

## REDUCTION IN GERMINATION AND SEEDLING GROWTH OF *THESPSIA POPULNEA* L., CAUSED BY LEAD AND CADMIUM TREATMENTS

M. KABIR, M. ZAFAR IQBAL, M. SHAFIQ AND Z.R. FAROOQI

Department of Botany, University of Karachi,  
Karachi-75270, Pakistan.

### Abstract

The effects of lead (Pb) and cadmium (Cd) on seed germination, root, shoot, seedling growth, seedling dry weight and seedling vigor index of *Thespesia populnea* L. were studied. Seed germination, seedling growth and seedling dry weights were significantly ( $p < 0.05$ ) affected by different concentrations (10, 30, 50 and 70  $\mu\text{mol/L}$ ) of lead as compared to control. Lead concentration at 10  $\mu\text{mol/L}$  significantly reduced seed germination, seedling growth and dry weight as compared to control. Cd treatment at 10  $\mu\text{mol/L}$  concentration also produced toxic effects on seed germination, seedling and root growth as compared to control. Increase in cadmium concentration up to 50  $\mu\text{mol/L}$  produced a significant reduction in seedling dry weight of *T. populnea* as compared to control. Seedlings vigor index of *T. populnea* gradually decreased with the increase in concentrations of lead and cadmium. Lead and cadmium treatments at 70  $\mu\text{mol/L}$  exhibited lowest percentage of tolerance as compared to control. It was concluded that inhibitory effects of cadmium treatments were more prominent than lead at its higher concentrations.

### Introduction

Metals have toxic effects and act as persistent pollutants in the atmosphere and add either by natural or anthropogenic activities. Although some metals are regarded essential nutrients, excess concentrations of all metals lead to various toxic effects such as oxidative stress and inhibition of enzyme activities (Dietz *et al.*, 1999; Pohlmeier, 1999). Most of the metal precipitates readily in soil can be observed. Those metals which immobilized are considered more hazardous to plants than those which can be dissolved. The former group includes Mn, Fe, Cd, As, Pb and Hg. The metals most damaging to crops are Cd, Cu, Mo, Ni, Pb and Zn (Lasat, 2002). Higher level of metal pollutants as Pb and Cd can pose lethal effects on plant growth. Metallic contaminations in environment and their accumulation in plants are very toxic since they affect germination and plants growth. Burhan *et al.*, (2001) suggested that there are about 50 metals which are toxic to human health, plants and animals. Metals can inhibit the activities of several enzymes, seed germination and seedling growth (Burzynski & Mereck, 1990; Hailing *et al.*, 1991; Singh & Srivastava, 1991; Iqbal & Siddiqui, 1992). Seed germination inhibition by heavy metals has been reported by many workers (Brown & Wilkins, 1986; Morzek & Funiceli, 1982; Shafiq & Iqbal, 2005). Javed & Sahar (1987) have reported reduction in germination of maize at 5-100 mM lead nitrate treatments. Iqbal & Mehmood (1991) reported a gradual decrease in plant growth of *Dalbergia sissoo* Roxb., with increasing cadmium levels.

Lead and cadmium are highly toxic elements. The harmful effects of Pb and Cd have been demonstrated by many workers (Breckle & Kahile, 1992; Lerda, 1992; Shafiq & Iqbal, 2005; Atiq-ur-Rehman & Iqbal, 2008). Toxic effects of lead and cadmium individually and in combination on germination and growth of *Leucaenia leucocephala* have been reported (Iqbal *et al.*, 2001). Deposition of Pb on the vegetation growing along the road sides not only affects growth and germination but also causes significant reduction in seed and fruit production of plants (Nasralla & Ali, 1985). Foliar applications of lead nitrate solution caused reduction in various growth indices and yield parameters of wheat (Rashid & Mukherji, 1993). The response of plant metabolism to heavy metals has become the subject of great interest in recent years because of their high toxicity to plants (Al- Helal, 1994).

*T. populnea* is a plant of the family Malvaceae with tropical and subtropical world wide distribution. The species comprised of trees, which are sometimes cultivated either for their usefulness to different traditional cultures or for ornamental purposes including their use as a medicine and in timber for craft. Dye can also be synthesized from this species. It is also used as a shade tree and as a wind breaker in order to control soil erosion.

*T. populnea* is planted along the busy roads and the impact of metals on this plant is not known. The present study was undertaken with a view to find out the toxic effects of lead and cadmium on seed germination and seedling growth of an important arid tree *Thespesia populnea*.

## Materials and Methods

Healthy seeds of *Thespesia populnea* L., were randomly collected from the Karachi University Campus. The seeds were air dried and stored at room temperature before treatments with metal solution. The micropyle end of seeds were cut with scissor to break the seed dormancy. The seeds were soaked in 0.2% solution of mercuric chloride for two minutes to avoid any fungal growth. The selected seeds were placed in 10 cm diameter Petri dishes lined with filter paper Whatman No. 42. Ten seeds were cultured in each Petri dish with six replications for each treatment. Lead (Pb) and cadmium (Cd) treatments were prepared using lead nitrate and cadmium nitrate with concentrations 10, 30, 50 and 70  $\mu$  mol/L, respectively. pH of different concentrations of lead and cadmium were determined by Keen (1931) which were in the range of 5.47-5.08 for lead and 6.27-5.96 for cadmium. At the start of experiment 3 ml of respective treatment was added in order to moisten the filter paper in each Petri dish and at every 2<sup>nd</sup> day, the old solution was sucked out and replaced with subsequently new solution. All the Petri dishes were kept at room temperature (25 $\pm$ 3°C). Distilled water was added to the Petri plates in place of metal solutions control. Seeds were considered to have germinated at radical emergence of 1mm. After 12 days, percentage germination was recorded and maximum root, shoot and seedling length were also obtained. Seedling dry weight was determined by placing the seedling in oven at 80°C for 24 hours. Seedling dry weight was measured with electrical balance. Seedling vigor index (S.V.I) was determined as per formula given by Bewly & Black (1982).

Tolerance indices (T.I.) were determined through use of the following formula given by Iqbal & Rahmati (1992):

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

Data were statistically analyzed by Analysis of Variance (ANOVA) (Steel & Torrie, 1984) and Duncan Multiple Range Test (DMRT) (Duncan, 1955) at  $p < 0.05$  level.

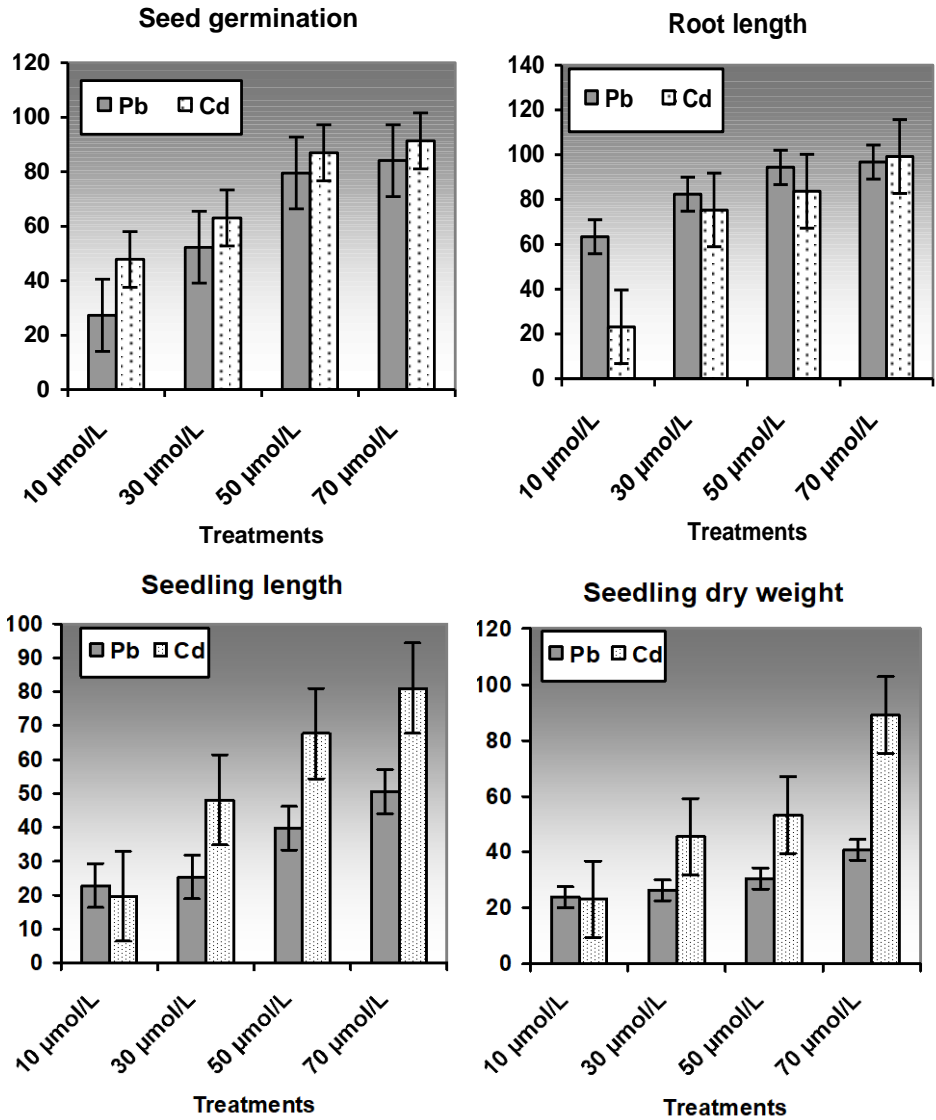


Fig. 1. Percent decrease in seed germination, seedling length, root length and seedling dry weight of *T. populnea* in various concentrations of lead and cadmium as compared to control.

**Results**

The results indicate that seed germination, seedling growth, dry weight and seedling vigor index of *Thespesia populnea* L., were reduced in all treatments (10, 30, 50 and 70 µmol/L) of lead and cadmium as compared to control (Table 1 & 2; Figs. 1-2). Lead treatment at 10 µmol/L produced significant ( $p<0.05$ ) effect on seed germination as compared to control. Lead treatments at 10, 30, 50 and 70 µmol/L concentrations showed adverse effects on the seedling length by lowering it from 6.39 to 3.16 cm (Table 1).



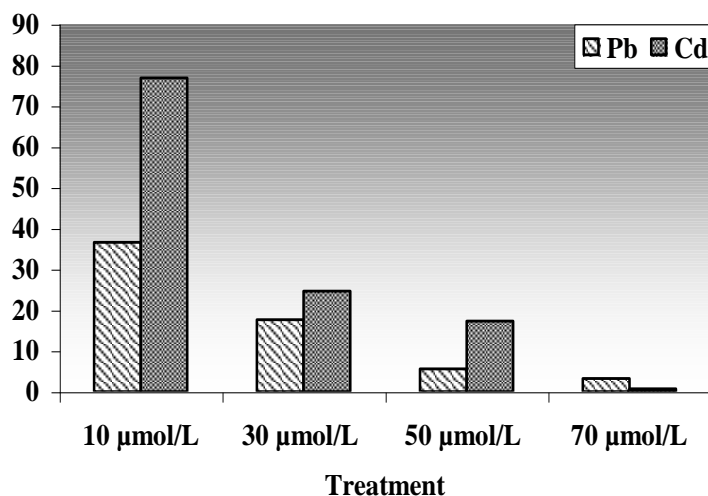
*Tolerance indices*

Fig. 2. Indices of tolerance of *Thespesia populnea* L.

Seed germination, seedling growth and dry weight of *T. populnea* got significant reduction at different concentration of cadmium treatment (10, 30, 50 and 70 µmol/L) as compared to control. Cd treatment at 10 µmol/L significantly reduced seed germination, seedling length and root length as compared to control (Table 2). Increase in concentration of Cd up to 50 µmol/L produced toxic effects on biomass production of *T. populnea* as compared to control. Lead and cadmium treatment at 10, 30, 50 and 70 µmol/L markedly decreased high percentage of seed germination, seedling length, seedling dry weight and root length. The assessment of early seedling growth as a comparison in terms of root length followed the same reduction pattern as observed for seed germination, in various applied concentrations of lead and cadmium. Root length was consistently reduced with increased concentrations of both metals. Maximum suppression of root length (0.08 cm) was recorded at the highest concentration of Pb at 70 µmol/L while at 70 µmol/L Cd treatment root length was reduced up to 0.02 cm as compared to control. The results for shoot length are not same as observed in other two earlier parameters. Lead treatments at 10 and 30 µmol/L increased the shoot length by 4.02 and 4.33 cm, respectively. However, increase in concentrations of Pb at 50, and 70 µmol/L reduced the shoot length. Shoot length was gradually reduced to 1.23 cm in 70 µmol/L of cadmium treatment. The data recorded on seedling dry weight revealed that application of Pb at 10, 30, 50 and 70 µmol/L suppressed the dry weight by 3.96, 3.83, 3.61 and 3.08 grams respectively as compared to control (5.20 g). While increased Cd treatments from 10-70 µmol/L reduced the dry weight from 4.75, 3.37, 2.89 and 0.67 grams as compared to control (6.18 g) and at the same concentrations of Pb.

Seedlings vigor index of *T. populnea* gradually decreased with increase in concentrations of lead and cadmium treatments. Seedlings of *T. populnea* also showed low percentage of tolerance to lead and cadmium treatment as compared to control. Tolerance to lead and cadmium treatment at 10, 30, 50 and 70 µmol/L exhibited the lowest level as compared to control (Fig. 2). It was concluded that inhibitory effects of Cd treatments were most severe on root length, shoot length, seedling length and seedling dry weight at higher concentrations of 70 µmol/L.

## Discussions

Metal toxicity is an important factor governing germination and growth of plants. During the last few years a significant increase in the amounts of metals has appeared in the environment. The constant increase of lead and cadmium over wide areas having more traffic density and metal processing industries raise serious questions as to its effects on the growth and vigor of trees. Germination and seedling establishment are vulnerable stages in the plant life cycle (Vange, *et al.*, 2004). We have investigated that how metal treatments affected germination and early growth performance of *T. populnea*. Lead and cadmium treatments at high concentration (70  $\mu\text{mol/L}$ ) found responsible for highest percentage of reduction in seed germination and seedling growth of *T. populnea*. Reduction in seed germination of *T. populnea* provided evidence that the element like Cd or Pb if present in excess is responsible in producing toxic effects which reduced plant growth and development. Similarly, Dalal & Bairgi (1985) have found reduction in seed germination, root, shoot and seedling length of jute varieties, *Corchorus olitorius* cv. JRO 524 and *Capsular corchorus* JRC 321 at different levels of Pb, particularly at 20 mg/l. The reduction in root length in *T. populnea* was more prominent in different concentrations of Pb and Cd treatments as compared to shoot and seedling length. It was also reported by Stefan *et al.*, (1991) in some other species. The reduced root length of *T. populnea* in metal treatments could be due to reduction in mitotic cell division in meristematic zone of root as suggested by Lerda, (1992) on *Allium cepa*. The reduction in root length occurred by accumulation of metals within the root, reducing the mitotic rate in meristematic zone specially blocking the metaphase in meristematic cells, therefore, roots show reduction in length (Goldbold & Kettner, 1991; Sharifah & Hishashi 1992). The other reason of reduced shoot and seedling length of *T. populnea* 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 radical and plumule tips e.g., enzyme amylase convert starch into sugar and protease act on protein. So when enzymatic activities were affected, the food did not reach to the radical and plumule and in this way shoot and seedling length were affected. Lead and cadmium treatments were found responsible for marked reduction in seedlings growth of *T. populnea*. It was also noted that inhibitory effects of Pb and Cd treatments at 70  $\mu\text{mol/L}$  concentration were more severe. Iqbal and Mahmood, (1991) had also found gradual decrease in plants growth with the increase in concentration of Cd. Tolerance to lead and cadmium in *T. populnea* was very low at higher concentrations. The reason of low tolerance to lead and cadmium might be due to occurrence of changes in physiological functions taking place during germination of *T. populnea* seedlings.

In the present investigation, it is concluded that lead and cadmium treatment produced toxic impact on germination, seedling growth and seedling dry weight and seedling vigor index of *T. populnea* as compared to control. Increase in the concentrations of both metals in the medium, brought up changes in most of the growth parameters of *T. populnea*. Therefore, there is a need to implement certain rules that help in the reduction of metal level from a wide range of sources such as from the metal processing industries and power generation plants. Seedling growth is considered as an indicator of metal stress on plant vigor. The findings can contribute to better ecological fragility, the potential of *T. populnea* tree in coordinating in land management programmes in metal contaminated

areas. Further plantation of *T. populnea* in lead and cadmium polluted area will help in reducing the burden of metal pollution. The identification of the toxic concentration of metals and tolerance indices of plant species would also be helpful for the establishment of air quality standard. Further more research studies with different metal stresses can be helpful in the solution of various problems associated with metal pollution in any region. Metal toxicity issues are of significant concern in many metal processing industries, including mining. There is a need to undertake further studies to establish the state of knowledge on the responses of plants to metal toxicities.

## References

- Al-Helal, A.A. 1995. Effects of cadmium and mercury on seed germination and early seedling growth of rice and alfalfa. *Journal of the University of Kuwait (Science)*, 22: 76-82.
- Ara, F., M.Z. Iqbal and M.S. Qureshi. 1996. Determination of heavy metals contamination of trees and soils due to vehicular emission in Karachi city. *Karachi University Journal of Science*, 24: 80-84.
- Attiq-ur-Rehman, S. and M.Z. Iqbal. 2008. Level of heavy metals in the foliage of naturally growing plants collected from Korangi and Landhi industrial area of Karachi city. *Pakistan Journal of Botany*, 40(2): 785-789.
- Bewly, J.D. and B.M. Black. 1982. Germination of seeds. In: *Physiology and biochemistry of seed germination*. (Ed.): A.A.Khan, Springer Verlag, New York, pp. 40-80.
- Breckle, S.W. and H. Kahile. 1992. Effects of toxic heavy metals Cd, Pb on growth and mineral nutrition of beech *Vegetatio*, 101: 43-53.
- Brown, M.T. and D.A. Wilkins. 1986. The effect of zinc on germination, survival and growth of *Betula*. (Series A), 41: 53-61.
- Burhan, N., S.S. Shaikat and A. Tahira. 2001. Effects of zinc and cobalt on germination and seedling growth of *Pennisetum americanum* L. *Pakistan Journal of Biological Sciences*, 4: 575-580.
- Burzynski, S. and K. Mereck. 1990. Effect of Pb and Cd on enzymes of nitrate assimilation in cucumber seedling. *Acta Physiology Plants*, 12: 105-110.
- Dalal, T. and P. Bairgi. 1985. Effect of Hg, As and Pb on germination and seedling growth of two Jute varieties. *Environmental Ecology*, 3: 403-407.
- Dietz, K.J., M. Baier and U. Krämer. 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: *Heavy Metal Stress in Plants: From Molecule to Ecosystems*. (Eds.): M.N.V. Prasad and J. Hagemeyer, Springer, Berlin, Germany. pp. 73-98.
- Duncan, D.B. 1955. Multiple Range and Multiple F-Test. *Biometrics*, 11: 1-42.
- Goldbold, D.L. and C. Kettner. 1991. Lead influences on root growth and mineral nutrition of *Picea abies* seedling. *Plant Physiology*, 139: 95-99.
- Hailing, L.I.U., L. I. Qing and Y. Peng. 1991. Effects of cadmium on seed germination, seedling growth and oxidase enzyme in crops. *Chinese Journal of Environmental Science*, 12: 29-31.
- Iqbal, M.Z. and T. Mehmood. 1991. Influence of cadmium toxicity on germination and growth of some common trees. *Pakistan Journal of Scientific and Industrial Research*, 34(4): 140-142.
- Iqbal, M.Z. and D. A. Siddiqui. 1992. Effect of lead toxicity on seed germination and seedling growth of some road side trees. *Pakistan Journal of Scientific and Industrial Research*, 35: 139-141.
- Iqbal, M.Z. and D.A. Siddiqui. 1992. Effects of lead toxicity on seed germination and seedling growth of some tree species. *Pakistan Journal of Scientific and Industrial Research*, 35: 139-141.
- Iqbal, M.Z. and K. Rahmati. 1992. Tolerance of *Albizia lebbek* to Cu and Fe application. *Ekologia (CSFR)* 11: 427-430.

- Iqbal, M.Z., M. Shafiq and A.S. Kausar. 2001. Toxic effects of lead and cadmium individually and in combination on germination and growth of *Leucaena leucocephala* (Lam.) de Wit. *Pakistan Journal of Botany*, 33: 551-557.
- Iqbal, M.Z., T. Mehmood and F. Ahmed. 1991. Influence of cadmium toxicity on germination and growth of some common trees. *Pakistan Journal of Scientific and Industrial Research*, 34(40): 140-142.
- Javed, I. and M. Sahar. 1987. Effects of lead germination, early seedling growth and soluble protein and acid phosphatase content in *Zea mays* L. *Pakistan Journal of Scientific and Industrial Research*, 30: 853-856.
- Keen, B.A. 1931. *The Physical Properties of Soil*. New York. Longman Green and company, pp. 380.
- Lasat, M.M. 2002. Phytoextraction of toxic metals. A review of biological mechanism. *Journal of Environmental Quality*, 31: 109-120.
- Lerda, D. 1992. The effect of lead on *Allium cepa*. *Mutation Research*, 231: 80- 92.
- Morzeck, J.R.E. and N.A. Funicelli. 1982. Effect of zinc and lead on germination of *Spartina alterniflora* Loisel., seeds at various salinities. *Env. Exp. Bot.*, 22: 23-32.
- Nasralla, M.M. and E.A. Ali. 1985. Lead accumulation in edible proteins of crop grown near Egyptian traffic roads. *Agriculture Eco. Environ.*, 13: 73-82.
- Pohlmeier, A. 1999. Metal speciation, chelation and complexing ligands in plants. In: *Heavy Metal Stress in Plants: From Molecule to Ecosystems*. (Eds.): M.N.V. Prasad and J. Hagemeyer, Springer, Berlin, Germany. pp. 29-50.
- Rashid, P. and A. Mukherjee. 1990. Effects of lead nitrate on some anatomical features of mung bean. *Bangladesh Journal of Botany*, 19: 149-154.
- Shafiq, M. and M.Z. Iqbal. 2005. The toxicity effects of heavy metals on germination and seedling growth of *Cassia siamea* Lamark. *Journal of New Seeds*, 7: 95-105.
- Shafiq, M. and M.Z. Iqbal. 2005. Tolerance of *Peltophorum pterocarpum* D.C. Baker Ex K. Heyne seedlings to lead and cadmium treatments. *Journal of New Seeds*, 7: 83-94.
- Sharifah, B.A. and O. Hishashi. 1992. Effect of lead, cadmium and zinc on the cell elongation of *Impatiens balsamina*. *Environ. Experi. Bot.*, 32: 439-448.
- Singh, D.N. and Srivastava. 1991. Effects of cadmium on seed germination and seedling growth of *Zea mays*. *Biol. Sci.*, 61: 245-247.
- Steel, R.G.D. and J.H. Torrie. 1984. *Principles and procedures of statistics*. Mc Graw Hill Book C., Inc., Singapore, p. 172-177.
- Stefan, A., I. Arundin and A. Onnes. 1991. Germination and initial growth in presence of heavy metals. *Ann. Bot. Fenn.*, 28: 37-43.
- Vange, V., I. Hevchand and V. Vandvik. 2004. Does seed mass and family affect germination and juvenile performance in *Knautia arvensis*? A study using failure time methods. *Acta Oecologia*, 25(3): 169-178.

(Received for publication 29 February 2008)