



The Toxic Effects of Lead on Seedling Growth Development of *Euphorbia Hirta* Forsk (Weed) and *Sporobolus Coromandelianus* (Retz.) Kunth (True Grass)

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ABSTRACT

Lead is considered a toxic heavy metal and released in the air, water and soils due to auto wheels, industrial and anthropogenic activities which influence on plant growth, fauna and environment. It is hypothesized that plants are capable of showing different responses of toxicity and tolerance to metals stresses. The objective of this study is to investigate the influence of one of the highly toxic heavy metal, lead (Pb) on the seedling growth of native plant species, *Euphorbia hirta* Forsk and *Sporobolus coromandelianus* (Retz.) Kunth in pots as there are very few data available in the literature on this toxicity subject. It is important to compare both genotype under various level of lead treatment. The seedlings of *E. hirta* and *S. coromandelianus* showed different effect when treated with 20, 40, 60, 80, 100 as compared without 0 ppm concentrations of lead. The results of the present studies showed the statistically significant ($p < 0.05$) influences of lead 40 ppm treatment on number of leaves, leaf area and seedling dry weight of *S. coromandelianus*. Lead treatment at 60 ppm significantly reduced shoot, root and total seedling height of *S. coromandelianus*. Lead treatment at 40 ppm also showed significant decreased on shoot, seedling length and number of leaves of *E. hirta*.

The seedlings of *E. hirta* and *S. coromandelianus* were also used for the development of tolerance indices percentage to different level of lead. The seedlings of *E. hirta* and *S. coromandelianus* showed changes in values of tolerance indices. Lead treatment at 20 ppm showed a positively decreasing influence on the tolerance indices of *E. hirta* and *S. coromandelianus*. Increase in lead level at 80 – 100 ppm highly decreased the tolerance indices percentage of *E. hirta* and *S. coromandelianus* as compared to control. The seedlings of *S. coromandelianus* showed tolerance (68.38%) as compared to *E. hirta* (50.79%) to lead at 100 ppm. The obtained data could be useful for a wide range of researchers working in this aspect.

1. Introduction

Pollution by heavy metals (Pb, Cd, Cr, Cu, Fe, Hg, Mn and Ni) in the environment is a nationwide and worldwide pollution problem due to industrial, anthropogenic and automobile activities. Lead is considered as a non essential element for plant growth and human beings. The accumulation of heavy metals in air, water and soil has serious influence on plant growth, vegetation and ecosystem balance. Among the heavy metals, Pb has proved toxic element for biota (Iqbal et al. 2001). There is about 235 million hectares land worldwide has been polluted by heavy metals (Bermudez et al. 2012). Air pollutants likewise lead compounds and heavy metals effects the respiratory, vegetation, photo synthetic pigments, root morphology, enzymatic activities of plants, changes in plant communities (Shahid, et al., 2012;

Zeeshan et al. 2016; Gautam et al. 2018; Verma et al. 2021). Effects of chronic stress of lead on anatomical structure of Tobacco roots, and tolerance mechanisms in *Salsola passerina* Bunge and *Chenopodium album* L were recorded (Yuan et al. 2011; Hu et al. 2012). The researchers have reported that the excess level lead (Pb) against recommended limit showed harmful effects on seed germination, seedling growth and yield of different plant species due to oxidative damage (Jaja and Odoemena 2004; Prasad et al. 2015; Pietrzykowski et al. 2018; Liu et al. 2020).

The presence of heavy metals in soil also influences on habitant, aquatic life, public health, ecosystem balance and plant growth (Giordani et al. 2005; Yang et al. 2005; Odilara et al. 2006; Joshi et al. 2009; Dribben et

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al. 2011; Shafiq and Iqbal, 2012; Kumari and Deswal, 2017; Gong et al. 2019; Shafiq et al. 2019).

Species description

Euphorbia hirta Forsk (garden spurge) is a weed, belongs to Euphorbiaceae family and characterized by white milky latex. The morphological, phytochemical, ethnopharmacological, and pharmacological information on *E. hirta* as an herb reviewed on account of medicinal purposes (Kumar et al. 2010). The phytochemistry, pharmacological aspects, toxicological potentials of crude ethanolic extracts of *E. hirta* reported in literature (Ogueke et al. 2007; Huang et al. 2012).

Sporobolus coromandelianus (Retz.) Kunth (true grass) is an annual species of grass and member of Poaceae family (eflora, 2020). *S. coromandelianus* is monocotyledon plant species. *Sporobolus*, Sporo derived from Greek word spora (seed) and bolus (throwing) alluding to the free seed and sometimes forcible manner of its release and common name is small drop seed (Aus-Grass, 2020). *S. coromandelianus* is a self-sprouting, simple and broad leaves plant species (EOL, 2020). *S. coromandelianus* clumps upto 25 cm tall (Cope, 1999) and its native range is from Eritrea to South Africa, West Indian Oceans, South central China, Jawa, New Guinea, Australia, Namibia, Oman, Sri Lanka, Sudan, Thailand, Yemen, Bangladesh, Afghanistan and native to Pakistan (Kewscience, 2020).

The ever increase of heavy metals in the environment is influencing on the germination and growth of plants. The response of plant growth to abiotic stress has become the subject of great interest in recent years by ecologist. Lead in excess level become toxic element for plants growth. The data on the development of tolerance indices for the native flora in the country is still insufficient. There is dire a need of development of high level of interest in selection of plant species and publication of data on the nature of toxicity and tolerance to metals in plants. Very few studies have been conducted on the effects of lead on the germination and growth of other plants species in Pakistan. The identification of metal tolerant species and plantation in polluted areas might be helpful to lessen the burden of pollutants from the environment. This study aims to evaluate and compare the effects of lead (Pb) on seedling growth performance of two different plant species, *Euphorbia hirta* Forsk and *Sporobolus coromandelianus* (Retz.) Kunth.

2. Materials and Methods

The seedling growth experiment was conducted in green house of the Department of Botany, University of Karachi, Pakistan. Three uniform size seedlings of *E. hirta* and *S. coromandelianus* were collected from the Karahi University campus after moon soon rain fall season in August. The selected two plant species were transplanted in plastic pots (9.0 cm in depth and 7.0 cm in diameter) having garden loam soil. The fraction of soil was one-part manure and two parts of fine sand. There were six metal treatment (0, 20, 40, 60, 80 and

100 ppm) for each plant species and the experiment was completely randomized. The seedlings were irrigated with fresh solutions of 0, 20, 40, 60, 80 and 100 ppm concentration of Pb prepared with lead acetate. Distilled water was applied to control group. 5 ml of respective treatment after the interval of two days was applied. Every week each pot was reshuffled to avoid the effects of shade, light or any other factors of environment. After two months, seedlings were removed from plastic pots and washed with distilled water. The root, shoot, seedling length, number of leaves, leaf area, and root, shoot, leaves and total seedling dry weight were recorded. The root / shoot, leaf weight ratio, specific leaf area and leaf area ratio were recorded (Rehman and Iqbal, 2009). Seedling dry weight was obtained after drying the samples in an oven at 80 °C for 24 hours.

Tolerance indice (T.I.) was determined on the basis of percentage using the following formula given by Iqbal and Rahmati (1992):

$$T.I. = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in distilled water}} \times 100$$

Statistical analysis

Statistical comparisons among all treatments for above and below ground dry plant biomass and proportion contributions thereof to overall dry plant biomass was evaluated using one-way Analysis of Variance (ANOVA) and Steffens post hoc test. Assumption of all tests were tested statistically verified. All statistical analysis was performed using SPSS 10.0 (SPSS) Inc., U.S.A.) for windows.

3. Results and Discussion

Despite knowing the phytotoxic effects of heavy metals, which is of global concern, there is a dire need to study about its impacts on native plants. Overall results of different seedlings growth parameters showed that lead is toxic element and deleterious for plant growth. The variable responses of a native plant *S. coromandelianus* and *E. hirta* to metal salt likewise lead (Pb) was recorded (Table 1-4; Fig. 1). In the present study the effects of different concentrations 0, 20, 40, 60, 80 and 100 ppm of lead on root, shoot, seedling length, number of leaves, leaf area and seedling dry weights (root, shoot, leaves and total seedling) of *E. hirta* and *S. coromandelianus* were recorded. The lead treatment of 20 ppm showed nonsignificant impact on the root and seedling length of *S. coromandelianus*. The lead treatment of 40 ppm significantly ($p < 0.05$) decreased number of leaves and leaf area of *S. coromandelianus*. A rise in concentrations of lead 60 ppm decreased root, shoot, seedling length and biomass production of *S. coromandelianus* as compared control. Lead treatment at 20 ppm indicated toxic effect on shoot, root and seedling height of *S. coromandelianus*. Reduced rate of seed germination and plant growth under stress is mainly due to Pb interference with enzymatic activities seedling dry weights (root, shoot, leaves and total seedling) of *S. coromandelianus* were recorded. Lead treatment at 20 ppm

significantly ($p < 0.05$) indicated adverse effect on number of leaves, leaf area, shoot, root, leaf, total plant dry weight, shoot/root, leaf weight ratio, specific leaf area and leaf area ratio of *S. coromandelianus*.

The reduction in root, shoot, seedling length and biomass production of *E. hirta* by concentrations 20, 40, 60, 80 and 100 ppm of lead as compared to control were noted. Air pollution due heavy metals present in soil constitutes negative impacts on tolerance indices of tree (Tripathi et al. 2009). Lead treatment at 20 ppm significantly indicated adverse effect on number of leaves, leaf area, shoot, root, leaf and total plant dry weight of *E. hirta*. A rise in concentrations of lead from 60 to 100 ppm further significantly ($p < 0.05$) decreased root and leaf growth of *E. hirta*. Ghani et al. (2010) studied the similar effect of different concentrations (0, 10, 20 and 30 ppm) of lead toxicity on the growth of two varieties of *Zea mize* (Neelam and Desi) in earthen pots. The different response on root / shoot, leaf weight ratio, specific leaf area and leaf area ratio of *E. hirta* to different concentrations 20, 40, 60, 80 and 100 ppm of lead as compared to control were noted.

The application of Pb at 20 ppm promoted shoot and seedling length of *E. hirta*. Maximum suppression of root length (32.30 cm), shoot length (9.50 cm) and seedling length (41.80 cm) of *E. hirta* were obtained at 80 ppm concentration of lead. Maximum suppressed in number of leaves (13.60) and leaf area (7.43) was found with the lead treatment of 100 ppm. Lead treatment at 60 to 80 ppm produced significant ($p < 0.05$) decrease on seedling growth performance as compared to control. The shoot (0.28 g), root (0.14 g), leaves (0.046 g) and total plant dry weight (0.466 g) of *E. hirta* seedlings exposed with concentrations of Pb at 100 ppm was recorded. These lead toxicity impact on the growth of *Thespesia populnea* were found similar with the results of Kabir et al. (2008). The results for tolerance indices of *Thespesia populnea* progressively decreased with the increasing concentration of heavy metal. Kabir et al.

Table 1

Effect of lead on seedling growth of *Sporobolus coromandelianus* (Retz.) Kunth and *Euphorbia hirta* Forsk

Treatment lead (Pb) ppm	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Number of leaf	Leaf area (sq. cm)
00	*49.00±1.08a	15.50± 0.51a	64.50±2.07a	25.10±1.20a	2.20±0.006a
	**36.30±2.15a	20.30± 0.52a	56.60±3.14a	34.50±0.46a	31.00±0.464a
20	44.50±1.07a	15.00±1.11a	59.50±2.22a	25.50±2.23ab	1.60±0.014ab
	40.16±1.20ab	19.60±1.08a	59.76±2.41ab	33.60±1.74ab	9.77±0.831ac
40	39.70±0.76ab	13.60±1.45bc	10.00±1.67ab	53.30±1.57ab	16.30±0.91b
	40.60±1.50b	7.14 ± 0.12a	50.60±1.46bc	18.60±4.64b	2.00±0.02b
60	46.60±3.17b	14.00±0.72ab	60.50±3.71b	14.80±2.21b	1.03±0.016bc
	36.30±2.36b	11.50±1.08b	47.80±3.31a	22.30±3.97bc	8.22±0.693b
80	38.80±1.47bc	7.14 ± 0.12a	50.40±2.74bc	15.30±1.31bc	0.48±0.005b
	32.30±1.68bc	11.60±1.74b	41.80±2.92b	22.50±3.12c	8.05±0.193bc
100	37.96±2.29c	9.50±1.84bc	48.56±2.90bc	12.00±1.02c	0.60±0.042c
	34.70±4.70c	10.60±0.92c	45.00±5.71c	13.60±2.27c	7.43±0.732bc

Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at $p < 0.05$ level. ± Standard Error; **Sporobolus coromandelianus* (Retz.) Kunth, ***Euphorbia hirta* Forsk

(2011) reported the different values of tolerance indices of *Samanea saman* for Cu, Fe, Pb and Zn. A high percent of decrease was found in seedling growth and above and belowground biomass of both species with the increase in lead treatment.

The tolerance in seedling growth of *E. hirta* and *S. coromandelianus* differed in their sensitivity to lead treatments in range of 20-100 ppm as compared to control (Fig. 1). The seedlings of both species were found resistant to lead tolerance at 20 ppm concentration as compared to control. An increase in concentration of lead treatment from 40 to 100 ppm increased the toxicity and lower the tolerance indices percentage in seedling growth performance of *S. coromandelianus*. *S. coromandelianus* showed tolerance indices (68.38%). It was noted that the lead treatment of 80 ppm showed (46.75%) tolerance indices in seedlings of *E. hirta* as compared to control.

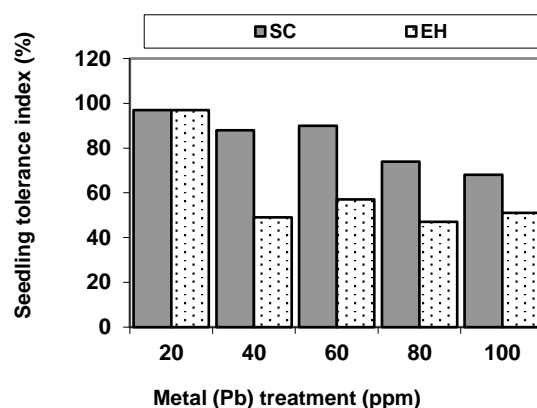


Figure. 1 Seedling tolerance index (%) of SC (*Sporobolus coromandelianus*) and EH (*Euphorbia hirta*) to different concentrations (20, 40, 60, 80 and 100 ppm) of Pb.

Table 2

Effect of lead on dry weight of *Sporobolus coromandelianus* (Retz.) Kunth and *Euphorbia hirta* Forsk

Treatment lead (Pb) ppm	Shoot dry weight (g)	Root dry weight (g)	Leaf dry weight (g)	Total dry weight (g)
00	*0.590±0.014a **0.290±0.01a	0.360±0.002a 0.200±0.003a	0.060±0.002a 0.051±0.001ab	1.01±0.002a 0.541±0.004a
20	0.420±0.03ab 290±0.01ab	0.185±0.003ab 0.116±0.004ab	0.024±0.002ab 0.034±0.001b	0.629±0.003ab 0.440±0.004ab
40	0.570±0.03bc 0.300±0.02bc	0.300±0.003b 0.640±0.001b	0.060±0.002b 0.045±0.002bc	0.930±0.003bc 0.985±0.004b
60	0.380±0.03ab 0.250±0.03bc	0.240±0.002ac 0.098±0.003bc	0.040±0.02bc 0.030±0.001ac	0.660±0.004ac 0.378±0.003bc
80	0.520±0.05c 0.240±0.02ac	0.210±0.003b 0.140±0.003bc	0.035±0.002ac 0.040±0.001bc	0.771±0.004bc 0.371±0.004bc
100	0.480±0.03bc 0.280±0.0bc	0.230±0.003bc 0.140±0.003c	0.060±0.002ab 0.046±0.002c	0.770±0.003c 0.466±0.003c

Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at $p < 0.05$ level.
± Standard Error;

Sporobolus coromandelianus* (Retz.) Kunth, *Euphorbia hirta* Forsk

Table 3

Effect of lead on root/shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio of *Sporobolus coromandelianus* (Retz.) Kunth and *Euphorbia hirta* Forsk

Treatment lead (Pb) ppm	Root / shoot ratio	Leaf weight ratio	Specific leaf area cm^2/g	Leaf area ratio
00	*0.610±0.0030a **0.680±0.0036a	0.059±0.760ab 0.094±0.003ab	36.66±2.29a 607.80±1.74a	2.17±0.158a 57.30±2.29a
20	0.440±0.0036ab 0.400±0.01ab	0.038±0.630b 0.077±0.004b	66.66±0.749ab 287.30±4.64ab	2.54±0.158ab 22.20±0.949ab
40	0.526±0.0035ac 2.13±0.02a	0.064±0.541b 0.045±0.001bc	33.33±2.29b 118.00±1.43bc	2.15±0.152b 5.39±0.158bc
60	0.631±0.0037bc 0.392±0.03ab	0.060±0.856ac 0.079±0.003ac	25.75±0.949bc 276.00±0.52ac	1.50±0.891bc 21.74±3.96ca
80	0.399±0.0036c 0.379±0.02b	0.045±0.145b 0.107±0.003c	13.71±0.714c 201.25±0.001bc	0.62±0.8564c 21.69±3.966b
100	0.479±0.0035ac 0.600±0.0036c	0.077±0.098ac 0.098±0.003c	10.00±0.922ab 161.5246±01.66c	0.77±0.098bc 15.94±0.158c

Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at $p < 0.05$ level.
± Standard Error;

Sporobolus coromandelianus* (Retz.) Kunth, *Euphorbia hirta* Forsk

Table 4

Percentage reduction in shoot length, root length, seedling dry weight and number of leaf of *Sporobolus coromandelianus* (Retz.) Kunth and *Euphorbia hirta* Forsk

Treatment lead (Pb) ppm	Shoot length (%)	Root length (%)	Total plant dry weight (%)	Number of leaf (%)
20	*9.10 **10.60 ⁺	3.20 3.40	37.70 2.60	1.50 ⁺ 18.66
40	18.00 11.84	12.20 50.70	7.90 46.00	35.00 82.0 ⁺
60	4.80 18.0	9.60 43.30	34.60 46.08	41.00 30.10
80	20.80 0.00	25.00 53.30	23.60 34.70	39.00 31.40
100	22.50 4.40	31.60 49.00	23.70 60.50	52.19 13.86

+ Percentage increase

The harmful effects of lead on seedling growth of *S. coromandelianus* and *E. hirta* was discussed. Seedlings of *S. coromandelianus* exhibited better tolerance to Pb treatments at all treatment as compared to *E. hirta*. The inhibitory impact of lead on seedlings growth were recorded in order *S. coromandelianus* > *E. hirta*. It can be

concluded that Pb treatment at higher level produced negative effects on the seedling growth performance of both species. Based on toxicity and tolerance indices (%), a great care is required by environmental managers while planting these species in metal contaminated areas.

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