Effects of Lead on Different Seedling Growth Attributes of Cowpea (*Vigna unguiculata* L.)

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Original Research Article

ABSTRACT

Aims: This study was conducted to determine the toxicity and tolerance to lead by the seedling growth of an important country legume crop cowpea (*Vigna unguiculata* L).
Study Design: The seedlings of cowpea were grown in sand culture at 0, 20, 40, 60, 80 and 100 ppm of metal ions of lead salt as lead acetate.
Place and Duration of Study: The experimental site is located in the Department of Botany at the Karachi University Campus, Pakistan in 2011.
Methodology: The healthy seeds of *Vigna unguiculata* L. were surface sterilised with 0.2% solution of sodium hypochlorite (NaOCL) for one minute to avoid any fungal contamination. The sand was collected from the construction site of the Karachi University washed 2-3 times with tap water, distilled water and also with 5% HCl to remove any types of impurities from the soil. Seedlings were grown in sand culture at 0, 20, 40, 60, 80 and 100 ppm of metal ions of lead salt as lead acetate. The Hoagland solution was used for the supply of nutrient elements. The experiment was conducted

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for six weeks. 5 ml of lead concentration were poured weekly and before given concentration of lead, the materials of the tray were drained out to avoid any algal contaminations. The experiment was completely randomized and consists of six treatments replicated six times. After six weeks the seedlings were harvested and morphological parameters shoot, root, seedling length (cm), number of leaves and leaf area (sq. cm) was noted. The biomass production such as shoot, root, leaf and total seedling dry weight (g) was also observed along with root/shoot, leaf weight, leaf area ratio and specific leaf area. The seedlings of cowpea were dried in an oven at 80° C for 24 hours until the seedlings were completely oven dried. Leaf area, Root/shoot ratio, leaf weight ratio, specific leaf area, leaf area ratio was determined, and a tolerance index was determined. The data obtained were statistically analyzed.

Results: The effects of different concentrations (0, 20, 40, 60, 80, 100 ppm) of lead on seedling growth performance of cowpea (*Vigna unguiculata* L.) as compared to control were observed. 100 ppm lead treatment in the form of lead acetate highly affected seedling growth and biomass production of *V. unguiculata* L. as compared to control Lead treatment at 40 ppm produce significant (P = .05). % reduction in seed germination of *V. unguiculata* L. as compared to control. Lead treatment at 20 ppm concentration produced a significant reduction in shoot length as compared to control. Root growth is an important growth variable and found negatively affected by the different concentration of lead treatment. The results also showed that lead treatment in the substrate at the same concentration (20 ppm) produced a significant effect on root and seedling growth of *V. unguiculata* L. The treatment of lead at 20 ppm produced significant ($p<0.05$) on the seedling dry weight of *V. unguiculata* L. as compared to control. 80 ppm concentration of lead treatment was found sufficient to cause significant % reductions in the seedling dry weight of *V. unguiculata* L. as compared with control. The seedlings of *V. unguiculata* L. were also tested for percentage of tolerance to lead. The results showed that *V. unguiculata* L. has a high tolerance to lead at 20 ppm and lowest at 80 ppm of lead. *V. unguiculata* seedlings showed the highest percentage of tolerance (92.50 %) to lead at 20 ppm. The lowest *V. unguiculata* seedlings were 64.50 % at 80 ppm of lead, but better tolerance of *V. unguiculata* L. seedlings was 73.25 % at 60 ppm of lead concentration.

Conclusion: In conclusion, lead was toxic causing significant reduction (P = .05). to the seedling and biomass production of cowpea. Increased in lead concentration decreased the tolerance indices of cowpea seedlings growth.

Keywords: Crop; germination; growth; lead;tolerance; toxicity.

1. INTRODUCTION

Plants are an integral part of life in many indigenous communities and modified the plant cover over wide areas [1-2]. Lead is considered highly toxic to the growth of plants. Lead (Pb) is an environmental pollutant extremely toxic to plants and other living organisms including humans [3]. High concentrations of Pb eventually may cause cell death [4]. Soil pollution with lead is a problem of concern [5].

The concentrations of heavy metals in the environment have increased at an alarmingly level. Metals are toxic to plants and fungi [6]. There are few detail reports on the impacts of metals on seed metabolism, germination, particularly roots and shoots recorded [7]. Lead is a global environmental pollutant that is present in the soil, water, air and biota. The increase in the concentration of heavy metal decreased plant growth and response for death [8]. Lead naturally occurs in substantial quantities in the earth's surface food, water urban soil and air and lead stress causes multiple direct and indirect effects on plant growth, metabolism and also alters some physiological processes [9-10]. The metals absorbed by plants and prove toxic to plants that can be observed as growth retardation, alteration in biochemical processes [11-13].

In Pakistan, substantial quantities of the agricultural chemical are used annually to enhance yield [14]. The ever increase of lead concentration over the wide areas of Karachi and rural areas raising serious questions as to its effects plant growth. Although the data on the effects of lead on cowpea seems scanty. The response of plant growth to toxic effects of heavy metals has become the subject of great interest in the field of ecology. Attention has been given in developed countries, about the effects of metal toxicities on crop growth. Therefore, this study was carried out with the aim to determine the effect of lead on the seedling growth of an important country legume crop cowpea (*Vigna unguiculata* L.).

2. MATERIALS AND METHODS

The healthy seeds of *Vigna unguiculata* L. were obtained from the market and were surface sterilised with 0.2% solution of sodium hypochlorite (NaOCl) for one minute to avoid any fungal contamination. The sand was collected from the construction site of the Karachi University and was sieved through a 2.0 mm sieve and after that, it was washed 2-3 times with tap water and later with distilled water. The sand was also washed with 5% HCl to remove any type of impurities. The pots with 7.3 cm in diameter and 9.6 cm in depth were filled with sand up to 2/3. All the pots were then placed in trays. Seedlings were grown in sand culture at 20, 40, 60, 80 and 100 ppm of metal ions of lead salt as lead acetate. In control, no treatment was given except distilled water. As nutrients elements were absent in sand, therefore, Hoagland solution was used for the supply of nutrients. The Hoagland solution was applied for 3-4 days. Three uniform size seedlings of cowpea were transplanted in each pot. Initially, 5 ml solution of lead acetate $Pb(CH_3COO)_2$ were applied. Every week the appearance of seedlings growth was recorded. The irrigation was carried out with the tap water on a daily basis. The experiment was conducted for six weeks. The nutrient solution was given after three days time interval for nutrition and proper growth of the plant. 5 ml solution of lead concentration were poured weekly and before given concentration of lead, the materials of the tray were drained out to avoid any algal contaminations. The experiment was completely randomized and replicated six times.

After six weeks, the seedlings were harvested. The shoot, root, seedling length (cm), number of leaves and leaf area, the specific leaf was recorded. The biomass production such as shoot, root, leaf and total seedling dry weight (g) was also determined along with root/shoot, leaf weight, leaf area ratio and specific leaf area. The seedlings of cowpea were dried in an oven at 80° C for 24 hours until the seedlings were completely oven dried.

Root/shoot ratio, leaf weight ratio, specific leaf area, leaf area ratio was determined by the following formulae, respectively.

Root/ shoot ratio = root dry weight / shoot dry weight

Leaf weight ratio = leaf dry weight/total plant dry weight
Specific leaf area = Leaf area/leaf dry weight
Leaf area ratio = Leaf area / Total plant dry weight

A tolerance index was determined by the following formulae as described by Iqbal and Rahmati [15]:

Mean root length in metal solution/Mean root length in distilled water X 100

Analysis of variance and Duncan's Multiple Range Test using personal computer software packages SPSS version 14.0 statistically analysed the data obtained.

3. RESULTS

Lead treatment was found toxic to all seedling growth parameters of *V. unguiculata* L. The seedling growth performance of *V. unguiculata* L.were tested in different concentrations (0, 20, 40, 60, 80 and 100 ppm) of lead as compared to control (Tables 1-3, Fig.1). Lead treatment was found highly toxic to all seedling growth parameters of *V. unguiculata* L. at 100 ppm concentration. Lead treatment at 40 ppm produced significant ($p<0.05$) effects on shoot length of *V. unguiculata* L. as compared to control (Table 1). Lead treatment at 20 ppm affected root growth of *V. unguiculata* L.. Lead treatment at 20 ppm did not produce significant effects on seedling length, while the increase in lead concentration at 40 ppm significantly decreased the seedling length of *V. unguiculata* L. as compared to control. Lead treatment at 40 ppm concentration produced toxic effects on a number of leaves of *V. unguiculata* L. and leaves were highly affected at 100 ppm lead concentration. The results also showed that lead treatment at similar concentration 40 ppm produced a significant effect on leaf growth of *V. unguiculata* L.

Lead treatment at all concentrations decreased high percentage of the seedling dry weight of *V. unguiculata* L. (Table 2). The treatment of lead at 40 ppm concentration produced significant ($p<0.05$) on shoot dry weight of *V. unguiculata* L. as compared to control. However, increase in the concentration of lead treatment at 80 -100 ppm further decreased shoot dry weight of *V. unguiculata* L.as compared with control. Lead treatment at 20 ppm concentration significantly affected root dry weight of cowpea. Lead treatment at 20 and 40 ppm concentrations were less toxic for the decrease in leaf dry weight of cowpea. Lead treatment at 40 ppm concentration showed more decrease in leaf dry weight of cowpea. While the Lead concentration of 20 ppm showed a high percentage of decrease in total plant dry weight of *V. unguiculata* L.

Lead concentration of 20 ppm also showed a high percentage of decrease in root/shoot ratio, leaf weight, specific leaf area and leaf area ratio of *V. unguiculata* L. (Table 3).

The seedlings of *Vigna unguiculata* L. were also tested for percentage of tolerance to lead. The results showed that *Vigna unguiculata* L. has greater tolerance to lead at 20 ppm and lowest at 100 ppm concentration Fig. 1. *V. unguiculata* L. showed higher tolerance of 94.46 % to lead at 20 ppm and lowest tolerance of 74.10 % at 100 ppm of lead concentration. *Vigna unguiculata* L. seedlings showed the better percentage of tolerance of 87.09 % at 60 ppm and 74.99 % at 80 ppm lead treatment Fig. 1.

Table 1. Effects of lead on seedling growth of cow pea (*V. unguiculata*)

Please arrange all table data in standard MS. Word table format as shown below

Treatments (ppm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	No of leaves	Leaf area (sq. cm)
0	48.03±0.28d	22.39±0.28e	70.33 ±0.52d	12.50 ±0.76c	28.43±0.281d
20	47.05±0.26d	21.15±0.13d	68.20 ±0.37d	11.00 ±0.77c	27.40±0.35bd
40	42.73±0.74c	19.50±0.25c	62.23 ±0.99c	9.33 ±0.95ab	25.10±0.58c
60	39.38±1.44b	17.81±0.14b	57.20±1.82b	8.66 ±0.80ab	20.31 ±1.35b
80	32.96±1.38a	16.78±0.53ab	49.75±1.89a	7.83 ±0.87a	17.63 ±0.40a
100	30.71±1.39a	16.60±0.45a	47.31±1.75a	7.66 ±0.42a	16.66 ±0.18a

Table 2. Effects of lead on seedling dry weight of cow pea (*V. unguiculata*)

Please arrange all table data in standard MS. Word table format as shown below

Treatments (ppm)	Shoot dry weight (g)	Root dry weight (g)	Leaf dry weight (g)	Total plant dry weight (g)
0	1.74±0.04b	0.86±0.02d	0.95±0.01c	3.55±0.06e
20	1.56±0.19b	0.84±0.03c	0.83±0.041c	3.24±0.21d
40	1.23±0.18a	0.74±0.02ab	0.77±0.04c	2.70±0.18c
60	1.03±0.07a	0.69±0.04ab	0.67±0.03ab	2.43±0.12bc
80	0.92±0.05a	0.64±0.03a	0.63±0.02a	2.20±0.08ab
100	0.86±0.03a	0.50±0.03a	0.48±0.04a	1.84±0.09a

Table 3. Effects of lead on root/shoot, leaf weight, specific leaf area and leaf area ratio cow pea (*V. unguiculata*)

Please arrange all table data in standard MS. Word table format as shown below

Treatments (ppm)	Root / shoot ratio	Leaf weight ratio	Specific leaf area	Leaf area ratio
0	0.49±0.01b	0.26±0.01b	27.16± 0.14a	7.27±0.09a
20	0.58±0.09a	0.26±0.02a	29.18±1.08b	7.64±0.64b
40	0.66±0.08a	0.29±0.03a	31.19±1.48bc	8.87±0.62d
60	0.67±0.01a	0.29±0.01a	31.14±1.38b	9.02±0.43c
80	0.70±0.02a	0.28±0.01a	30.62±1.43b	8.78±0.36bc
100	0.58±0.02a	0.26±0.01a	36.61±2.79c	9.30±0.37bc

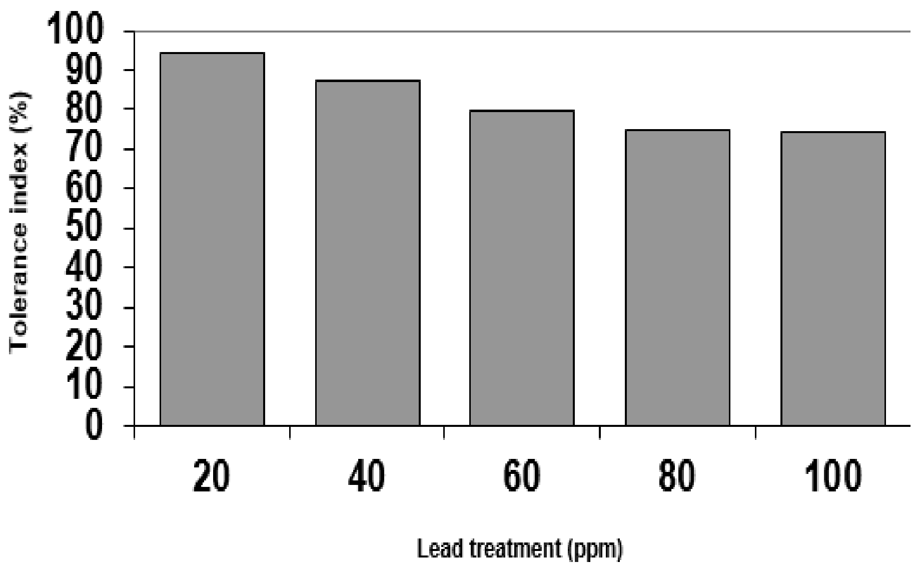

Fig. 1. Percentage tolerance index in seedling of cow pea (*Vigna unguiculata*) to different concentration of lead

4. DISCUSSION

The plants under stress condition are likely may be affected by high concentrations of pollutants. The growth of plants or any other living organisms affected directly or indirectly in the presence of heavy metals [16]. Lead is a toxic heavy metal. In the present study, the effect of different concentrations of lead on the root, shoot, seedling length and seedling dry weight of an important bean crop, cowpea *Vigna unguiculata* L. were recorded. Kasim et al. [17] also showed that lead treatment at 150 mM produced significant physiological, photosynthetic and ultra structural changes in seedlings of *Vigna unguiculata* L.

The seedling growth parameters of cowpea responded differently to lead treatment at a higher concentration as compared to control. The high percentage decrease in shoot growth of cowpea at 40-60 ppm lead treatments provided evidence that the treatment of lead in excess was inhibitory to plant growth and development. In the present study, a significant decrease in the number of leaves and leaf area of cowpea at 100 ppm lead treatment was also observed. An excessive amount of toxic elements usually caused a reduction in plant growth [18]. The permeability of metals can decrease the growth of plants. The reduction in the seedling growth of *V. unguiculata* L. with the increase in the concentration of lead provides further evidence that the lead in excess may be inhibitory to plant growth and development. Excessive concentrations of metals result in phytotoxicity [19]. Similarly, a drastic decrease in seedling growth of *Vigna radiata* (L.) Wilczek cv. Pusa Baisakhi in the presence of 1.0 mM lead acetate [20]. Root elongation tests have been used to evaluate the damage caused by toxic compounds [21] and many species including cabbage, lettuce, carrot, cucumber, tomato and oats have been recommended for the phytotoxicity test [22]. The roots have the ability to supply water and nutrients to the plants. They are also required to produce hormones, which may regulate the growth and performance of both root and shoot [23]. In the present, the study, the root growth of *V. unguiculata* L. was found to be highly decreased at 80 ppm lead concentration. These findings also agree with the work of [24] who reported that a low concentration of 1 µM lead (Pb) reduced root, shoot growth of cowpea (*Vigna unguiculata* L.). The primary site of Pb^{2+} toxicity was the root, where it caused severe reductions in root growth, loss of apical dominance and the formation of localised swellings behind the root tips.

The significant decrease in seedling dry weight of *V. unguiculata* L. due to the metal toxicity of lead was also recorded in the present work. The treatment of lead in *V. unguiculata* L. provided evidence that the trace element in the nutrient medium when present in excess may be inhibitory to plant growth and development especially at a concentration greater than 60 ppm. Toxicants accumulate in the plant when soluble forms are present in high quantities. The exact amount of accumulation depends upon the solubility of the pollutants in the soil [25]. The biomass production of *V. unguiculata* L. was initially non-significant and decreased and significantly with increasing the lead concentrations up to 100 ppm. Heavy metals at higher concentrations are toxic to plant growth [26] by modifying metabolic processes, oxidative stress in plants [27] and excessive accumulation in plant tissue may disturb the physiological processes such as inhibition of root growth recorded in *Vigna mungo* (L.) [28].

5. CONCLUSION

According to the tolerance test, it could be seen that tolerance to lead was higher at low concentration of lead in the seedlings of *Vigna unguiculata* L. These results showed that the reason of tolerance against heavy metals might be a physiological association of the tolerance mechanism to these metals. The seedling growth of *V. unguiculata* L. showed a high percentage of tolerance to lead at 20 ppm concentration. The treatment of 100 ppm concentration of lead produced the lowest percentage of tolerance in the cowpea the seedlings in the present study.

It is concluded, that concentration of heavy metals like lead and other metals in the environment has been increased due to automobiles, industries, agrochemicals and anthropogenic activities and are responsible for limiting the crop yield. Lead is found to be a highly toxic pollutant for plants. The results showed that treatment of different concentrations of lead to the seedlings of *V. unguiculata* L. responded differently with the increasing contamination exposure as 20, 40, 60, 80 and 100 ppm. The response of *V. unguiculata* L. seedlings in the form of tolerance indices to lead treatment was found suitable pollutant indicator to study the deleterious effects of the lead. The present findings proved the deleterious effects of lead at a higher concentration to the seedlings of *V. unguiculata* **L**. The information from the present studies would be helpful in understanding the level of lead tolerance in seedlings of *V. unguiculata* **L.** while growing in lead polluted areas. Heavy reliance on metals containing agrochemicals such as fungicides, nematicides, and pesticides (Lead arsenate) should be discouraged. The continuous release of lead into the immediate environment may endanger the growth performance of other crops. Current research shows that lead treatment at different concentration has produced an important effect on seed germination and seedling growth of *V. unguiculata* **L**.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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