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Effect of Cadmium and Lead Stress on Seed Germination and Seedling Growth of *Jatropha curcas L.*

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Heavy metal pollution in the biosphere has become a worldwide problem. Metal industry effluents, mining sites, municipal and agricultural waste are important sources of metal dispersion in environment. Heavy metal imposed phytotoxicity affects seed germination, seedling growth, photosynthesis and other physiological processes. Exposure of seeds to cadmium (Cd) and lead (Pb) has deleterious effects resulting into inhibition of germination, delayed germination time and retardation of seedling growth due to toxicity. The aim of this research is to investigate the toxic effects of Cd and Pb on seed germination and seedling growth of Jatropha curcas L. and evaluate its tolerance for heavy metal stress. The experimental treatments included exposure to five concentrations of cadmium nitrate and lead acetate (ranging from 25 to 125 μM/L), under which the germination and seedling growth parameters were determined periodically. The germination and growth of J. curcas L. was affected by cadmium and lead supplemented at different concentrations and the toxicity effects were found to be concentration dependent. Tolerance indices declined sharply with increasing concentrations of lead and cadmium treatments. Germination was inhibited upto 50% by 100 μmol/L of Cd, 125 μmol/L of Pb. Mean germination time and seedling vigour index also gradually decreased with increase in Cd and Pb concentration. Cadmium proved to be more toxic than lead in all considerations.

Keywords: Cadmium, Lead; Germination; Heavy Metal Stress; Jatropha curcas L; Seedling Growth.

Environmental contamination due to heavy metals have become a matter of great concern over few decades as it poses toxic effects on plants and animals inhabiting the affected region. These pollutants are added in soil and water through industrial discharge, mining, pesticides, fertilizers and automobile exhausts. High contamination of heavy metals in soil and water also pose a potential threat to ecosystem; while their toxic effects limit the agricultural yield, their uptake by plants incorporates them in the food chain causing hazardous health effects. Heavy metals such as lead, cadmium, arsenic, chromium and mercury are extremely toxic, causing oxidative burst and tissue damage, leading to acute or chronic poisoning in humans and animals.

Metal toxicity in plants has been reported by various authors[1,2]. Trace amounts of heavy metals are potential enough to damage the vital functions of plants such as seed germination, seedling

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growth, photosynthesis and other physiological events. The plants respond to heavy metal stress through various mechanisms involving change in cell membrane permeability, oxidative burst, and excessive production of anti-oxidants. Growth inhibition is a general phenomenon associated with most of heavy metal toxicity[3, 4]. Severe impact on seed germination percentage and seedling growth parameters due to heavy metals like lead has been reported in *Brassica pekinensis*[5], *Cassia siamea*[6] and some tree species[7,8]. Heavy metal concentration levels between 10 to 200 ppm have been reported to affect the seed germination parameters like germination percentage, germination index, emergence and growth of plumule and radicle, root and shoot lengths, root and shoot dry matter, with variable impacts. Lead toxicity effects on cytological parameters of roots impacting their growth and architecture were studied in *Allium cepa* and *Zea mays* L.[9,10].

Jatropha curcas L. belonging to family Euphorbiaceae, is a plant with multiple benefits. Its seeds contain approximately 40-45% oil and therefore it is a potential oilseed crop to be used as feedstock for biodiesel production[11]. It is also known for its ethno- pharmacological uses as an antimicrobial agent, a potential antioxidant and for its anti-inflammatory activity, due to the presence of large number of bioactive phytochemicals[12]. It is also being explored for its phytoremediation potential as it has shown high tolerance towards heavy metals when grown in contaminated soils[13]. *J. curcus* is a stress-resistant, perennial plant, growing well in marginal or poor soil, therefore there is special interest in its cultivation in barren and arid regions[14]. The aim of the present research was to investigate the effects of heavy metals Cd and Pb on seed germination and seedling growth of *J. curcas* L. and determine the tolerance of this plant towards these heavy metals.

MATERIAL AND METHOD

Seed Collection and Preparation

Seeds of *J. curcas* L, which were 36 days old, were obtained from Jatropha Research Centre, Bawal, Haryana. Approximately 2000 seeds were screened for selection of healthy seeds. Aborted, damaged and infected seeds were discarded and healthy seeds of uniform weight were selected for the experiment.

Seed germination and growth conditions

The study was conducted in the green house in botanical garden of Manav Rachna International Institute of Research & Studies, Faridabad. Germination beds of 20 x 12 cm were prepared in polythene bags and filled with fertile soil collected from an agricultural field of Faridabad. It was mixed with organic compost in ratio 2:1 and tap water was added to each bag to bring the soil to field water capacity. Soil pH was maintained at 7.2. Healthy and intact seeds were screened and surface sterilized using 1% sodium hypochlorite followed by washing thrice with distilled water before sowing. Soils in the bags were then raked using fingers and one seed was sown in each bag at a depth of 20-25 mm. Immediately after sowing, soil was watered and thereafter watering was carried out on alternate days to keep the beds with adequate moisture.

Heavy Metal Treatment

Heavy metal treatment solutions were prepared using cadmium nitrate and lead acetate in concentrations of 25, 50, 75, 100 and 125 μmol/L. Each set of treatment consisted of 6 bags, each of which was supplied with 10 ml of respective treatment at every alternate day. There were 12 replicates per treatment. Control set was left untreated.

Seed germination and growth assessment

Germination was marked by the rupturing of seed coat and subsequent emergence of the radicle and plumule. Germination was recorded every day until complete emergence was achieved. Dry biomass of seedlings was determined after harvesting 10 random samples. Germination percentage and seedling vigor index (SVI) were calculated by applying the formula. First leaf emergence indicated the seedling growth. The seedlings were said to be established on emergence of the second leaf when the plantlets from each batch were uprooted and washed to remove soil particles adhered to roots. Dry matter and moisture content (mg g-1 dry weight) in all plant samples were also calculated. Tolerance index (TI) were determined on the basis of mean root length [15] using the formula given below-

TI (%) = ($MLR_{treatment}$ / $MLR_{control}$) X 100
here,
$MLR_{treatment}$ = mean length of the radicle in heavy metal treatment
$MLR_{control}$ = mean length of the radicle in control

Statistical Analysis

The experiments were conducted in randomized block design in triplicate. The data was analyzed using one-way analysis of variance (ANOVA) to determine the effect of heavy metal treatments and least significant difference (LSD at P=0.05) tests were performed to determine the statistical significance of the differences between means of treatments.

RESULTS AND DISCUSSION

The effect of Cd and Pb exposure to *J. curcas* L. seeds during germination and seedling growth was assessed on the basis of various growth parameters.

Effect on Germination

Germination parameters of *J. curcas* L. seeds treated with different heavy metal solutions are presented in Table 1. A statistically significant decrease in germination rate, as compared with control seeds, was observed for seeds treated with 75 and 100µmol/L solutions of cadmium and lead salts respectively. After a prolonged exposure to high amounts of heavy metals some plants showed symptoms of phytotoxicity such as stunted growth, depletion of chlorophyll, shrunken and deformed leaves. Exposure to 100 µmol/L of Cd, 125 µmol/L of Pb resulted in approximately 50% inhibition of germination or early death of the majority of seedlings within 8 days (Figure-1, 2 and 3). The growth of seedlings was evidently affected by both the heavy metals.

The low percentage germination of the treated seeds, especially at the higher levels of the applied heavy metals, is comparable with the results of studies reporting that cadmium considerably inhibits the germination of *Cassia siamea* and *Leucaena leucocephala*[16, 17]. The elevated concentrations of heavy metals impact water uptake by germinating seeds that hamper nutrient mobilization, thereby retarding metabolic activities and inhibiting cell division. Concentration dependent decline in seedling vigour index was also noticed in all the cases. The inhibitory effects of lead on seed germination and seedling growth have also been reported in wheat[18]. Similar physiological effects were observed in this study where exposure to Cd and Pb not only caused inhibition of germination in *J. curcas* L. seedlings but also lead to chlorosis, necrosis and retardation of growth due to toxicity.

Effect on Seedling growth

The root and shoot length of the treated seedlings were also found to be considerably reduced at all levels of the heavy metals applied,

Table 1. Effects of various concentrations of Cadmium and Lead on Seed Germination parameters of *J. curcas* L.

Heavy Metal Treatment (µmol/L)		Germination Percentage	Mean Germination Time (Days)	Seedling Vigour Index	Dry Matter (mg/gdw)	Moisture Content (%)
Cd	25	77.08±1.95	6.40	602.73±10.0	38.58±0.64	65.01+1.55
	50	73.96±9.25	6.55	577.29±10.94	37.81±0.72	64.23+1.11
	75	68.75±2.85	7.00	529.68±14.59	37.08±1.02	49.20+1.50
	100	56.25±5.46	7.00	412.32±12.82	28.86±0.90	49.20+1.50
	125	40.63±4.98	7.05	391.31±11.77	27.59±0.83	49.20+1.50
Pb	25	87.50±4.42	6.45	602.73±7.12	38.58±0.46	64.60±1.33
	50	86.46±2.92	6.51	582.63±18.97	38.16±1.34	63.80±1.96
	75	81.25±2.44	6.66	568.89±16.12	38.10±1.06	63.72±1.74
	100	72.92±1.95	6.71	478.93±18.68	33.53±1.31	56.14±2.38
	125	58.33±3.29	6.96	430.50±9.92	30.35±0.70	50.79±1.34
Control		93.75±2.44	5.12	648.97±8.31	39.74±0.51	65.14±1.09

± Standard Error, All values significant at P<0.05

except at 25 µmol/L of Pb, where root length were more or less comparable with those of untreated seedlings (Figure-3). Root and shoot growth reduced to approximately 50% in J. *curcas* L. seedlings exposed to Cd and Pb concentrations > 50 µmol/L. The adverse effects on root and shoot growth were much more pronounced at the higher concentration of Cd and Pb, which may be one aspect of the role of metabolic inhibitors in the overall phenomenon of plants growth. The response of the test plants to the high levels of the Pb was reflected in overall decrease of growth. Lead is reported to adversely affect the growth and accumulate in aerial parts in *Epipremnum aureum*[19].

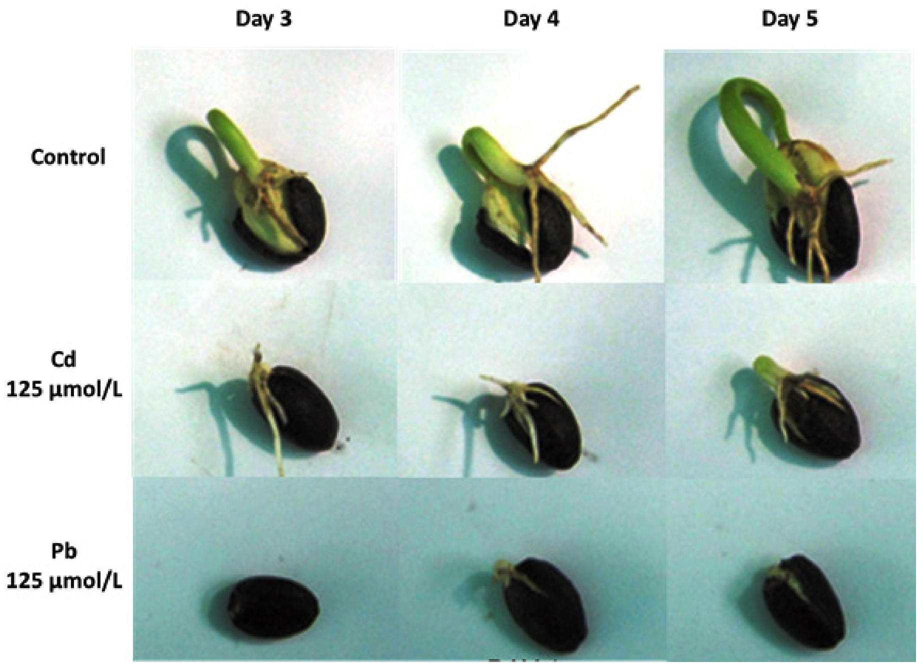

Fig. 1. Seed germination in *J. curcas* L. on exposure to Cd and Pb

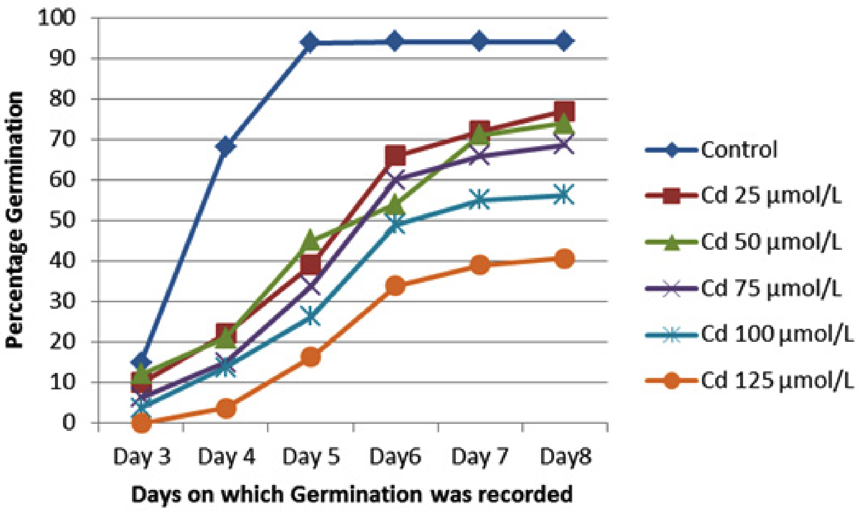

Fig. 2. Effect of Cd on Germination of *J. curcas* L

Adverse effects of Pb toxicity on seed germination and seedling growth have also been observed in *Spartma alterniflora*[20]. A very rapid reduction in root growth was observed when treated with various concentrations of lead in of *Allium cepa*[9] and *Zea mays*[10].

Effect on Moisture content and dry matter

The exposure to Cd and Pb decreased the moisture content and attenuated the dry matter significantly in experimental plants (Table-1). The considerable reduction in dry matter yields of the plants under the influence of high concentrations of heavy metals is in agreement with significant reduction reported in dry biomass of *Albizzia lebbeck* under the toxic effect of lead and cadmium[21]. The heavy metals Pb and Cd induced toxicity greatly reduced dry matter in *Thespesia populnea* L.[22]. Fresh weight, dry weight and relative water content were also found to be greatly decreased in mung bean on exposure to 200 ppm of Cd[23]. Exposure to 25 ppm of Cd and Pb caused significant decrease in dry weight of seedlings of *Leucaena leucocephala*[17].

Effect on Tolerance Indices

The tolerance index was calculated on the basis of mean root length attained in cadmium

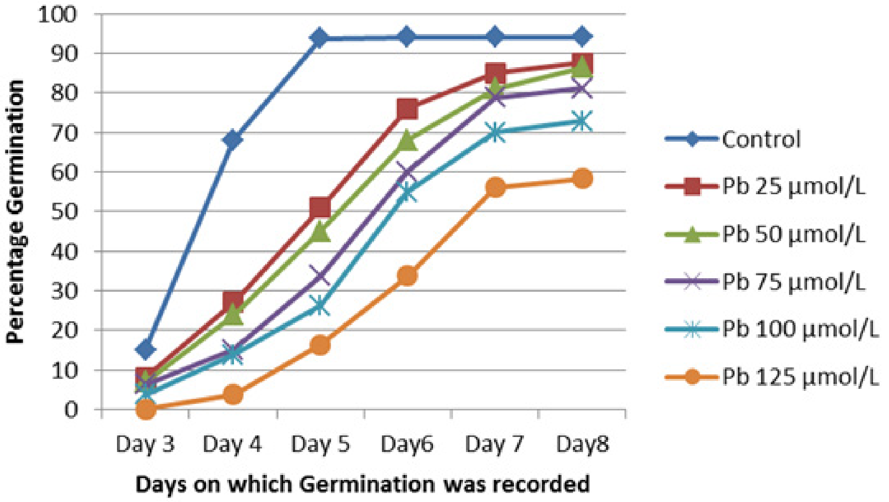

Fig. 3. Effect of Pb on Germination of *J. curcas* L

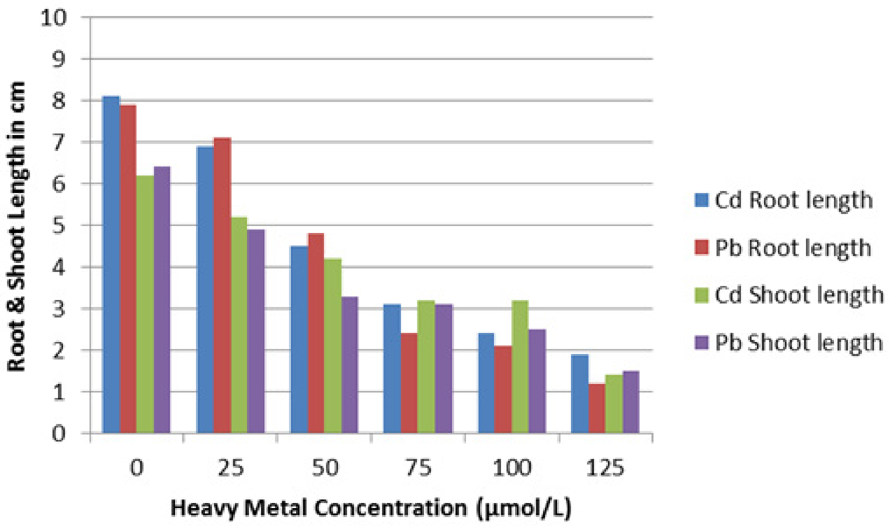

Fig. 4. Effect of Cd and Pb on Seedling growth of *J. curcas* L.

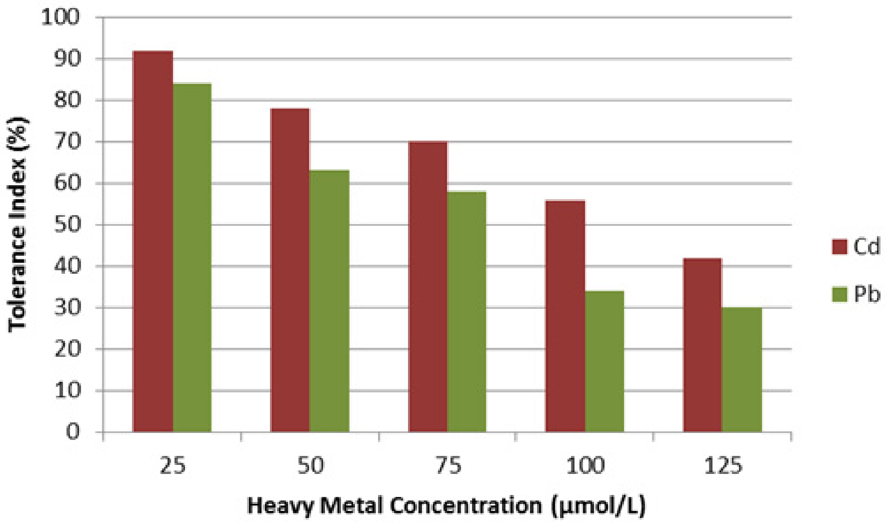

Fig. 5. Effect of Cd and Pb on Tolerance index of *J. curcas* L.

and lead treatment groups with respect to control. The *J. curcas* L. seedlings were found to be more tolerant to cadmium as compared to lead. Cadmium concentration of 100 µmol/L and Pb concentration of 125 µmol/L decreased the tolerance index to less than 50 percent, which is a direct indication of heavy metal toxicity in seedlings (Figure-4). Low tolerance indices have been attributed to the physiological alterations during growth in wheat under heavy metal stress[24]. Effect of cadmium, iron and zinc on wheat and bean species was evaluated and cadmium was found to be more toxic than iron and zinc. The metal tolerance of germinated seeds was also found to differ significantly between species and bean was more tolerant to metal stress than wheat[25].

CONCLUSIONS

Heavy metal pollutants like Cd and Pb undoubtedly cause deleterious effects in plants. In this study, the tolerance limits and mechanisms of heavy metal tolerance of *J. curcas* L. were assessed against five levels each of Cd and Pb exposure, at seed germination and seedling development stage. Cd and Pb stress caused delay in germination and it was observed that the mean germination time in both the cases was higher as compared to the control. The germination percentage was seriously affected due to heavy metal stress. The Cd level 100 µmol/L and Pb concentration 125 µmol/L inhibited approximately 50% of germination due to severe toxicity leading to seed browning and tissue necrosis. All the growth parameters were affected at this concentration therefore, these levels were also determined as the tolerance limits for *J. curcas L.* These findings are relevant to evaluate the effect of heavy metal pollutants Cd and Pb on *J. curcas* L. seed germination and seedling growth and assess the tolerance levels of the plant. This plant is widely recognized for its potential to produce biodiesel, besides it is also reputed as a pharmacologically important plant. The results from this study would certainly be useful to find strategies to alleviate *J. curcas* L. from heavy metal stress and also to explore and enhance its phytoremediation potential.

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Conflict of Interest

The author declares that there is no conflict of interest.

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