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The Influence of Lead on Seedling Growth of Wheat (Triticum Aestivum L.)

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Abstract
The concentration of heavy metals such as lead (Pb) in the environment has been increased due to automobiles, industries, agro chemicals and anthropogenic activities and responsible for limiting the crop yield. The effects of different concentrations (0, 20, 40, 60, 80, 100 ppm) of lead on seedling growth performance of wheat (*Triticum aestivum*) were recorded. Lead treatment in the form of lead acetate at 100 ppm highly affected seedling growth and biomass production of *T. aestivum* as compared to control. Lead treatment at 40 ppm concentration produced significant ($p<0.05$) reduction in shoot length. Root growth is an important growth variable and found greatly reduced at different concentrations of lead treatment. The results also showed that lead treatment at 20 ppm produced significant effect on shoot length and number of leaves, leaf area of *T. aestivum*. The treatment of lead at 20 ppm produced significant ($p<0.05$) reduction on seedling dry weight of *T. aestivum* as compared to control. The seedlings of *T. aestivum* were also tested for percentage of tolerance to lead. The results showed that *T. aestivum* has high tolerance to lead at 20 ppm (90.26 %) and lowest at 100 ppm (62.36 %) of lead. *T. aestivum* seedlings showed better percentage of tolerance (80.81 %) to lead at 60 ppm.
Keywords: Germination, growth, lead, specific leaf area, tolerance, wheat, toxicity.

1. Introduction

The discharge of untreated chemicals from industries, household, fertilizers and automobiles are responsible for spreading of different types of toxic chemical compounds in the air, water, soil affecting the environment and growth of plants. Among the toxic elements release in the environment, lead is highly toxic for growth of plants. Lead (Pb) is an environmental pollutant extremely toxic to plants, fungi and other living organisms including humans (Lamhamdi, et al., 2011; Crane et al. 2012; Shafiq et al., 2008; Shafiq and Iqbal, 2012). Heavy metal contamination levels in agricultural soil are of major significance because of the potential to accumulate in soil for a long period of time (Iwegbue et al. 2013). High concentration of metal ions in soil environment may pose a significant risk to the quality of soils, plants, natural waters and human health (Wu and Zhang, 2010). Heavy metals in soil has the potential of transfer through soil infiltration down to ground water aquifer or plant – root uptake and subsequent transfer into the food chain and excessive accumulation of heavy metals in agricultural soils may result not only in soil contamination but also consequences for food quality and public health safety issues (Haliru et al., 2014).

Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage (Yadav 2010). Given the ever increasing environmental pollution with metal(loid)s, few reports regarding impacts of metals on seed metabolism, viability and germination, vegetative tissues, roots and shoots are available (Kranner and Colville, 2011).

Attention has been given, in developed countries, about the effects of metal toxicities on germination and growth of plants. Lead is a global environmental pollutant that is present in soil, water, air and biota. The increase in concentration of heavy metal decreased plant growth and responsible to death (Kumar *et al.,* 2011). Lead is the heaviest of the nonradioactive metals and also naturally occur in the earth's surface of the soil (DeAbrreu et al., 1998). Lead is an important industrial heavy metal, which contaminates environment and ultimately, food, water urban soil and air (Haq et al., 2013). Lead stress causes multiple direct and indirect effects on plant growth and metabolism and alters some physiological processes (Diaz et al., 2001). The metals have their atomic mass more than 50 are considered heavy metals. In western countries, the toxicity was assessed because of the impact of heavy metals/metalloids on biological objects in soils and soil solutions (Vodyanitskii, 2016).

Large quantities of agricultural chemical used annually to enhance yield in Pakistan (Nuzhat *et al.,* 2005). The ever increase concentration of toxic pollutants over the wide areas of Karachi and rural areas raising serious questions as to its effecting growth of agricultural crops. The response of crop growth to heavy metals has become the subject of great interest in recent years globally because of their nature of toxicity to plants. Therefore, the aim of the paper was to determine the effect of lead on seedling growth of an important country crop wheat (*Triticum aestivum*).

2. Materials and methods

The healthy seeds of *Triticum aestivum* L. were obtained from the local market and surface sterilized with 0.2% solution of sodium hypochlorite (NaOCl) for one minute to avoid any fungal contamination. The experimental site is located in the Department of Botany at the Karachi University Campus. The sand was collected from the construction site of the Karachi University. The sand was sieved through 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 types of impurities. The pots with 7.3 cm in diameter and 9.6 cm in depth were filled with sand upto 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 wheat (*Triticum aestivum*) 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 daily basis. The experiment was conducted for six weeks. The nutrient solution (The Hoagland solution) was given after three days time interval for nutrition and proper growth of plant. Five 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, specific leaf was recorded. The biomass production such as shoot, root, leaf and total seedling dry weight (g) was also determined alongwith root / shoot, leaf weight, leaf area ratio and specific leaf area. The seedlings of wheat 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 were 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 (1992):

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 analyzed the data obtained.

3. Results

Lead treatment was found toxic to the all seedling growth parameter including (shoot, root and seedling length, number of leaves, leaf area) of wheat (*Triticum aestivum* L.). The seedling growth performance of *T. aestivum* were tested using different concentrations (0, 20, 40, 60, 80 and 100 ppm) of lead as compared to control (Table 1-6, Fig. 1). Lead treatment in the form of lead acetate at 100 ppm was found highly toxic to all seedling growth parameter of *T. aestivum*. Lead treatment at 20 ppm did not produce any significant effects on root and shoot length of *T. aestivum* as compared to control (Table 1).

Table 1. Effects of lead on seedling growth of wheat (*Triticum aestivum*)

Treatments (ppm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	No of leaves	Leaf area
0	32.00±1.09c	10.58±1.04c	42.58 ±2.04b	4.83 ±0.16b	6.16 ±0.41c
20	30.83±0.79bc	9.55±0.79bc	40.38 ±1.51ab	4.66 ±0.21ab	4.93 ±0.67bc
40	30.33±1.33 abc	9.16±1.21bc	36.92 ±4.52ab	4.66 ±0.21ab	4.13±0.14b
60	28.00±19.8ab	8.55±0.78abc	36.55 ±2.75ab	4.66 ±0.33ab	3.51 ±0.55b
80	27.08±0.63a	7.55±0.14ab	34.63 ±0.77a	4.16 ±0.16ab	2.13 ±0.47a
100	26.66±0.66a	6.60±0.34a	33.26 ±1.00a	4.00 ±0.25a	1.86 ±0.41a

Numbers followed by the same letters in the same column not significantly different ($p<0.05$) according to Duncan's multiple range test. ± Standard error

Increase in concentration of lead treatment at 40 ppm concentration produced toxic effects on shoot length as compared to control. Root growth is an important growth variable and found significantly ($p<0.05$) affected at 60 ppm lead treatment. The results also showed that lead treatment at 80 ppm produced significant effect on number of leaves of *T. aestivum* as compared to control. The treatment of lead at 40 ppm produced significant ($p<0.05$) reduction on seedling dry weight of *T. aestivum* as compared to control (Table 2).

Table 2. Effects of lead on seedling dry weight of wheat (*Triticum aestivum*)

Treatments (ppm)	Shoot dry weight (g)	Root dry weight (g)	Leaf dry weight (g)	Total plant dry weight (g)
0	0.20±0.01b	0.07±0.01b	0.04±0.003c	0.32±0.02a
20	0.20±0.02b	0.07±0.02b	0.04±0.001c	0.31±0.08b
40	0.16±0.01 b	0.06±0.01ab	0.03±0.003c	0.25±0.07b
60	0.15±0.01a	0.05±0.01ab	0.02±0.004ab	0.23±0.01a
80	0.13±0.01a	0.04±0.01a	0.02±0.002a	0.19±0.01a
100	0.12±0.01a	0.03±0.01a	0.02±0.002a	0.17±0.01a

Numbers followed by the same letters in the same column not significantly different ($p<0.05$) according to Duncan's multiple range test. ± Standard error

The treatment of lead at all concentration did not produce any significant effect on root / shoot ratio and specific leaf of *T. aestivum* as compared to control (Table 3). The treatment of lead at 20 ppm produced significant ($p<0.05$) effect on leaf weight ratio and leaf area ratio of wheat (*T. aestivum*) as compared to control (Table 3).

Table 3. Effects of lead root /shoot, leaf weight, specific leaf area and leaf area ratio on seedling of wheat (*Triticum aestivum*)

Treatments (ppm)	Root / shoot ratio	Leaf weight ratio	Specific leaf area	Leaf area ratio
0	0.35±0.02a	0.13±0.01b	149.55± 8.99a	19.42±1.43c
20	0.36±0.03a	0.03±0.02a	119.41±17.64a	3.36±1.97a
40	0.36±0.03a	0.01±0.02a	124.44±13.36a	1.46±0.38a
60	0.37±0.07a	0.12±0.02b	127.05±20.37a	15.93±3.01bc
80	0.34±0.06a	0.12±0.01b	97.38±25.96a	11.03±2.17b
100	0.30±0.03a	0.11±0.01b	96.83±20.05a	11.04±2.80b

Numbers followed by the same letters in the same column not significantly different ($p<0.05$) according to Duncan's multiple range test. ± Standard error

Lead treatment at all concentration decreased high percentage of shoot, root, and seedling length, number of leaves and leaf area (Table 4). While, lead treatment also highly decreased shoot, root, leaf and total plant dry weight of *T. aestivum* as the concentration of lead increased (Table 5).

Table 4. Percent reduction in seedling growth of wheat (*Triticum aestivum*) using different concentration of lead.

Treatments (ppm)	Shoot length	Root length	Seedling Length	No of leaves	Leaf area
20	3.65	9.73	5.16	3.51	19.96
40	5.21	13.42	13.29	3.51	32.95
60	12.50	19.18	14.16	3.51	43.01
80	15.37	28.63	18.67	13.87	65.42
100	16.68	37.61	21.88	17.18	69.80

Table 5. Percent reduction in seedling dry weight of wheat (*Triticum aestivum*) under different concentration of lead.

Treatments (ppm)	Shoot dry weight	Root dry weight	Leaf dry weight	Total plant dry weight
20	0	0	0	3.12
40	20	14.28	25	21.87
60	25	28.27	50	28.12
80	35	42.85	50	40.62
100	40	57.14	50	46.87

Lead treatment at 20 ppm concentration was less toxic for decrease in shoot length (3.65 %), root length (9.73 %), seedling length (5.16 %), number of leaves (3.51 %), leaf area (19.96%) and total plant dry weight (3.12 %). Lead treatment at 40 ppm concentration showed more decrease in shoot length (5.21 %), root length (13.42 %), seedling length (13.29 %) and seedling dry weight (21.87 %) of *T. aestivum*. Lead concentration of 60 ppm further decreased shoot length (12.50 %), root length (19.18 %), seedling length (14.16 %) and total seedling dry weight (28.12 %) of *T. aestivum* as compared to control. Lead concentration of 80 ppm decreased shoot length (15.37 %), root length (28.63 %), seedling length (18.67 %) and plant dry weight (40.62 %) of *T. aestivum* as compared to control. Lead concentration of 100 ppm showed highest percentage of decease in shoot length (16.68 %), root length (37.61 %), seedling length (21.88 %) and number of leaves (17.18 %) of wheat. The treatment of lead at 100 ppm decreased shoot dry weight (40 %), root dry weight (57.14%) and leaf dry weight (50%). Lead treatment at 100 ppm , root / shoot ratio (14.28 %), leaf weight ratio (15.38 %), specific leaf area (35.25%) and leaf area ratio (43.15 %) of *T. aestivum* as compared to control (Table 6).

Table 6. Percent reduction in root / shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio of wheat (*Triticum aestivum*) using different concentration of lead.

Treatments (ppm)	Root / shoot ratio	Leaf weight ratio	Specific leaf area	Leaf area ratio
20	+2.85	76.92	20.15	82.60
40	+2.85	92.30	16.79	92.48
60	+5.71	7.69	15.04	17.97
80	2.85	7.69	34.88	43.20
100	14.28	15.38	35.25	43.15

+= Percent increase

The seedlings of *T. aestivum* were also tested for percentage of tolerance to lead. The results showed that *T. aestivum* has greater tolerance to lead at 20 ppm and lowest at 100 ppm of lead (Fig. 3). *T. aestivum* showed higher tolerance (76.27 %) to lead at 40 ppm and lowest (34.74 %) at 100 ppm of lead. *T. aestivum* seedlings showed better percentage of tolerance 72.20 % to lead at 60 ppm and (58.98 %) at 80 ppm lead treatment (Fig. 1).

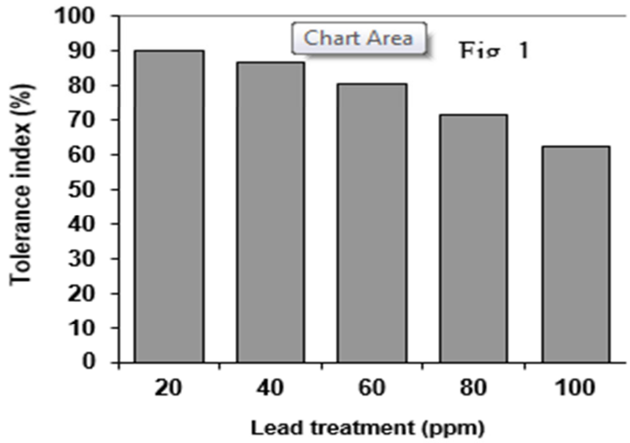

Fig. 1. Percentage tolerance index in seedlings of wheat (*T. aestivum*) to different concentration of lead treatment.

4. Discussion

Plants play a unique role for the existence of all heterotrophic organisms (Kralova and Masarovieova, 2006). Researchers have studied the influence of abiotic stress on plants. The direct or indirect stress of heavy metals, salt, chilling, drought or UV-B radiation may affect the development, growth and basic metabolisms in plants (Szollosi 2014). Lead is a toxic and dangerous heavy metal. The lead treatment at 150 mM showed a significant physiological, photosynthetics and utltra structural changes in seedlings of *Vigna unguiculata* (Kasim *et al.,* 2014). In present study, the effect of lead on root, shoot, seedling growth and seedling dry weight of an important crop wheat (*T. aestivum*) was recorded. The seedling growth of wheat responded differently to lead treatment at higher concentration as compared to control. High percentage of decrease in root, shoot, seedling length, number of leaves and leaf areas of wheat at higher concentration of lead at 40-60 ppm and provided evidence that treatment of lead in excess inhibitory to plant growth and development. Metal toxicity is an important factor governing germination and growth of plants. The reduction in the seedling and root growth of *T. aestivum* with the increase in concentration of lead at 100 ppm provides further confirmed that the lead in excess may be inhibitory to plant growth and development. Excessive amount of toxic element usually caused reduction in plant growth (Kubota and Allaway, 1972). Toxic metal ions accumulate in plants same as uptake processes of essential micronutrient metal ions. The amounts of metal absorbed by a plant depend on the concentrations and speciation of the metal in the soil solution result in phytotoxicity (Patra *et al.* 2004). The plant reaction with respect to it stresses are complex and involves in many kinds of physiological and biochemical processes. Singh *et al.,* (2003) recorded the reduction in seedling growth of *Vigna radiata* (L.) Wilczek cv. Pusa Baisakhi when treated with 1.0 mM lead.

The root elongation tests have been found to evaluate the damage caused by toxic compounds present in various composts (USEPA, 1982). Many species including cabbage, lettuce, carrot, cucumber, tomato and oats have been recommended for the phytotoxicity test (F.D.A., 1982). The root growth of *T. aestivum* was found decreased more than 35 percent at 100 ppm lead concentration. The results of this investigation have shown that lead treatment is more toxic for *T. aestivum* for root development. These findings are also agree with the work of (Kopittke et al., 2007). A low concentration of 1 µM lead (Pb) reduced the root and shoot of cowpea (*Vigna unguiculata*). The primary site of Pb^{2+} toxicity was the root, caused severe reductions in root growth, loss of apical dominance, the formation of localized swellings behind the root tips (due to the initiation of lateral roots), and the bending of some root tips (Kopittke *et al.,* 2007).

The significant decrease in seedling dry weight of *T. aestivum* due to metal toxicity of lead was also recorded. The treatment of lead in *T. aestivum* provided evidence that the trace element in nutrient medium if present in excess may be inhibitory to plant growth and development especially at more than 80 ppm. Toxicants accumulate in the plant when soluble forms are present in high quantities (Treshow, 2010). The biomass production of *T. aestivum* was initially non significant and decreased significantly with increasing the lead concentrations upto 100 ppm.

Studies on differential tolerance of mung bean cultivars to metalliferous mine wastes and tannery effluents were observed (Samantaray and Rout, 1999; Bera and Kanta-Bokria, 1999). The toxicity and tolerance to lead depends on the concentration, type of salt, soil properties and plant species. According to tolerance test it could be seen that tolerance to lead was higher at low concentration of lead in the seedlings of *T. aestivum*. 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 *T. aestivum* showed high percentage of tolerance to lead at 20 ppm concentration. The treatment of 100 ppm concentration of lead produced lowest percentage of tolerance in seedling of wheat.

5. Conclusion

It is concluded that treatment of different concentrations of lead to seedlings of *T. aestivum* responded differently. The present findings proved the deleterious effects of lead at higher concentration to the seedlings of *T. aestivum.* The information from the present studies would be helpful in understanding the level of lead tolerance in seedlings of *T. aestivum* while growing in lead polluted areas. The findings may contribute understanding the potential of wheat seedlings plantation in land management programs. Heavy reliance on metals containing agrochemicals such as fungicides, nematicide, 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 biomass production of wheat. The accumulation of such types of toxic pollutants in larger concentrations by crop can produce harmful effects to other agricultural crops and ecosystems. The response of *T. aestivum* seedlings in the form of tolerance indices to lead treatment was found suitable pollutant indicator about the deleterious effects of the lead.

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