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Phytotoxic effects of mercury on seed germination and seedling growth of *Albizia lebbeck* (L.) Benth. (Leguminosae)

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Abstract. A study was conducted to determine the phytotoxic effect of mercury on seed germination and seedling growth of an important arid legume tree *Albizia lebbeck*. The seeds germination and seedling growth performance of *A. lebbeck* responded differently to mercuric chloride treatment (1 mM, 3 mM, 5 mM and 7 mM) as compared to control. Seed germination of *A. lebbeck* was significantly ($p < 0.05$) affected by mercury treatment at 1 mM. Root growth of *A. lebbeck* was not significantly affected by mercury treatment at 1 mM, and 3 mM. Shoot and root length of *A. lebbeck* were significantly ($p < 0.05$) affected by 5 mM concentration of mercury treatment. Increase in concentration of mercury treatment at 5 mM and 7 mM significantly ($p < 0.05$) reduced seedling dry weight of *A. lebbeck*. The treatment of mercury at 1 mM decreased high percentage of seed germination (22%), seedling length (10%), root length (21.85%) and seedling dry weight (9%). Highest decrease in seed germination (51%), seedling (34%), root length (48%) and seedling dry weight (41%) of *A. lebbeck* occurred at 7 mM mercury treatment. *A. lebbeck* showed high percentage of tolerance (78.14%) to mercury at 1 mM. However, 7 mM concentration of mercury produced lowest percentage of tolerance (51.65%) in *A. lebbeck*. The seed germination potential and seedling vigor index (SVI) clearly decreased with the higher level of mercury. Plantation of *A. lebbeck* in mercury-polluted area will help in reducing the burden of mercury pollution. *A. lebbeck* can serve better in coordinating in land management programs in metal contaminated areas. The identification of the toxic concentration of metals and tolerance indices of *A. lebbeck* would also be helpful for the establishment of air quality standard.

Keywords: mercury; seed germination; growth; seedling vigor index; tolerance; toxicity; Albizia lebbeck; tree

1. Introduction

Rapid increase in the industrial and anthropogenic activities and discharge of untreated chemicals in the immediate environment is responsible for spreading of different types of chemical compounds in the air, soil and water, affecting the environment and growth of plants. Among the toxic elements released in the environment, mercury is considered highly toxic for the growth

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of .plants. The effects of mercury on plants have been well documented. Mercury may enter plant foliage through two primary pathways: (1) uptake of the oxidized from (Hg (II) or methyl mercury); (2) adsorbed onto soil particles and/or dissolved in soil water through roots (Boszke *et al*. 2008, Rea *et al*. 2002). Che *et al*. (2003) reported toxicity in cottonwood trees between 8 and 40 ppm of soil mercury and plant death at 400 ppm of soil mercury. Toxic metal ions enter cells by means of the same uptake processes as 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, its movement successively from the bulk soils to the root surface, then into the root and finally into the shoot (Kacálková *et al*. 2009). Excessive concentrations of metals result in phytotoxicity through: (i) changes in the permeability of the cell membrane; (ii) reactions of sulphydryl (-SH) groups with cations; (iii) affinity for reacting with phosphate groups and active groups of ADP or ATP; and (iv) replacement of essential ions (Patra *et al*. 2004). Toxic metal ions enter cells by means of the same uptake processes as 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, its movement successively from the bulk soils to the root surface, then into the root and finally into the shoot. Excessive concentrations of metals result in phytotoxicity through: (i) changes in the permeability of the cell membrane; (ii) reactions of sulphydryl (-SH) groups with cations; (iii) affinity for reacting with phosphate groups and active groups of ADP or ATP; and (iv) replacement of essential ions (Patra *et al*. 2004, Rodrigues *et al*. 2010, Rodríguez *et al*. 2009).

Mercury is a silvery metal and is present in the environment in organic and inorganic forms. Toxicity of metals on plant growth has been observed in certain isolated areas where excessive amount occurred. Mercury at low concentrations represents major hazards to living organism. In Pakistan, substantial quantities of agricultural chemicals are used annually to enhance yield (Nuzhat *et al*. 2005). Mercury used for eliminating various pests causes harmful effects on agricultural plants. They produce toxic effects on the leaves where crucial functions such as photosynthesis and transpiration are carried out, cause morphological, anatomical and physiological changes, inhibits pollen germination and pollen tube formation and thus affected fruit production (Gill and Garg 2014, Tort *et al*. 2005). Seed health plays an important role for successful cultivation and yield exploitation of a crop species (Rajput *et al*. 2005). Mercury (5 μM $HgCl_2$), a general blocker of aquaporins in various organisms, reduced the speed of seed germination and induced a true delay in maternal seed coat (testa) rupture and radicle emergence, by 8-9 and 25-30 hours, respectively **(**Willigen *et al*. 2006). Mercury stress may result in decreased foliar chlorophyll content and/or damage to internal leaf structure (Dunagan *et al*. 2007). Arsenic (As) and mercury (Hg) are among the most dangerous heavy metals to humans and the environment because of their toxicity towards all living organisms and their related accumulation capability (Comino *et al*. 2009). The results showed that concentrations of As and Hg accumulated in *Poa annua* increase with the increasing contamination exposure as 0.25, 0.5 and 5 mg L^{-1}, for Hg 0.1, 0.2 and 2 mg L^{-1}. The molecular response of wheat (*Triticum aestivum* L. cv. Yangmai 13) seedlings to heavy metal (Cd, Hg) and 1, 2, 4-trichlorobenzene (TCB) stresses were examined by two-dimensional gel electrophoresis, image analysis, and peptide mass fingerprinting. The results showed inhibitions of root and shoot growth of wheat by Cd, Hg, and TCB. Hg stress inhibited protein synthesis while Cd and TCB stresses induced or up-regulated more proteins in the leaves (Ge *et al*. 2009). The changes in growth and antioxidant enzyme activities in *Jatropha curcas* seedlings using varying concentrations of mercury were reported (Gao *et al*. 2010). Its content was found greater in the radicles than those of in the cotyledons and hypocotyls. The biomass in the cotyledons, hypocotyls and radicles increased gradually with increasing mercury concentrations,

peaking in seedlings exposed to mercury concentration of 50 μM, and then decreased.

Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage. In addition, plants accumulate metal ions that disturb cellular ionic homeostasis. To minimize the detrimental effects of heavy metal exposure and their accumulation, plants have evolved detoxification mechanisms (Yadav 2010). Heavy metals effects on ecosystems may be difficult to pinpoint in the field and the toxic effects of mercury depend on its chemical form and concentration (Jean-Philippe *et al*. 2011). The potential bioavailability of the Hg from the soil might by characterized by variety of chemical processes, differing in the extraction agent, its concentration, the sample weight or the time of extraction (Šípková *et al*. 2012). Metals are toxic to both plants and fungi, and elevated soil metal concentrations have been documented to change the structure of ectomycorrhizal communities and high concentrations of mercury (0-366 μg g^{-1} Hg) in soil decreased survival of *Pinus rigida* seedlings (Crane *et al*. 2012). Up to now numerous studies have investigated the influence of abiotic stress factors on plants. Due to their direct or indirect presence they may affect the development, growth, basic metabolisms in plants or any other living organisms. Each kind of these, such as heavy metals, salt stress, chilling, drought or UV-B radiation, might induce the formation or the overproduction of reactive oxygen species (Szollosi 2014).

Mercury is a highly toxic pollutant with expensive clean up, because of its accumulative and persistent character in the biota (Pérez-Sanz *et al*. 2012). Plants are an integral part of life in many indigenous communities (Bhatia *et al*. 2014). Attention has been given in developed countries about the effects of metal toxicities on germination and growth of plants. Metal toxicity is an important factor governing germination and growth of plants. The permeability of metals can decrease the growth of plants. Mercury chloride ($HgCl_2$), the main representative of mercury compounds, is the target of numerous investigations, not only because of its intrinsic toxicity but also because it accounts for the toxicity of elemental mercury since the latter is converted to Hg^{+2} by oxidation (Sobral-Souza *et al*. 2014). Therefore, a study was carried out to determine the effect of mercury on seed germination and seedling growth of an important arid zone tree *Albizia lebbeck* (L.) Benth. (Leguminosae) commonly known as Siris. It is commonly planted in Sind, Baluchistan and the Punjab plains of Pakistan.

2. Materials and methods

Locally collected healthy seeds of *Albizia lebbeck* were surface sterilized with dilute concentration of sodium hypo-chloride for one minute to avoid any fungal contamination. The seeds were washed with distilled water and transferred in petri dshes (90 mm diameter) and placed on Whatman filter paper No. 42 at room temperature (28°C ± 2). Four 40-Watt tube lights were used as a continuous light source. The experiment was conducted in completely randomized design with each treatment replicated three times. Initially 5 ml solution of mercuric chloride in different range 1, 3, 5 and 7 mM was applied. Solutions were daily changed. Treatment supplied with distilled water served as control. After 10 days, seed germination percentage, shoot and root length were noted. The seedling dry weight was determined by drying the plant materials in an oven at 80°C. The data collected for various growth parameters were statistically analyzed by Duncan's Multiple Range Test and the analysis of variance techniques (ANOVA) on personnel computer using COSTAT version 3. The germination potential was determined by computing a seedling vigor index (SVI) as per the formula given below (Bewly and Black 1982). Tolerance

indices was determined by the following formula as given by Iqbal and Rahmati (1992):
Mean root length in metal solution/Mean root length in distilled water X 100.

3. Results

The seed germination and seedling growth performance of *A. lebbeck* was tested in different concentrations (1, 3, 5 and 7 mM) of mercury as compared to control (Fig. 1-3). Mercury

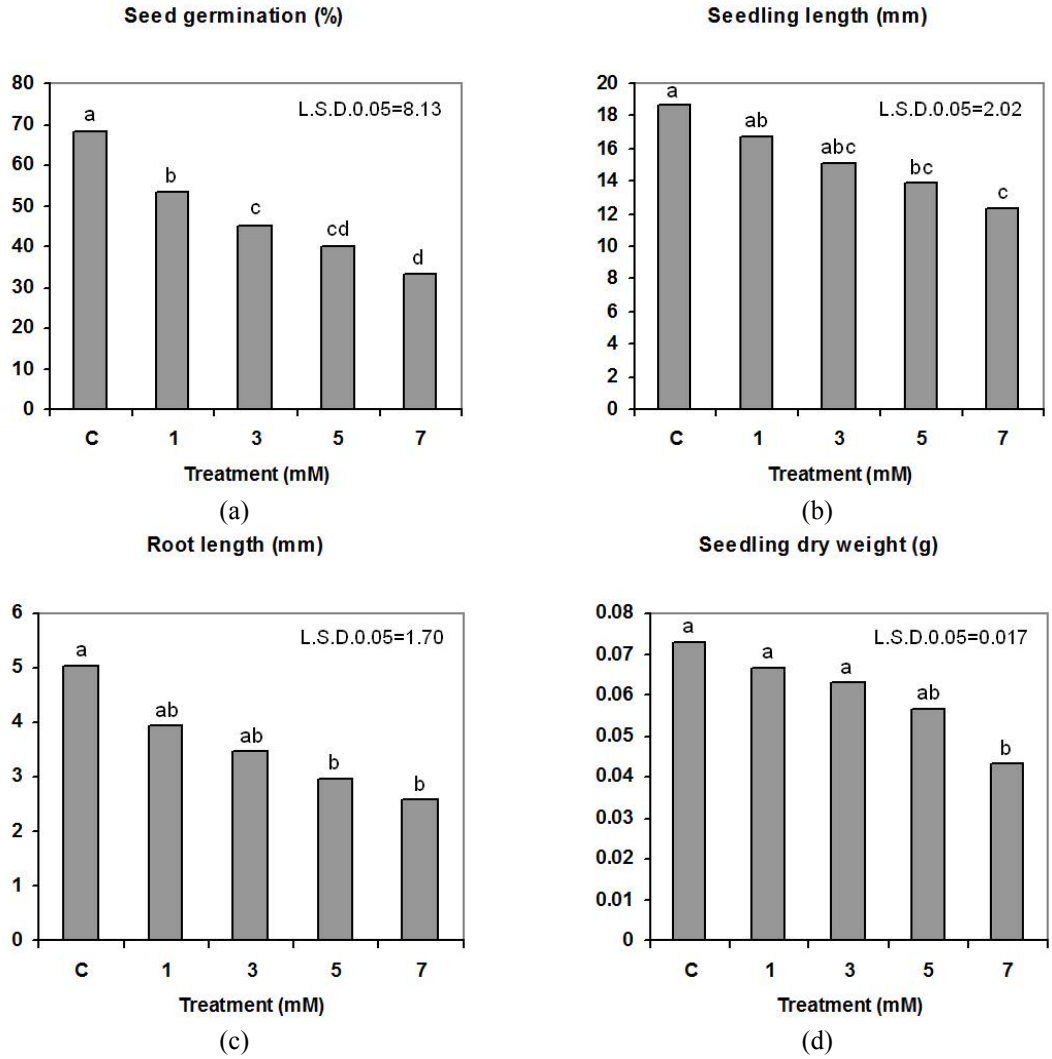

Fig. 1 Effects of different concentration of mercury treatment on seed germination (%), shoot length (mm), root length (mm) and seedling dry weight (g) for *Albizia lebbeck* as compared to control. Number followed by the same letters in the same bar are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test

treatment in the form of mercuric chloride at 1 mM showed a significant ($p < 0.05$) reduction in seed germination of *A. lebbeck* as compared to control (Table 1). Increase in concentration of mercury treatment at 5 mM significantly reduced the shoot and root growth of *A. lebbeck* (Fig. 1). The results showed that mercury treatment in the substrate from 1 to 3 mM did not produce any significant effect on seedling dry weight as compared to control. However, increase in concentration of mercury treatment at 5-7 mM was found sufficient to cause significant reductions in seedling dry weight of *A. lebbeck* when compared with control.

Mercury treatment at all concentration decreased high percentage in seed germination, shoot, root length and seedling dry weight of *A. lebbeck* (Fig. 2). Mercury treatment at 1 mM concentration

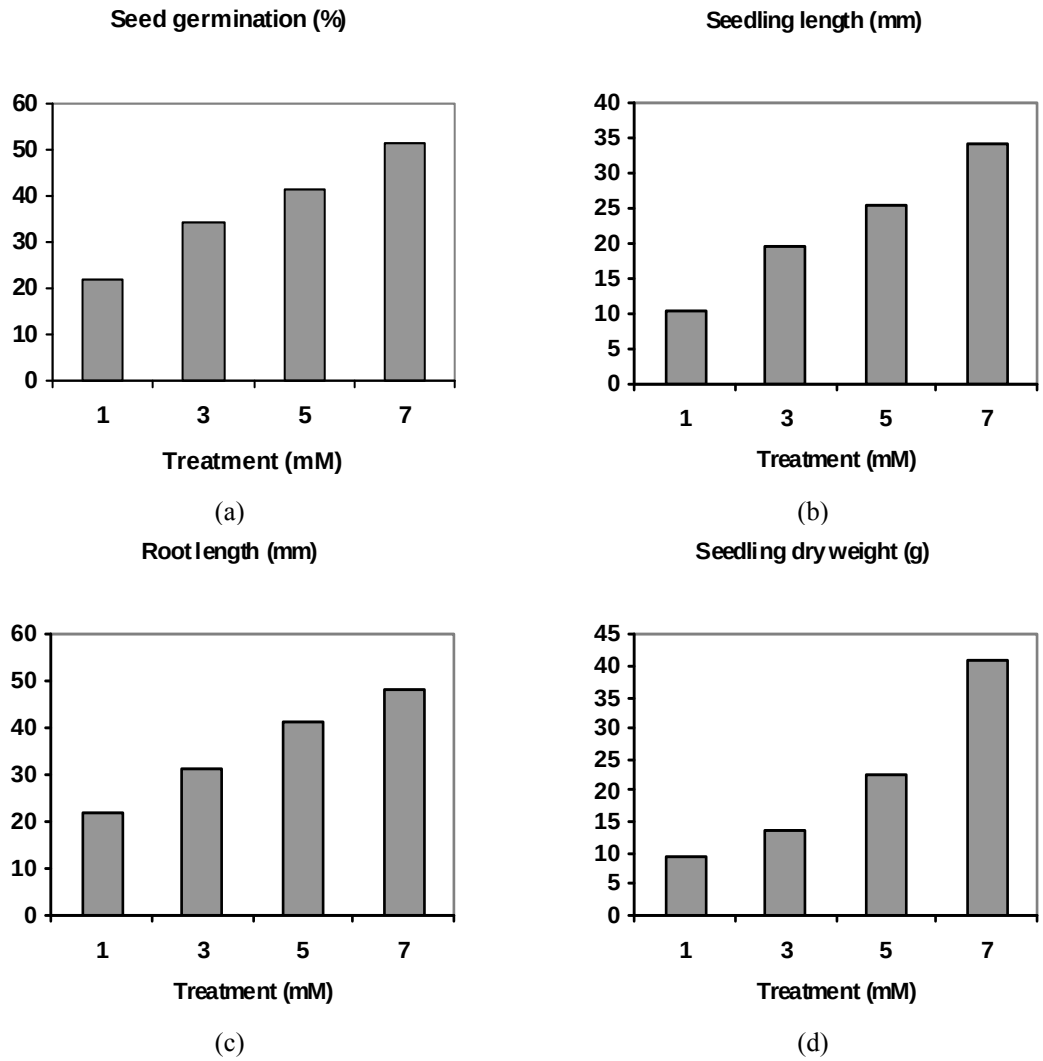

Fig. 2 Percentage decrease in seed germination, seedling length, root length and seedling dry weight of *Albizia lebbeck* using different concentration of mercury

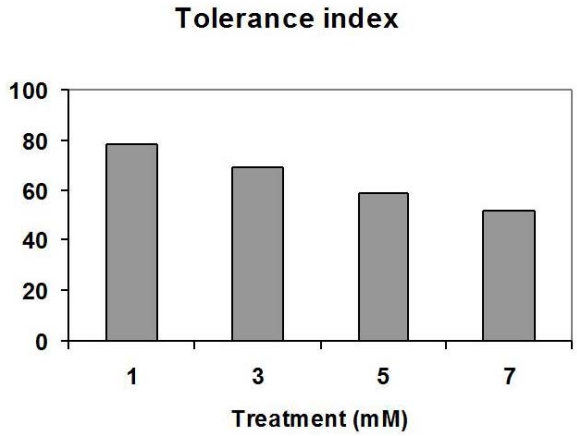

Fig. 3 Percentage of tolerance in *Albizia lebbeck* using different concentration of mercury

Table 1 Significance level of various growth parameters of *Albizia lebbeck*. (LSD. $p < 0.05$)

Growth parameter	L.S.D. value
Seed germination (%)	8.13
Seedling length (mm)	1.70
Root length (mm)	2.02
Seedling dry weight (g)	0.017

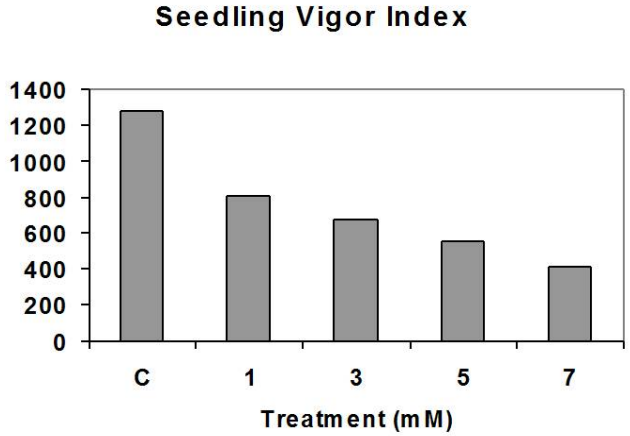

Fig. 4 Seedling vigor index in *Albizia lebbeck* using different concentration of mercury

was found responsible for decrease in seed germination (22%), seedling length (10%), root length (22

for percentage of tolerance to mercury. The results showed that seedlings of *A. lebbeck* have gre%) and seedling dry weight (9%) of *A. lebbeck* as compared to control, while mercury treatment at 5 mM concentration was found responsible for further decrease in seed germination (41%), seedling length (25%), root length (41%) and seedling dry weight (23%). Mercury

treatment at 7 mM concentration reduced highest percentage of decrease in seed germination (51%), seedling length (34%), root length (48%) and seedling dry weight (41%) of *A. lebbeck* as compared to control.

The seedlings of *A. lebbeck* were also tested ater tolerance (78.14%) to mercury at 1 mM (Fig. 3). Similarly *A. lebbeck* seedlings showed better percentage of tolerance (68.86%) to mercury at 3 mM. The results also showed that seedlings of *A. lebbeck* have lowest percentage of tolerance (51.65%) at 7 mM to mercury.

The seed germination potential and seedling vigor index (SVI) clearly decreased with the higher level of mercury (Fig. 4). The seedlings of *A. lebbeck* showed maximum seedling vigor index in control. The results showed that seedlings vigor index of *A. lebbeck* were lowest to mercury at 7 mM.

4. Discussion

World vegetation is an important component of our planet. Plants have a unique role for the existence of all heterotrophic organisms including human population (Kralova and Masarovieova 2006). Various types of human and industrial activities, lack of the proper pollutant control devices and discharge of pollutant prior to any treatment are the common causes of the environmental degradation and affecting germination and growth of plant. Metal toxicity is an important factor governing germination and growth of plants. During the past two decades increased amounts of metals have appeared in the environment (Bini and Bech 2014). The constant increase of mercury over the wide areas raises serious questions as to its effects on the growth and vigor of trees. The effects of mercury metals on *A. lebbeck* trees have not been intensively studied. However, the present investigation suggested that increase in mercury concentration might cause a decrease normal development in plant species growing in the mercury-contaminated areas.

The response of plant growth to heavy metals treatment has become the subject of great interest due to their nature of toxicity to plants. Attention has been given, in many countries, about the effects of metal toxicities on plants growth. A little attention has been given on this species. In present investigation, the effect of mercuric chloride on seed germination, seedling growth, root and seedling dry weight of an important arid tree *A. lebbeck* was recorded. The seed germination and seedling growth responded differently to mercuric chloride treatment as compared to control. High percentage of decrease in seed germination, seedling growth and seedling dry weight of *A. lebbeck* provided evidence that the treatment of mercury in excess may be inhibitory to plant growth and development. Seeds contain the embryo as a new plant in miniature and have two major functions, reproduction and dispersal. Seed formation completes the process of plant reproduction and, with seed germination, the next plant generation starts. Given the ever-increasing environmental pollution with metal(loid)s, it is perhaps surprising that relatively few reports detail the impacts of metals on seed metabolism, viability and germination in comparison to the numerous publications on the effects of metals in vegetative tissues, particularly roots and shoots (Kranner and Colville 2011).

The seedling growth of *A. lebbeck* showed high percentage of tolerance to mercury at 1 mM concentration. A concentration of 7 mM of mercury produced a significant ($p < 0.05$) inhibition to seedling length as compared to control. The permeability of metals can decrease the growth of plants. Reduction in seed germination, seedling growth and dry weight of *A. lebbeck* was observed when treated with different concentration of mercury. Mercury at high concentration decreased the

seed germination of *A. lebbeck*. Inhibition due to the presence of mercury in the substrate provided evidence that the element in medium if present in excess may be inhibitory to plant growth and development. The root growth of *A. lebbeck* was found decreased by 48% at 7 mM mercury concentration. Similalrly, alfalfa plants (*Medicago sativa*) pretreated with 0.2 mM Salycylic Acid for 12 h and subsequently exposed to 10 μM Hg^{2+} for 24 h displayed attenuated toxicity to the root (Zhou *et al*. 2009). In another investigation, plants of *Chilopsis linearis* grown with 0, 50, 100, and 200 μM Hg [as $Hg(CH_3COO)_2$] and 0 and 50 μM Au (as $KAuCl_4$) in hydroponics showed that seedling grown with 50 μM Au + 50 μM Hg and 50 μM Au + 100 μM Hg had roots 25 and 55% shorter than control roots, respectively (Rodríguez *et al*. 2009).

The results of this investigation have shown that mercury is more toxic to *A. lebbeck* root development than other growth parameters. The reduction in root growth of *A. lebbeck* provides further evidence that the mercury in excess may be inhibitory to plant growth and development. The root elongation tests have been used as simple, rapid, reliable and reproducible techniques to evaluate the damage caused by toxic compounds present in various composts. Many plant species have been recommended for the phytotoxicity test. The roots are normally considered in relation to their 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. The significant decrease in seedling growth of *A. lebbeck* agrees with the conclusion that the excessive amount of toxic element usually caused reduction in plant growth.

Plant growth and development are the result of many physiological processes. Plant growth under stress condition is most likely to be adversely affected by heavy metals. Mercury content and distribution as well as its effects on growth and oxidative stress in 30-day-old tomato seedlings (*Lycopersicon esculentum* Mill.) observed. The content of Hg increased with external Hg concentrations, and was considerably higher in roots than in shoots. Excess Hg suppressed biomass production of both roots and shoots and reduced chlorophyll content in leaves (Cho and Park 2000).

High concentration of heavy metals produced toxic effects on seedling growth and can severely limit the yield. In another investigation, the treatment of mercury (2 and 5 mg L^{-1}) found responsible for decreased in hill activity, chlorophyll, protein and dry weights and increased tissue permeability over control values in *Azolla pinnata* (Sakoar and Sasdhar 1986). According to tolerance test it could be seen in our results that tolerance to mercury in *A. lebbeck* was higher at low concentration 1-3 mM. Increase in concentration of mercury at 5-7 mM showed lowest percentage of tolerance in *A. lebbeck*. The reason of tolerance might be a physiological association of the tolerance mechanism at this level.

5. Conclusions

It is concluded that mercury treatment produced toxic effect on seed germination, seedling growth and seedling dry weight of *A. lebbeck*. Increase in the concentration of mercury at 7 mM in the medium, brought up different changes in the all growth parameter performance of *A. lebbeck*. According to tolerance test, tolerance to mercury in *A. lebbeck* was lower as compared with control. Plantation of *A. lebbeck* in mercury-polluted area will help in reducing the burden of mercury pollution. However, the plantation of *A. lebbeck* is suggested in urban areas to overcome the burden of mercury pollution to some extent. *A. lebbeck* can serve better in coordinating in land management programs in metal contaminated areas. 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. Its importance increases where the soil is acidic in nature and prolonged input of such types of pollutants can move to ground water and streams. This may endanger biota and drinking water reservoirs for humans. Plants growing on metal-contaminated sites need to develop some degree of tolerance to metal toxicity in order to survive. Since all plants contain at least some metal in their tissues, they clearly are incapable of completely excluding potentially toxic elements, but simply of restricting their uptake and/or translocation. The mechanisms for metal tolerance are: (a) metal sequestration by specially produced organic compounds; (b) compartmentalization in certain cell compartments; (c) metal ion efflux; (d) organic ligand exudation. Inside cells, proteins such as ferritins and metallothioneins, and phytochelatins, participate in excess metal storage and detoxification. When these systems are overloaded, oxidative stress defense mechanisms are activated (Patra *et al*. 2004). The development of novel phytoremediation strategies to mitigate mercury contamination, an increasingly important worldwide threat, could be enhanced by identifying Hg-tolerant legume cultivars (Torre *et al*. 2013). *Albizia lebbeck* can be used for such studies.

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