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TOXIC EFFECTS OF LEAD AND CADMIUM INDIVIDUALLY AND IN COMBINATION ON GERMINATION AND GROWTH OF *LEUCAENA LEUCOCEPHALA* (LAM. DE-WIT)

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Abstract

Leucaena leucocephala is economically an important plant. Seeds collected from two different populations of *L. leucocephala* responded differently to metal treatments. Germination of *L. leucocephala* showed significant ($p<0.05$) effect when treated with 25, 50, 75 and 100 μg^{-cm^3} of Pb and Cd individually and in combination. It was observed that Cd was more toxic than Pb on all measured variables. With increasing concentrations of Pb and Cd, the rate of seed germination was reduced for seeds collected from the polluted area. Cd treatment at 100 μg^{-cm^3} caused a significant reduction in root growth for seeds collected from the polluted area. At combined treatments, the seed germination and seedling growth decreased than individual treatments of Pb and Cd. The seeds of *L. leucocephala* collected from the polluted area caused significant ($p<0.05$) reduction in shoot length at combined treatment of 100 μg^{-cm^3} Pb and Cd. Reduction in seed germination and seedling growth at 25 μg^{-cm^3} of Pb treatment for seeds collected from the polluted area was also observed. Increased in concentration of Pb upto 75 μg^{-cm^3} suppressed root length for seeds collected from the polluted area. Root growth was greatly reduced due to heavy metals than shoot growth. The shoot length of *L. leucocephala* showed a significant reduction in combined treatment at 50 μg^{-cm^3}Pb + 50 μg^{-cm^3} Cd, than individual treatment of Pb and Cd.

Introduction

Among the heavy metals, Cd and Pb are highly toxic pollutants. They are generally added in the environment through automobile exhaust (Lagerwerff and Specht 1970), fertilizer impurities (William and David 1973) and through industrial effluents (Campbell 1976). Deposition of Pb on the vegetation growing along the roads not only affects growth and germination but also causes significant reduction in seed and fruit production of plants (Nasralla and Ali 1985). Foliar application of lead nitrate solution resulted in a reduction in various growth indices and yield parameters of wheat (Rashid and Mukherji 1993). Diameter foliage and new stem growth of sycamore appeared to be synergistically affected by combined Pb plus Cd treatment (Carlson and Bazzaz 1977). Trees in cities are subject to widespread pressure, which suppresses performance and shortens life span (Gilbertson and Bradshaw 1985). Roadside plants and crops grown near the roads were found to contain more lead (Albasel and Cottenie 1985).

In industrialized cities like Karachi, automobile emission is a major source of atmospheric pollution (Qadir and Iqbal 1991). Vehicular traffic releases detrimental exhaust gases and toxic pollutant, which are affecting the city environment. 100 millions ton gasoline powered vehicles on the road today are a mobile source of number of air pollutants, including unburnt and partially burnt hydrocarbons, lead compounds and other elements that are contained in petrol and lubrication oils. These pollutants are also harmful since, they may combine with the other pollutants

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to cause greater or synergistic effects. These air pollutants may interfere with the biological processes relating to general metabolism, photosynthetic activities, mitochondrial respiration and stomatal clogging of plants (Miles et al. 1972; Miller et al. 1973; Ahmed and Qadir 1975).

The response of plant growth and metabolism to heavy metals has become the subject of great interest in recent years because of their high toxicity to plants (Al-Helal 1994). Pb and Cd toxicity has become important due to their presence in the environment and found responsible for producing detrimental effect on plant growth (Iqbal and Shafiq 1998). In view of the destructive role of heavy metals derived from the auto vehicular exhaust emissions on plants, an investigation was carried out to determine the toxic effects of lead and cadmium on seed germination and growth of *L. leucocephala.*

Materials and methods

The healthy seeds of *Leucaena leucocephala* (Lam. de-Wit were collected from two different habitats, the University campus, a clean site and a polluted site in Gulshan-e-Iqbal (University Road). The top end of the seeds were slightly cut with the clean scissor. The seeds were placed in petridish (90 mm in diameter) on Whatman No. 1 filter paper and treated with 25, 50, 75 and 100 μg^{-cm^3} Pb in the form of lead nitrate and cadmium as cadmium sulfate individually and in combination. The experiment was completely randomized with three replicates. Petridishes were kept at room temperature 25±2C°. In control, no treatment was given except distilled water. 2 ml of solution of each treatment was added to the respective Petridish. The number of germinated seeds were counted after 10 days of treatment. Germination was scored as protrusion of the radicle through the testa. Seedling dry weight was determined by drying the plant materials in an oven at 80 degree centigrade for 24 h. The data obtained were statistically analyzed to work out ANOVA. Duncan Multiple Range Test (DMRT) was applied to compare the mean values.

Tolerance indices were determined using the following formula:

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

Results

The toxicity and tolerance of Pb and Cd individually and in combination in terms of seed germination, seedling growth were found different for both populations of *L. leucocephala* (Fig. 1-6 and Table 1). Twenty-five μg^{-cm^3} Pb treatment significantly ($p<0.05$) reduced the seed germination of *L. leucocephala* collected from the polluted and clean areas. Similarly, the seedling length of both populations was greatly reduced. The treatment of Pb at 25 μg^{-cm^3} significantly reduced the root growth for seeds collected from the polluted area, whereas no such reduction in root growth was observed at the same lead level for seeds collected from the clean area. Significant ($p<0.05$) reduction in shoot length was observed at 75 μg^{-cm^3} treatment of Pb in population from the polluted area. The seed germination and seedling growth of *L. leucocephala* showed significant reduction at 25 μg^{-cm^3} Pb treatment for seeds collected from the polluted and clean areas (Fig. 1-2). The effects of Pb treatment at 75 μg^{-cm^3} on shoot length were more pronounced for population collected from the polluted area. Root growth was highly affected at 100 μg^{-cm3} Cd treatment for population collected from the polluted area. The seed germination of *L. leucocephala* also showed a significant reduction in combined treatment at 25 μg^{-cm3}Pb + 25 μg^{-cm3} Cd for population collected from both areas (Fig. 1). The increase in the combined treatment of Pb and Cd showed great reduction in shoot and root growth of population collected from the polluted as compared to clean area. The combined treatment of Pb+Cd at 100 μg^{-cm3} showed highest decrease in seed germination and seedling growth for population collected from the polluted area. The tolerance to Pb and Cd treatment individually and in combination were found different for population collected from the polluted and clean area. The population of *L. leucocephala* collected from the clean area showed high percentage of tolerance to lead and cadmium treatment as compared to that from the polluted area.

Discussion

The inhibition of seed germination and seedling growth of *L. leucocephala* appeared to be due to toxicity of Pb and Cd in solution. Seed germination inhibition by heavy metals has also been reported by some workers (Brown and Wilkins 1986; Morzek and Funiceli 1982). Pb and Cd treatments caused toxic effects on the root growth for seeds collected from the polluted and clean areas. The reason for reduced root length in metal treatments could be due to reduction in mitotic cells in meristematic zone of root as suggested by Lerda (1992) on *Allium cepa*. These findings confirm that Pb reduced the frequency of mitotic cell in meristematic zone and Pb causes inhibition of root growth. Chao and Wang (1990) showed that percentage length of infected roots and dry biomass of maize plant were reduced by the addition of heavy metals. The seedling growth of *L. leucocephala* showed a significant reduction with increase in concentration of Cd solution. Iqbal and Mehmood (1991) had also found gradual decrease in plant growth with the increase in concentration of Cd. Heavy metals had also inhibited the seedling growth of *Phaseolus aureus* (Sharma 1982). The shoot and seedling length of both seeds collected from the polluted and clean area were found decreased at 100 μg^{-cm3} Cd than the other treatments. The reason of reduced shoot and seedling length in metal treatments could be the reduction in meristematic cells present in this region and some enzymes contained in the cotyledon and endosperms. Cells become active and begin to digest and store food which is converted into soluble form and transported to the radicle and plumule tips e.g. enzyme amylase convert starch into sugar and protease act on protein. So when activities of hydrolytic enzyme were affected, the food did not reach to the radicle and plumule and in this way shoot and seedling length were affected.

The situation is rare when only one pollutant is present at a time. In most of the circumstances more than one pollutant is known in the atmosphere, already contaminated by a large number of substances (Iqbal 1987). At combined treatments, all the measured variable were also decreased in similar to the individual treatments of Pb and Cd. At the combined treatments of 100 μg^{-cm3} Pb + 100 μg^{-cm3} Cd, the seedling growth and germination percentage of both seeds were greatly reduced. It showed that Pb, when combined with Cd has got adverse effects on plants as compared to individual treatments. The reason for growth reduction at both combined treatments than individual treatments of Cd could be the interaction of Pb and Cd. These results were supported by Carlson and Bazzaz (1977); they determined that the growth was synergistically affected in the combined Pb plus Cd treatments. Kahle and Breckle (1989) had studied that when metals were applied on the roots of young beech in combined treatment, 20 ppm Pb and 1 ppm Cd, the effect was great. The seeds of *L. leucocephala* collected from the clean area were more tolerant than polluted area. The degree of effectiveness of seeds was varied with their genetic ability. Mathur et al. (1987) have found that higher concentration of Cd and Cr (100-250 ppm) had affected germination and early growth performance of *Allium cepa.*

It is concluded that Pb and Cd individually and in combination had produced adverse significant effect on all the growth phases of both populations particularly on the root growth. Cd showed comparatively more effects on the seedling growth than Pb at individual treatments for seeds collected from the polluted area. Cd in mixture with Pb was more toxic. The inhibitory effect of both metals on seed germination may be due to ionic toxicity. Both metals produced toxic effects on root than shoot growth. The reason might be due to rapid accumulation of heavy metals in root than shoot. According to tolerance test it could be seen that tolerance to Pb and Cd individually and in combination for seeds of *L. leucocephala* collected from the polluted area was lower than clean area. The reason of low tolerance against both metals might be due to changes in the physiological association of the tolerance mechanism in seed germination and seedling growth. The treatment of heavy metals (Hg, As, Pb, Cu, Cd and Cr) 2 and 5 mg L^{-1} decreased the hill activity, chlorophyll, protein and dry weights and increased the tissue permeability over control values in *Azolla pinnata* (Sakoar and Sasdhar 1986). A great deal of effort is required to prevent this economically important plant for being damaged to pollution. The imbalance in the environment might leads to eliminate this species. The treatment of lead and cadmium on seeds of *L. leucocephala* collected from the polluted environment had proved the loosing viability of the seeds deriving from these automobiles and their ultimate impact on the plant growth.

Table 1. Different growth attributes of seeds of *Leuacena leucocephala* collected from clean area (a) and polluted (b) areas in different treatment of lead and cadmium.

Variables	Pb	Cd	Pb+Cd
Seed germination (%)	9.39 a 19.92 b	16.93 a 19.92 b	23.48 a 29.33 b
Seedling length (mm)	27.40 14.51	26.84 17.18	15.60 19.87
Shoot length (mm)	12.57 14.32	15.07 19.52	12.91 17.08
Root length (mm)	12.57 14.32	15.07 14.92	12.91 17.08
Seedling dry weight (g)	0.04 0.01	0.03 0.02	0.04 0.04

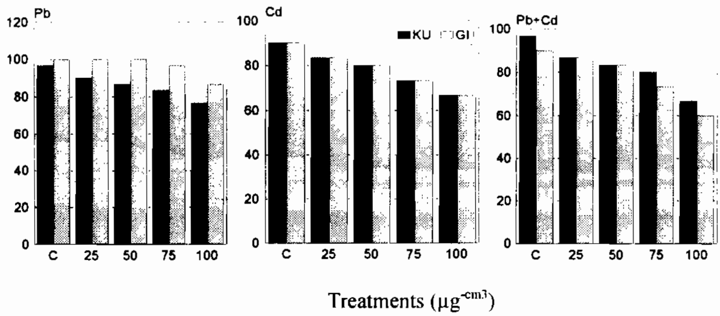

Figure 1. Effects of lead and cadmium on seed germination of *Leucaena leucocephala.*
KU= Karachi University Campus
GI= Gulshan-e-Iqbal

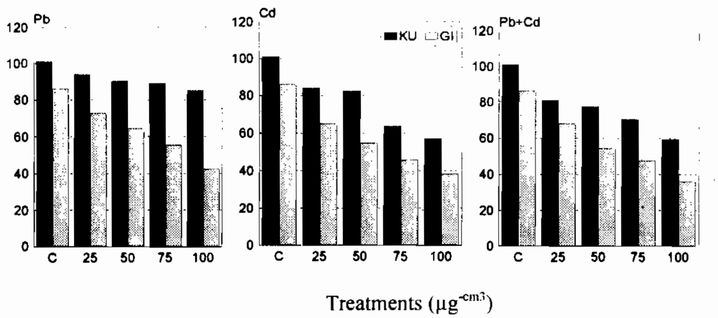

Figure 2. Effects of lead and cadmium on seedling length of *Leucaena leucocephala.*
KU= Karachi University Campus
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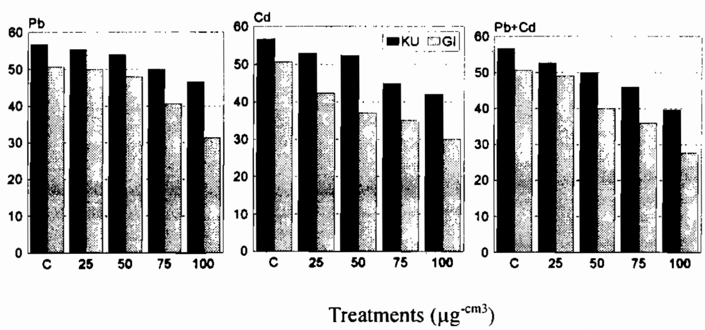

Figure 3. Effects of lead and cadmium on shoot length of *Leucaena leucocephala.*
KU= Karachi University Campus
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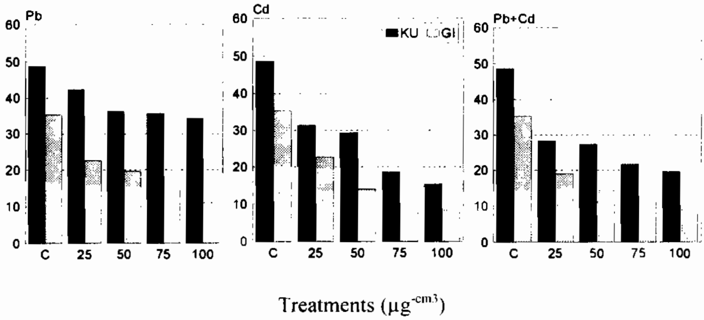

Figure 4. Effects of lead and cadmium on root length *Leucaena leucocephala*.
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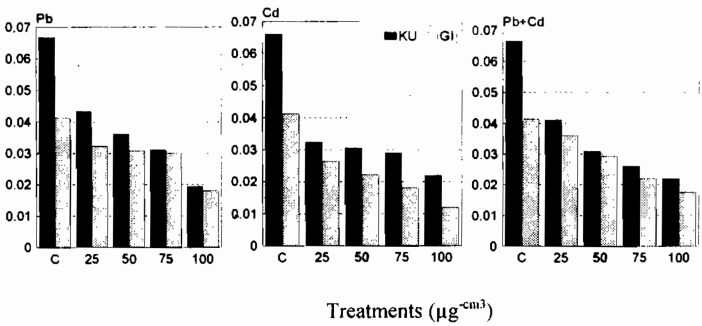

Figure 5. Effects of lead and cadmium on seedling dry weight of *Leucaena leucocephala*.
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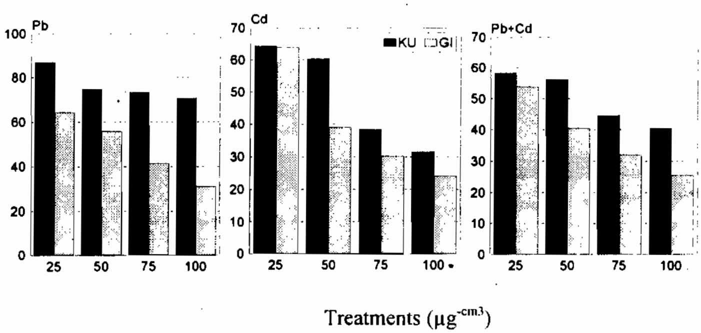

Fig.6. Tolerance index of *Leucaena leucocephala*.
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