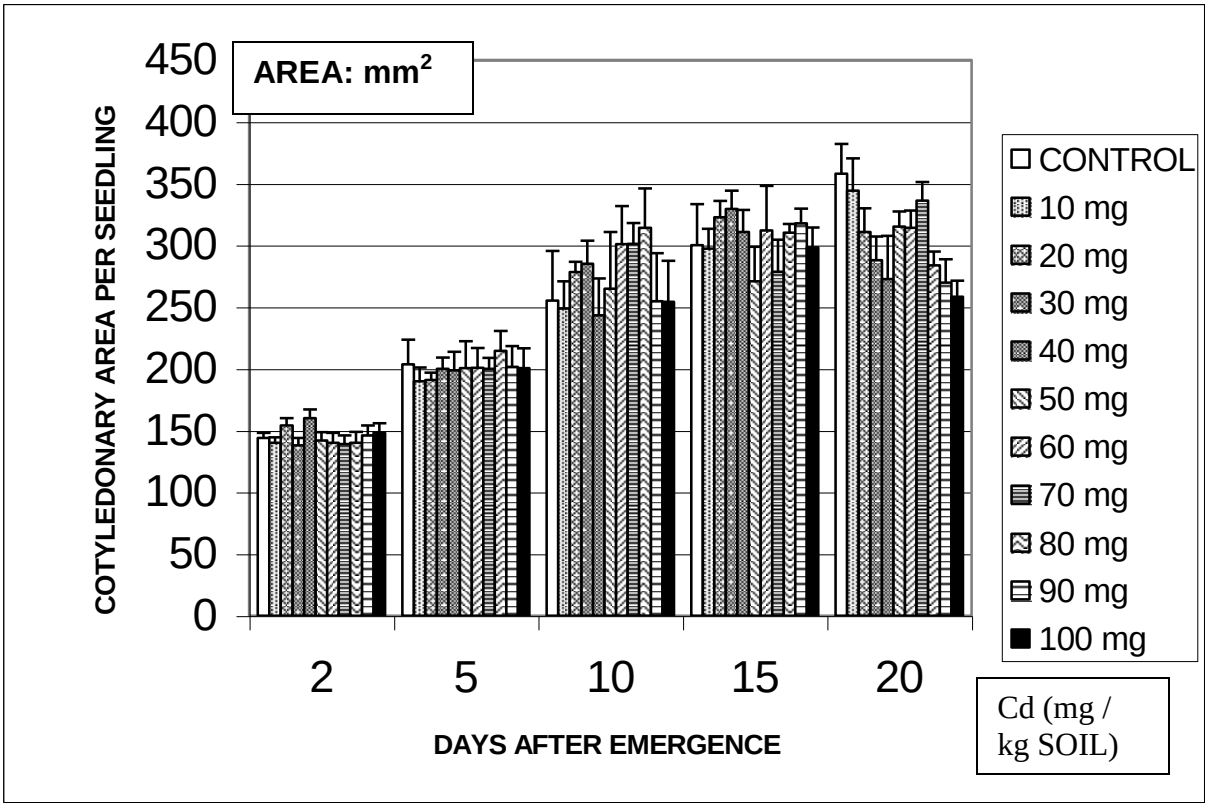

Fig. 4. Effect of cadmium in soil on cotyledonary expansion, in terms of one-sided cotyledonary area, in *P. juliflora.*

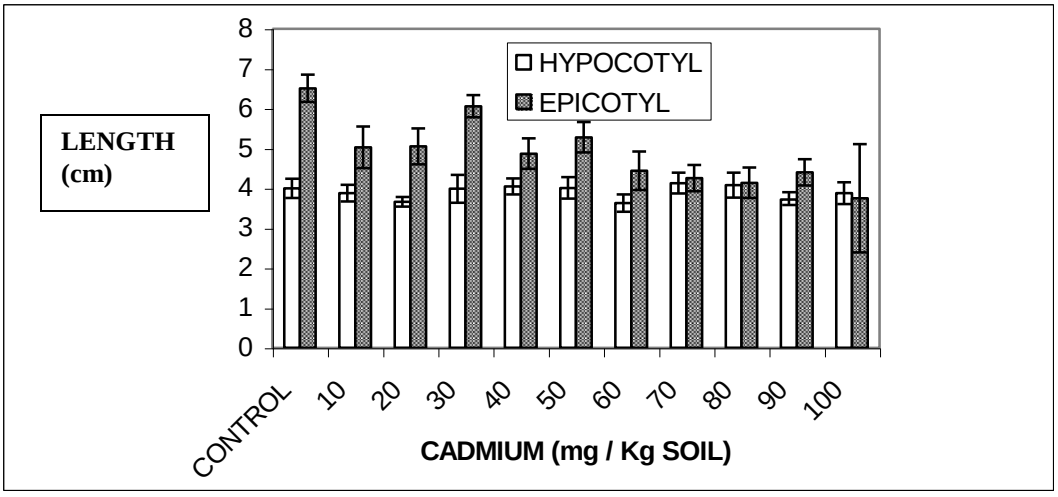

Fig. 5. Effect of cadmium on hypocotyl and epicotyl length of 50-day old *P. juliflora* seedlings.

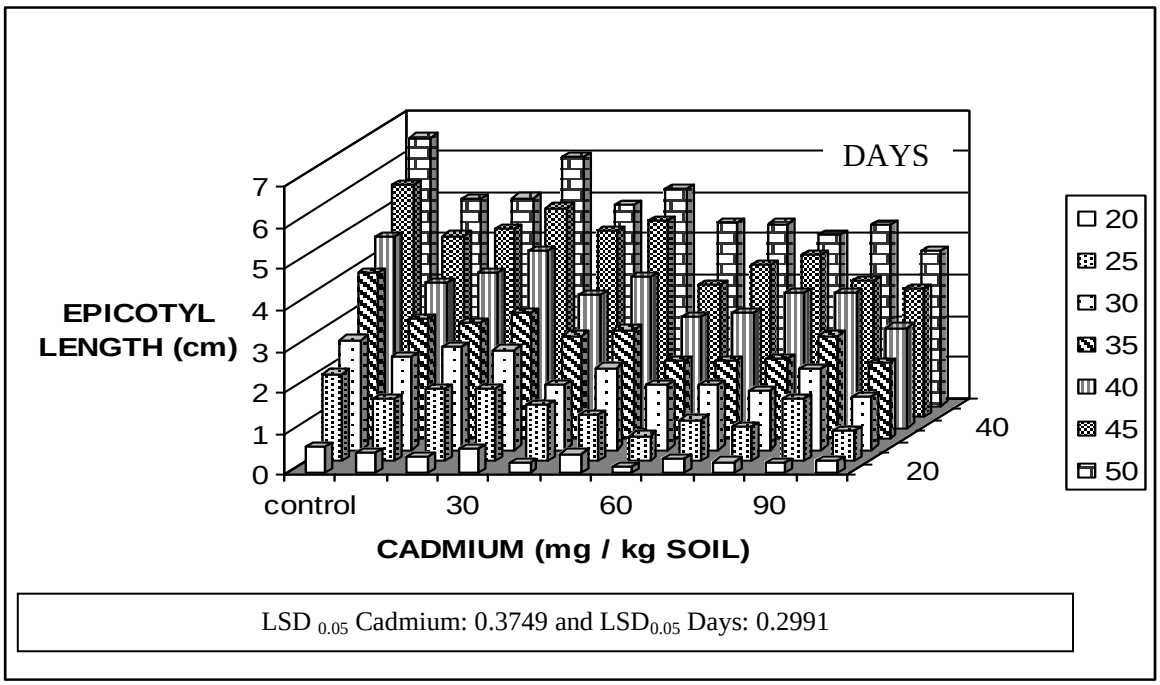

Fig.6. Effect of cadmium on epicotyl elongation in 20 – 50 days old seedlings of *P. juliflora*.

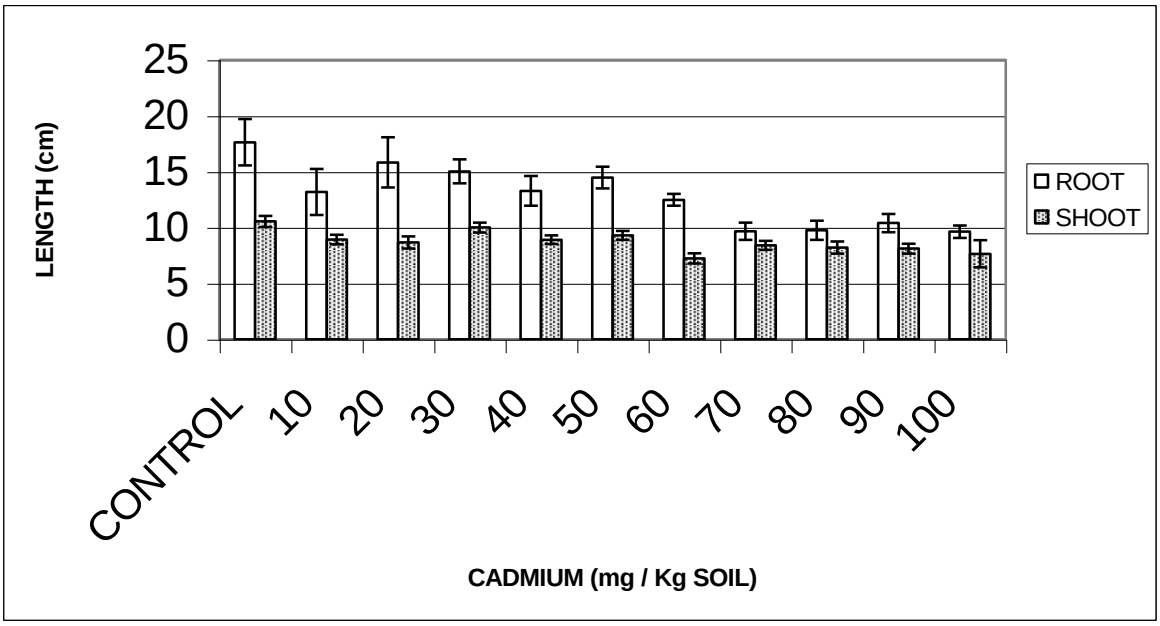

Fig. 7. Effect of cadmium concentration on root and shoot lengths of *P. juliflora* seedlings.

Effect of Cadmium on Number of Leaves per Seedling

Like other parameters of seedling growth, the number of leaves in 25 as well as 50-day old seedlings declined significantly. The maximum number of leaves produced by an individual was 08 in 10 mg Cd/kg soil treatments and minimum number of leaves produced by any individual was 04 in some plants under higher Cd concentration. In 50-day seedlings average number of leaves declined from 6.66 ± 0.21 in control to 3.8 ± 1.43 under 100 mg cadmium / kg soil (Fig.8) and 50% reduction in number of leaves in these seedlings as per following equation corresponded with Cd concentration of 169.2 mg per kg of soil.

Number of leaves = 6. 243 – 0.01844 Cd (mg / kg soil) ± 0.8724
t = 30.9 t = - 5.284
p < 0.001 p < 0.001
r = - 0.5572; df = 62; p < 0.001; F = 27.92 ……. (5)

Interestingly, the development of primary leaf was substantially suppressed in number of seedlings under high Cd concentration. In such seedlings secondary or even tertiary leaf had developed before the development of primary leaf (Table 3).

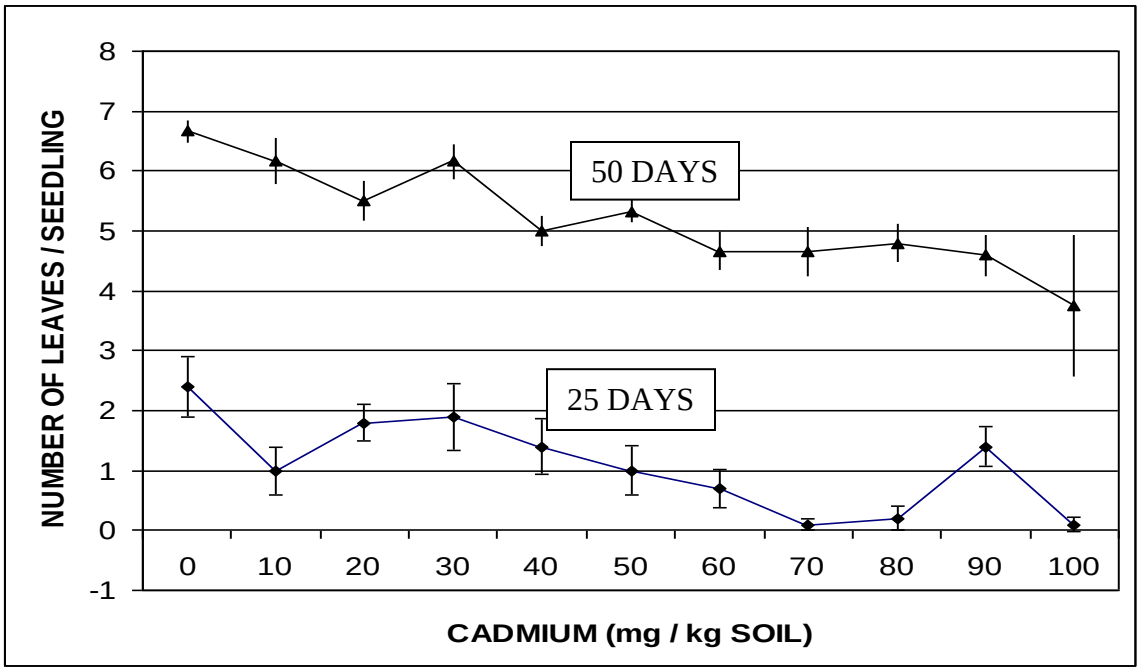

Figure 8. Effect of Cd on number of leaves per seedling on 25th and 50th day of growth.

Table 3. Per cent proportion of 30-day old seedlings of *P. juliflora* bearing fully expanded leaves under various Cd concentrations.

Treatment	Leaves			
	I	II	III	IV
Control	100	100	100	66.6
10 mg Cd/kg soil	80	100	50	-
20 mg Cd/kg soil	66.6	100	56.0	-
30 mg Cd/kg soil	100	100	86.0	42.86
40 mg Cd/kg soil	71.4	100	66.6	-
50 mg Cd/kg soil	55.5	100	33.3	3.4
60 mg Cd/kg soil	37.5	62.5	37.5	-
70 mg Cd/kg soil	12.5	62.5	37.5	-
80 mg Cd/kg soil	-	50	-	-
90 mg Cd/kg soil	33.3	100	14.3	-
100 mg Cd/kg soil	-	75	25	-

Effect of Cadmium on Number of Leaflets per Seedling

The number of leaflets per seedling, in both 30-day and 40-day old seedlings grown under various cadmium concentrations, declined significantly and gradually with increasing Cd concentration (Fig.9) and related to Cd as follows:

30-day old seedlings:

Number of leaflets / seedling = 66.4519 – 0.32593 Cd (mg / kg soil) ± 19.03

t = 15.91 t = - 4.48

p < 0.001 p < 0.001

r = - 0.4643; df = 73; p < 0.001; F = 20.07 …….. (6)

40-day old seedlings:

Number of leaflets / seedling = 109.708 – 0.522481 Cd (mg / kg soil) ± 23.98

t = 19.75 t = - 6.07

p < 0.001 p < 0.001

r = - 0.6107; df = 73; p < 0.001; F = 26.53 … …. (7)

TL_{50} values calculated as per Davis *et al.* (1972) for number of leaflets per 30-day and 40-day old seedlings were 61.7 and 76.6 ppm of Cd, respectively. Such values calculated on the basis of equation # 6 and 7 turned out to be 101.9 and 104.9 ppm of Cd per kg of soil, respectively.

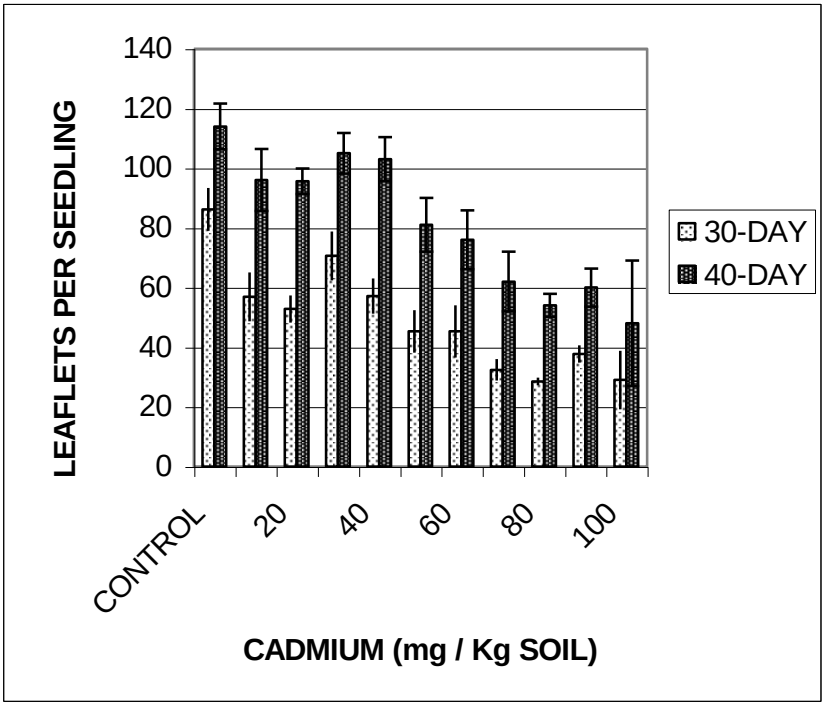

Fig.9. Effect of cadmium concentration on number of leaflets per seedling of *P. juliflora*.

Effect of Cadmium on Number of leaflets per Leaf

The variation in number of leaflets per leaf of 40-day old seedlings grown under various Cd concentrations is presented in Table 4. The primary leaf is always unipinnate but the subsequent leaves are bipinnate. Leaflets are small –around 2-3 mm^2 at seedling stage and their number per leaf vary with the two types of leaf. The number of leaflets in primary, secondary or other leaves presented no discrete pattern of change against cadmium. Decline in number of leaflets per seedling against increasing Cd concentration should, thus be the function of decline of number of leaves, which is evident from the data in Table 4. Fifth leaf couldn't develop in 40-day old plants under

70 mg Cd / kg soil or higher concentrations of Cd. No plants other than controls could develop sixth leaf at 40th day of growth.

Table 4. Number of leaflets per expanded leaf in 40-day old *P. juliflora* seedlings grown under various Cadmium concentrations.

Treatments	LEAVES					
	I	II	III	IV	V	VI
Control	11 ± 1.0 (10-14) *	30 ± 1.40(28-36)	29.30 ± 2.2 (20-36)	28.00 ± 1.03 (24-32)	29.00 ± 1.0 (28-32)	32.0± 0.0 (32)
Cd (10 mg / Kg Soil)	11.3 ± 0.66 (10-12)	29.0 ± 2.70 (18-36)	27.33 ± 2.99 (20-36)	26.40 ± 2.71 (20-36)	24.0 ± 0.00 (24) **	- ---
Cd (20 mg / Kg Soil)	10.66 ± 0.67 (10-12)	29.60 ± 0.97 (28-32)	29.33 ± 2.23 (28-36)	25.60 ±± 0.98 (24-28)	28 ± 0.0 (28) **	-----
Cd (30 mg / Kg Soil)	12.0 ± 0.0 (12-12)	30.0 ± 0.90 (28-32)	27.33 ± 1.90 (20-32)	26.66 ± 0.84 (24-28)	28.00 ± 0.0 (28-28)	-----
Cd (40 mg / Kg Soil)	10.66 ± 0.42 (10-12)	29.50 ± 1.09 (26-33)	26.67 ± 2.23 (19-32)	25.00 ± 1.23 (20-28)	29.0 ± 1.00 (28-32)	-----
Cd (50 mg / Kg Soil)	11.33 ± 0.67 (10-12)	27.33 ± 1.23 (24-32)	26.67 ± 1.98 (20-32)	25.00 ± 1.00 (24-28)	28.0 ± 0.00 (28) **	-----
Cd (60 mg / Kg Soil)	10.5 ± 0.50 (10-12)	30.33 ± 1.20 (24-36)	29.66 ± 2.33 (24-36)	28.0 ± 0.00 (28) **	24.0 ± 0.0 92 **	-----
Cd (70 mg / Kg Soil)	9.0 ± 1.0 (8-10)	26.40 ± 1.60 (24-32)	27.00 ± 2.42 (20-36)	20.50 ± 0.44 (20-22)	-----	-----
Cd (80 mg / Kg Soil)	10.0 ± 0.0 (10)*	29.50 ± *	26.6 ± 2.2 (26-36)	24.0 ± 2.8 (20-33)	----- 4.00 (20-28)	-----
Cd (90 mg / Kg Soil)	11.0 ± 1.00 (8-12)	27.33 ± 1.22 (24-32)	25.66 ± 0.97 (24-28)	28.00 ± 0.0 (28) **	-----	-----
Cd (100 mg / Kg Soil)	10.0 ± 0.0 (10)*	26.66 ± *	23.50 ± 2.90 (22-32)	28.0 ± 0.50 (23-24)	----- 0.0 (28-28)	----

*, Figures in parenthesis denote range of leaflets per leaf; ** The very leaf present in one individual only.

Effect of Cadmium on Internodal Elongation in 50-Day Old Seedlings

Table 5 represents data on internodal elongation of 50 – day old *P. juliflora* seedlings under Cd environment. Higher Cd concentrations suppressed the development of leaves and thus the higher order internodes (7th and in particular the 8th), which were drastically shortened with increasing Cadmium concentration. Internodal length shortened significantly ($F = 112.56$, $df = 7$, $p < 0.001$; $LSD_{0.05}$: 0.0785) from base to apex in control as well as treated seedlings and also declined significantly with increasing Cd concentration ($F = 9.66$, $df = 10$, $p < 0.001$; $LSD_{0.05}$: 0.0921). Both internodal position and cadmium concentration interacted significantly ($F = 2.0$, $df = 70$, $p < 0.001$).

Effect of Cadmium on Biomass of 50-Day Old Seedlings

Cadmium significantly and gradually reduced biomass of *P. juliflora* seedlings and the 50% reduction in biomass per seedling as per regression equation (Fig. 10) corresponded with Cd concentration of 180 mg per kg of soil.

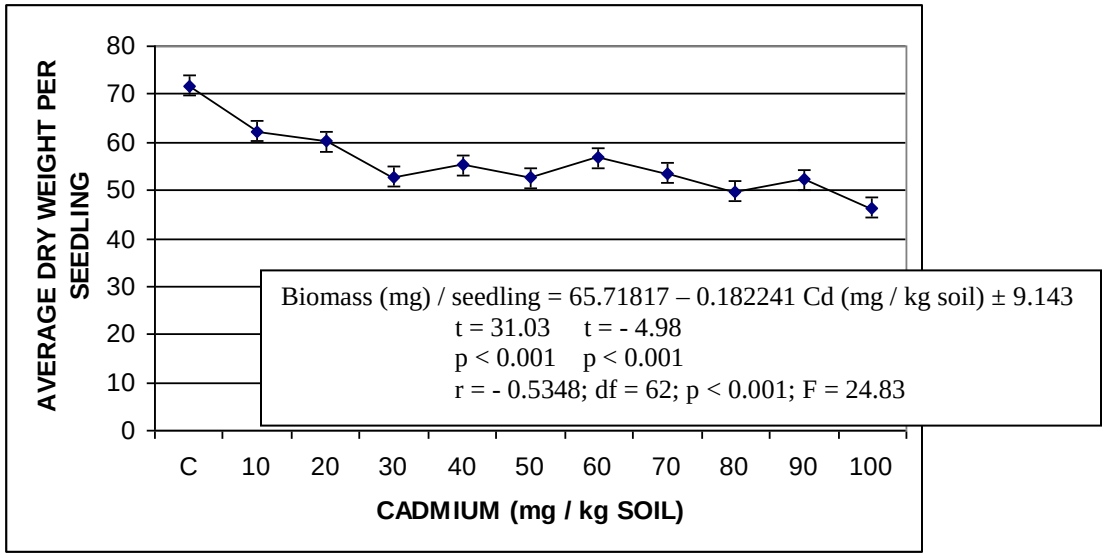

Figure 10. Effect of cadmium on average seedling weight (mg) of 50 – day old seedlings of *P. juliflora.*

Table 5. Effect of cadmium concentration on internodal elongation of *P. juliflora* seedlings at 50th day of growth after emergence.

Treatment	Internodal* Length (cm)							
	I	II	III	IV	V	VI	VII	VIII
Contrtol	0.87 ± 0.095	0.95 ± 0.096	0.75 ± 0.022	0.85 ± 0.062	0.83 ± 0.049	0.76 ± 0.080	0.52 ± 0.183	0.28 ± 0.070
Cd (10 mg / Kg Soil)	0.81 ± 0.127	0.87 ± 0.117	0.77 ± 0.102	0.82 ± 0.060	0.67 ± 0.102	0.48 ± 0.159	0.23 ± 0.150	0.10 ± 0.100
Cd (20 mg / Kg Soil)	0.85 ± 0.109	0.72 ± 0.107	0.83 ± 0.112	0.82 ± 0.090	0.65 ± 0.102	0.31 ± 0.114	0.15 ± 0.096	----
Cd (30 mg / Kg Soil)	0.95 ± 0.081	0.85 ± 0.890	0.76 ± 0.092	0.86 ± 0.072	0.85 ± 0.081	0.67 ± 0.042	0.42 ± 0.114	0.10 ± 0.082
Cd (40 mg / Kg Soil)	0.81 ± 0.041	0.64 ± 0.084	0.69 ± 0.039	0.66 ± 0.058	0.58 ± 0.574	0.48 ± 0.117	0.58 ± 0.163	0.083 ± 0.083
Cd (50 mg / Kg Soil)	1.04 ± 0.069	0.76 ± 0.061	0.90 ± 0.062	0.80 ± 0.049	0.73 ± 0.081	0.23 ± 0.120	0.18 ± 0.120	0.067 ± 0.067
Cd (60 mg / Kg Soil)	0.82 ± 0.070	0.75 ± 0.034	0.64 ± 0.078	0.64 ± 0.086	0.33 ± 0.080	0.083 ± 0.083	0.067 ± 0.083	0.066 ± 0.066
Cd (70 mg / Kg Soil)	0.72 ± 0.065	0.93 ± 0.077	0.68 ± 0.074	0.65 ± 0.076	0.57 ± 0.167	0.25 ± 0.092	----	----
Cd (80 mg / Kg Soil)	1.080 ± 0.086	0.74 ± 0.087	0.83 ± 0.141	0.84 ± 0.140	0.38 ± 0.132	0.04 ± 0.040	----	----
Cd (90 mg / Kg Soil)	0.93 ± 0.056	0.72 ± 0.075	0.83 ± 0.061	0.60 ± 0.137	0.45 ± 0.131	0.033 ± 0.033	----	----
Cd (100 mg / Kg Soil)	0.80 ± 0.108	0.38± 0.130	0.69± 0.247	0.50± 0.240	0.40± 0.230	0.48± 0.293	0.10± 0.070	----

*, I, from cotyledonary insertion point to primary leaf; II, primary leaf node to secondary leaf node; III, secondary leaf node to third leaf node; …….. and so on.

DISCUSSION

Germination of abraded seeds of *P. juliflora* in 25 - 400 ppm of cadmium in petriplates and emergence of seedlings from abraded seeds in soil containing 10 - 100 mg Cd/kg soil indicated substantial degree of tolerance against cadmium in *P. juliflora* seeds. The velocity of germination or emergence, of course, was impeded by a lag of around 24h. Reports regarding germination of seeds of many plants in response to varying concentrations of cadmium are generally those of differential degree of germination inhibition e.g., *Medicago sativa* (Peralta-Videa *et. al.,* 2000, 2002), *Achillea millifolium* (Pasquale *et. al*., 1988a), *Matricaria recutita* (Pasquale *et. al*., 1988b), *Albizzia lebback* and *Thespesia populnoides* (Iqbal and Khalid, 1998); *Delonix regia* (Iqbal *et. al.,* 2000); *Leucaena leucocephala* (Iqbal *et. al.*, 2001), *Parkinsonia aculeata* and *Pennisetum americanum* (Shaukat *et. al.,* 1999), which could be due to osmotic and

toxic effects of metal salt or decreased levels of auxins as a result of destruction of auxins by the metals ions (Mukharjee and Das Gupta, 1972). On the other hand, *in vitro*, Cd concentration below 10 µM has been reported not to affect germination and cotyledon development in *Arabidopsis thaliana* (Perfus-Barbeoch *et. al*, 2000). *Brassica juncea* genotype Vardan is reported to be highly tolerant to Cd (Singh and Brar, 2002). Iqbal and Khalid (1998) have reported germination in *Peltophorum pterocarpum* to be a process insensitive to cadmium. Seed germination of *Triticum aestivum*, *Sorghum bicolor* and *Cucumis sativus* is also reported to be insensitive to Cd toxicity (An, 2004). Singh *et al.* (2004) have studied Cd effects in its dose-range of 10^{-9} to 10^{-3}M in *Phaseolus vulgaris* and found physiologically non-toxic and even a potentially beneficial role of Cd at concentration below 10^{-5} M. Cadmium toxicity at germination level is, therefore, a species-specific phenomenon.

In *P. Juliflora*, germination is epigeous, and early growth of seedling is marked by expansion of cotyledon. Besides nutrient reserves in them, the occurrence of light stimulated processes in them also provides added photosynthetic products for the metabolism of developing seedlings. For around 2 weeks after emergence, in pot culture experiment, much of the aboveground activity in shoot of *P. juliflora* was in form of growth of hypocotyl and expansion of cotyledons and no appreciable development of epicotyl had taken place up till 20^{th} day of growth after emergence. The seedling growth of *P. juliflora*, i*n vitro*, appeared to be sensitive to cadmium. Under Cd influence of 25 – 400 ppm concentrations, *in vitro*, although cotyledonary expansion after six days of growth, remained almost unaffected; the hypocotyl was affected significantly but to a lesser extent than radicle, which was inhibited drastically. In Cd concentrations higher than 50 ppm, radicle appeared dehydrated, turned brown and almost burnt gradually in higher concentrations. It declined curvilinearly in response to increasing Cd-concentration. Arduini *et al.* (1994) have also reported browning of roots of pine seedlings due to Cd-toxicity. In our experiment, radicle elongation of *P. juliflora, in vitro*, declined by 50% in as low as 36 – 45 ppm Cadmium concentration. Hypocotyl was relatively much resistant and declined by 50% in Cd concentration as high as 288 – 300 ppm, comparatively much higher than that reported for shoot growth of *Parkinsonia aculeata* (38.9 ppm) by Shaukat *et. al.*(1999).

In pot culture experiment, 50% decline in root and shoot growth corresponded with 129.5 and 249.8 ppm Cd per kg of soil. More adverse effect of Cd-toxicity on radicle / root elongation has been reported by several workers (Iqbal and Khalid, 1998; Shaukat *et. al.*, 1999; An, 2004). Many species of plants are known to accumulate larger amounts of Cd in roots e.g., *Arabidopsis thaliana* (Perfus-Barbeoch *et. al.*, 2000), *Brassica juncea* genotype Vardan (Singh and Brar, 2002), maize (Jatimliansky *et al.*, 2004) and various cultivars of *Brassica compestris* L. ssp. *Chilensis* (Zhu-JunZhu *et. al.,* 2004). Tobacco roots are reported to accumulate 50-fold more Cd than aboveground parts of the seedlings (Gichner *et. al.,* 2004). The reason for the differential response of root and shoot to Cd is not exactly known in *P. juliflora* but it might, in part, be due to more rapid accumulation of metal in root than in shoot. Vladimirovich (1997) have reported root endodermis to play a barrier role in limiting Cadmium and lead penetration into central vascular cylinder. Senthikumar *et. al.* (2005) have shown larger accumulation of Cd in roots of *P. juliflora* growing in Cd-contaminated soils of metal-based foundry units of Coimbatore, India. Higher Cd concentrations are reported to cause cell death in root zone in *Arabidopsis thaliana* (Suzuki, 2005). Faster rate of detoxification of metal in shoot compared to root (Al-Helal, 1995) may yet be another reason for relative cadmium resistance in shoot. The decline of rate of cotyledon expansion, after 15 days of growth under increasing Cd stress may probably be attributed to the nutrient exhaustion, decline of photosynthesis and metabolic disturbance in cotyledons. Disturbance of almost all metabolic processes under severe Cadmium stress have been reported by a number of workers (Vassilev *et. al.*, 1995; Vassilev and Yardanov, 1997).

In short, the effects of Cadmium on seedling growth were differentially varying and organ-specific. Length of hypocotyl and number of leaflets per primary or subsequent leaf didn't vary significantly but all other structural components viz. root and shoot length, epicotylar growth, number of leaves and leaflets, cotyledonary area and biomass accumulation per seedling, with little variation in their responses, happened to, more or less, constantly and

significantly decline under Cd-stress. Phytotoxic effects of Cd on such parameters as studied here are well exemplified from literature (Aidid and Okamoto, 1993; Shaukat *et. al.*, 1999; Sandalio *et. al.*, 2001; Dube *et. al.*, 2003); Ghos *et. al.*, 2004; Zhu-JunZhu *et. al.*, 2004; Mediouni *et al.*, 2006). Differential degree of response of various morphological structures to cadmium may probably be attributed to differential degree of Cadmium accumulation in them or differential rates of metal detoxification in various tissues involved.

There were distinct stages of leaf development in P. *juliflora*. Normally, the first leaf of the seedling is unipinnate and following leaves are bipinnate. Besides, reduction in number of leaves and leaflets per seedling under Cd-stress, there was a tendency of delay in formation of primary leaf progressively with increasing Cd concentration. In this respect *P. juliflora* resembled to *Brassica rapa* (Ghos *et. al*., 2004) wherein Cd also induced delay in primary leaf formation and *Arabidopsis thaliana* where first leaf development was strongly inhibited at Cd concentration over 1µM, *in vitro* (Perfus-Barbeoch *et. al.*, 2000).

The most limiting effect of Cadmium to *P. juliflora* in our studies was the inhibition of radicle elongation. Fifty per cent reduction in root elongation after 50-day growth in pot culture experiment corresponded to Cd concentration of 129.5 mg / kg soil, higher in comparison to 36 – 45 ppm Cd, *in vitro*. This difference in growth response may be hypothesized to be due to some protection provided by the soil particles against Cd or competitive exclusion of Cadmium by ions present in the soil. Calcium-induced alleviation of root growth against cadmium is recently reported by Suzuki (2005). Better performance of the plant against cadmium toxicity in soil may also be possibly due to the development of mycorrhiza, which is generally associated with roots of this species (Tarafdar and Rao, 1998; Mohan *et. al*., 1998). Plants in certain mycorrhizal associations (e.g., *Paxillus-Pinus* mycorrhizal symbiosis) are reported to be less sensitive to Cadmium stress than non-mycorrhizal plants (Schützendübel and Polle, 2001). Arbuscular mycorrhiza inocula isolated from *P. juliflora* rhizosphere were found to accelerate the growth of some agro-forestry and social forestry legumes in perturbed ecosystems (Kalippan, 2000; Rai *et. al.*, 2004; Senthilkumar *et. al.*, 2005).

The most widely accepted mechanism for Cd-tolerance include such processes as 1) biochemical detoxification, 2) Compartmentalization of the metal ions within cell, 3) restricted uptake of metal ions (low uptake of ions by tolerant species), 4) restricted transport of metal ions from root to shoot, 5) formation of polypeptides which may bind metal, 6) chelation by organic acids, and 7) role of plasmalemma (Mehrag, 1993). We need to develop our understanding of the mechanism of cadmium tolerance in *P. juliflora.* In spite of the mortality of two seedlings during experimentation in higher cadmium concentrations, it is obvious that *P. juliflora* is a fairly tolerant plant to cadmium. It is a potential metallophyte and may be a crucial plant-tool in remediation of heavy metal contaminated soils. It is reported to accumulate Barium (Nagaraju and Rajesh, 2003). It can tolerate Aluminum and to a little extent Chromium also (Jamal *et. al.*, 2006). It can substantially accumulate Cd, Ni and Cr (Prasad, 2006) and Pb (Aldrich *et. al.*, 2004). It possesses capabilities to withstand Cd-toxicity in field conditions. It is a miraculous plant of very wide ecological amplitude – fast growing phreatophyte and salinity tolerant too (Khan *et. al.*, 1987). Studies pertaining to its eco-physiology with reference to its heavy metal tolerance at tissue and cellular levels may further elucidate its phytoremediation potential.

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