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ANALYSIS OF EFFECTS OF LEAD AND IRON TREATMENT ON EARLY SEEDLING GROWTH OF *ALBIZIA LEBBECK* L. (BENTH.) AND *EUCALYPTUS GLOBULUS* LABILL. *IN VITRO* STUDIES

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Abstract: Pollution by heavy metals in the environment is a worldwide problem. The aim of the research study was to record the effect of lead (Pb) and iron (Fe) elements on early seedling growth of *Albizia lebbeck* L. (Benth.) and *Eucalyptus globulus* Labill. The obtained results showed that higher level of lead (Pb) and iron (Fe) elements treatment present in the substrate had wide a spectrum of toxicity activity against seedlings growth performance of *A. lebbeck* and *E. globulus* as compared to control in lab conditions. Statistically analyzed data showed that seedling growth of *A. lebbeck* and *E. globulus* were reduced significantly ($p<0.05$) with increased concentrations of Pb and Fe 5 to 20 ppm as compared control (0 ppm). The percentage of seedling tolerance index of *A. lebbeck* and *E. globulus* showed different level of iron and lead. *A. lebbeck* showed greater tolerance indices (61.19%) in the presence of Fe than Pb treatments (50.39%). It was also noted that tolerance indices of *E. globulus* was reduced more in Fe (41.19%) as compared to Pb treatments (55.32%).

Keywords: Aromatic tree, heavy metals, seedling growth, tolerance, toxicity, tree.

Introduction

Heavy metals have a density of greater than 5 g/cm^3 [STOBRAWA & LORENC-PLUCINSKA, 2008]. The total amount and complexity of toxic pollutants in the environment are increasing day by day due to discharge of untreated chemicals from automobile and industries are a serious threat to the growth and quality of plant life [IQBAL & al. 2001; MEHBOOB & al. 2018; SHAFIQ & IQBAL, 2012; SHAFIQ & al. 2019]. The response of plant growth and metabolism to heavy metals has become the subject of great interest in recent years by plant ecologist. Among the heavy metals, lead and iron at higher levels are toxic element for germination and growth of plants. Soil pollution by heavy metals is a global environmental problem as has been affected about 235 million hectares land worldwide and on soil microbial properties [CHANDER & al. 2001; BERMUDEZ & al. 2012].

Lead (Pb^{2+}) is a wide spread dangerous heavy metal and strongly depends on its chemical speciation and toxicological potential [DRIBBEN & al. 2011; YUAN & al. 2011; SHAHID & al. 2012]. An inhibition in seedling growth of *Vigna radiata* (L.) Wilczek in the presence of 1.0 mM lead acetate was recorded [SINGH & al. 2003]. Pb level of 200 μM posed adverse effects on root morphological organization and root activity of *Elsholtzia argyi* H. Lév. plants [ISLAM & al. 2007]. The physiological responses and tolerance mechanisms to Pb stress in concentration of 50, 150, 300, 600, 800 and 1000 mg/L) for the *Salsola passerina* Bunge

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exhibited higher Pb tolerance in terms of the seed germination rate and bioactivities [HU & al. 2012]. Lead treatment at 05, 10, 15, 20 and 25 µmol L^{-1} declined seed germination, root, shoot and seedling length, seedling dry biomass and plant circumference of *Thespesia populnea* (L.) Soland. ex Corréa [KABIR & al. 2011]. The effect of different concentrations (0, 10, 20 and 30 ppm) of lead resulted reduction in root growth of two varieties of *Zea mays* L. [GHANI & al. 2010] commonly referred as maize.

Fe is essential element and moves to the seeds, most likely via the phloem, as the flow of the xylem is driven by transpiration [GRUSAK, 1994; YONEYAMA & al. 2015]. Fe is considered highly reactive and toxic via the Fenton reaction [MORRISSEY & GUERINOT, 2009]. $Fe^{(2+)}$ play a key role of in epithiospecifier protein activity [WILLIAMS & al. 2010].

Iron (Fe) is a metallic element and essential for plant growth. When Fe gets absorbed it binds to cell wall and other macromolecules in the cell. High concentration of Fe is found in roots, leaves and in stems [BHATTI & IQBAL, 1988]. Iron treatment significantly ($p<0.05$) reduced growth of plants [MORZECK & FUNICELLI, 1982; AL-HELAL, 1995; SHAFIQ & IQBAL, 2006]. Reduction in seed germination of all selected plants provided confirmation that Fe if available in excess is accountable in producing harmful effects. The work of VANGE & al. (2004) showed that higher concentrations of metals put adverse impacts on the growth of plants as seed germination is the most vulnerable stages in the development of plants. OZTURK & al. (2003) also observed that deceased in seedling fresh and dry weight in the presence of Fe treatment. Physiological effect on thirty three old days *Arabidopsis thaliana* seedlings to the interaction of iron deficiency and nitrogen form recorded [KARRAY-BOURAOUI & al. 2010].

Albizia lebbeck and *Eucalyptus globulus* are multipurpose economic value trees from which wood is used for both the industrial and medicinal purposes. *A. lebbeck* and *E. globulus* is widely used as raw material for the manufacturing of paper, timber, and packaging material, aromatic and in oil medicines industry in Pakistan. This study aims to evaluate the toxic effects of lead (Pb) and iron (Fe) salt on seed germination and early seedling growth performance of *A. lebbeck* and *E. globulus* under laboratory conditions.

Materials and methods

The different concentration of lead (Pb) and iron (Fe) salt in lead nitrate ($PbNO_3$) and ferrous sulphate ($FeSO_4$) for metal treatment were prepared. Stock solutions were prepared by weighting the 1.29 g and 2.71 g of $PbNO_3$ and $FeSO_4$ respectively and put them into two separate volumetric flask (1000 ml) and filled with distilled water up to mark on volumetric flask. To prepare the 5, 10, 15 and 20 ppm concentrations of both metals (Pb and Fe) 0.5, 1.0, 1.5 and 2.0 ml was sucked respectively from both stock solutions and added into volumetric flask (100 ml) and filled with distilled water up to the mark on flask. The certified seeds of *A. lebbeck* and *E. globulus* were collected from National seed store of Bhakkar. The seeds were surface sterilized with 2% of sodium hypochlorite (NaOCl) solution for two minutes to prevent fungal infection after that all the sterilized seeds were thoroughly washed with distilled water. Because of hard seeds coat pretreatments of seeds by soaking them in fresh water for 15 hours was carried out. The top trimmings of seeds were cut with the help of hygienic scissors to reduce any achievable seed dormancy. This mechanical scarification is a best treatment for first rate seed germination as well as best progression to break hard seed coat. The Petri dishes were washed to drop off the chances of further fungal infectivity. The Petri dishes and filter papers were sterilized in autoclaved. Afterward the five seeds on the filter paper (Whatman No. 42) were placed in medium size Petri dishes (90 mm) and had two replicates for each treatment of metals. Initially, 3 ml of distilled water was given to control group and 3 ml of each concentration of both metal solutions were given to each set of respective treatment. In a while, on daily basis the old solution was removed by sucking from Petri dishes in order to reduce the chances of seed turgidity and later on 2 ml of fresh solution of each metal concentration was added to each set of respective treatment. The experiment was kept in laboratory conditions at room temperature (38 ± 4 °C) and experiment was lasted for 15 days. The experiment was completely randomized and replicated twice. The germination was scored as protrusion of the radical through the testa. The seed germination percentage, root, shoot and seedling length of *A. lebbeck* and *E. globulus* were measured. Seedling dry weight was obtained after drying the samples in an oven at 80 °C for 24 hours.

Tolerance index (T.I.) was determined using the following formula given by IQBAL & RAHMATI (1992):

T.I. = (Mean root length in metal solution / Mean root length in distilled water) x 100

Statistical analysis

Data of different growth parameters were analyzed statistically (SPSS 20) on personal computer by analysis of variance and Duncan Multiple Range Test at $p<0.05$ level.

Results and discussion

High concentrations of metals in the environment can create harmful effects on germination and growth of plants. In present time most heavy metals especially lead (Pb) and iron (Fe) became important due to their regular increase in the atmosphere. Most of the plant species under applied stress conditions are probably to be badly influenced by heavy metals. The influence of heavy metals on vegetation depends upon the quantity of that toxic metal taken by the plant [HAILING & al. 1991]. Sensitivity of plants and toxicity of heavy metals are affected by the concentration of given metal, length of exposure period and mechanism of biological processes occurring in the plant species [ERNST & al. 1992].

In present study the seedling growth of *A. lebbeck* and *E. globulus* was carried out in different concentrations of lead and iron under laboratory conditions (Table 1-4, Figure 1). Statistically analyzed data showed that seed germination, seedling growth and tolerance indices of seedlings were reduced significantly ($p<0.05$) with increased concentrations of both applied metals (Pb and Fe) as compared control. Results presented in Table 1-2 revealed that lead treatment at 05 ppm application created significant ($p<0.05$) results on seedling length (9.35 cm) of *A. lebbeck* as compared to control (13.04 cm). The root length (2.74 cm) and shoot length (5.74 cm) of *A. lebbeck* showed further $p<0.05$) reduction with lead treatment at 10 ppm. Pb toxicity found responsible for causing a detrimental effect on seedling growth *Leucaena leucocephala* (Lam.) de Wit small fast growing [IQBAL & SHAFIQ, 1998]. KOPITTKE & al. (2007) found a concentration as low as 1 µM lead (Pb) highly toxic to plants. In an experiment, the relative fresh mass of cowpea (*Vigna unguiculata* L. Walp.) was reported reduced by 10% at a Pb^{2+} activity of 0.2 µM for the shoots and at a Pb^{2+} activity of 0.06 µM for the roots. The primary site of Pb^{2+} toxicity was the root, causing severe reductions in root growth, loss of apical dominance, the formation of localized swellings behind the root tips and the bending of some root tips of *V. unguiculata*. Pb was found to accumulate primarily within the cell walls and intercellular spaces. Maximum suppression of root length (1.71 cm), shoot length (4.07 cm) and seedling length (5.78 cm) were recorded at highest concentrations of Pb (20 ppm). Results of selected plants species showed that seedling growth, seedling weight (fresh and dry) of *A. lebbeck* were reduced in all treatments (05, 10, 15 and 20 ppm) of Pb as compared to control (0 ppm). Lead treatment at 10 and 15 ppm progressively decreased seedling dry weight 0.34 and 0.29 g as compared to control (0.46 g) of *A. lebbeck*. The assessment of seedling dry weight of *A. lebbeck* followed the same reduction array as observed for seedling fresh weight in different applications of lead.

Table 1. Effects of Lead (Pb) on root, shoot, seedling growth and seedling fresh and dry weight of *Albizia lebbeck*

Treatment Lead (Pb) ppm	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh Weight (g)	Seedling dry Weight (g)
00	5.47 ± 0.13a	7.57 ± 0.49a	13.04 ± 0.52a	0.70 ± 0.005a	0.46 ± 0.005a
05	3.54 ± 0.47b	5.81 ± 0.27b	9.35 ± 0.35b	0.50 ± 0.007b	0.37 ± 0.005ab
10	2.74 ± .35bc	5.74 ± 0.53bc	8.48 ± 0.70bc	0.46 ± 0.009bc	0.34 ± 0.008b
15	2.24 ± 0.09c	5.24 ± 0.21bc	7.48 ± 0.26c	0.40 ± 0.004bc	0.29 ± 0.006bc
20	1.71 ± 0.27cd	4.07 ± 0.56c	5.78 ± 0.84d	0.36 ± 0.003c	0.26± 0.005c
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error					

The seedling length (root, shoot), seedling weight (fresh and dry) and tolerance indices of *A. lebbeck* were decreased with the application of iron (Fe) at 05, 10, 15 and 20 ppm concentrations as compared to control (0 ppm). With increasing 05, 10, 15 and 20 ppm concentrations of iron treatment also markedly reduced the root, shoot, seedling length, seedling fresh and seedling dry weight of *A. lebbeck* (Table 2). Chemical compounds exhibit toxicity via many mechanisms of toxic action [REN, 2003]. Some of morphological parameters, root, shoot and seedling length of *A. lebbeck* got significant ($p<0.05$) reduction at varying concentrations of Fe treatment than control. Maximum suppression of root and shoot growth of *A. lebbeck* with Fe (20 ppm) treatment with relation to root length (2.31 cm), shoot length (5.17 cm) and seedling length (7.48 cm) were recorded. Plant responses to metals can be considered as dose dependent. Toxicity appears to be the results of several interactions. While increased iron treatments 5, 10, 15 and 20 ppm reduced the fresh weight from 0.68, 0.65, 0.56 and 0.48 g as related to control (0.70 g) of *A. lebbeck*. The assessment of seedling dry weight of *A. lebbeck* followed the same reduction array as observed for seedling fresh weight in different applications of iron. Heavy metal, cadmium inhibited biomass production as well as the absorption of K, Ca, Mg, Fe and dramatically increased Cd accumulation in both roots and shoots of *Linum usitatissimum* L. [BELKHADI & al. 2010].

Table 2. Effects of Iron (Fe) on root, shoot, seedling length, seedling fresh and dry weight of *A. lebbeck*

Treatment Iron (Fe) ppm	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh Weight (g)	Seedling dry Weight (g)
00	5.47 ± 0.13a	7.58 ± 0.49a	13.05 ± 0.52a	0.70 ± 0.005a	0.46 ± 0.005ab
05	4.07 ± 0.33b	6.79 ± 0.09ab	10.86 ± 0.31b	0.68 ±0.003ab	0.52 ± 0.003a
10	3.34 ± 0.24bc	6.44± 0.19b	9.78 ± 0.43c	0.65 ±0.009ab	0.45 ± 0.004ab
15	2.34 ± 0.09c	5.22 ± 0.16c	7.56 ± 0.25d	0.56 ± 0.008b	0.41 ± 0.009b
20	2.31± 0.07cd	5.17 ± 0.16cd	7.48 ± 0.20de	0.48 ±0.001bc	0.33 ± 0.002c
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error					

Lead (05, 10, 15 and 20 ppm) application responded variably with seedling growth performances parameters of *E. globulus* than control (Table 3). Lead concentrations at 20 ppm created significant ($p<0.05$) effects on length of root (0.91 cm), shoot (1.03 cm) and seedling length (1.94 cm) of *E. globulus.* Seedling fresh weight of *E. globulus* was highest in control seedlings (0.0345 g) and gradually declined with the increase in Pb concentration from 05 to 20 ppm. The decrease in seedling fresh weight 0.0320, 0.0311 and 0.0231 g of *E. globulus* was recorded for those seedlings which were treated with 05, 10 and 15 ppm concentrations of Fe. The lowest seedling fresh weight (0.0178 g) was recorded for those seedlings which were treated with 20 ppm concentrations Pb. Similarly, the seedling dry weight of *E. globulus* was also reduced 0.244, 0.0216 and 0.0132 g in Pb applications at 05, 10 and 15 ppm as associated to control (0.0269 g). It reduced spontaneously with increasing Pb concentration in substrate. The lowest seedling dry weight (0.0100 g) was recorded for those seedlings of *E. globulus* which were treated with 20 ppm concentrations Pb.

Table 3. Effects of lead (Pb) on seed germination, seeding growth and seedling dry weight of *Eucalyptus globulus*

Treatment lead (Pb) ppm	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
00	1.22 ± 0.04a	2.44 ± 0.02a	3.66 ± 0.21a	0.0345 ± .003a	0.0269 ± 0.003a
05	1.16 ±0.03ab	2.43 ± 0.08a	3.59 ± 0.09ab	0.0320 ± .003ab	0.0244 ± 0.002b
10	1.09 ± 0.02b	2.19 ± 0.09b	3.28 ± 0.11b	0.0311 ± .003ab	0.0216 .003bc
15	0.97 ± 0.03c	1.32 ± 0.15c	2.29 ± 0.15c	0.0231 ±0.002b	0.0132 ±0.001d
20	0.91 ± .03cd	1.03 ± 0.10d	1.94 ± 0.17cd	0.0178 ±0.003c	0.0100 ±0.003e
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Err					

Similarly, iron (05, 10, 15 and 20 ppm) application variably reduced the selected seedling growth performances parameters of *E. globulus* than control (Table 4). An increase in the concentrations of iron up at 20 ppm created significant ($p<0.05$) effects on length of root (0.79 cm), shoot (1.00 cm) and seedling length (1.79 cm) of *E. globulus*. Seedling fresh weight of *E. globulus* was highest in control seedlings (0.0348 g) and gradually declined with the increase in Fe concentration from 05 to 20 ppm. The decrease in seedling fresh weight 0.0307, 0.0291 and 0.0286 g of *E. globulus* was recorded for those seedlings which were treated with 05, 10 and 15 ppm concentrations of Fe. Similarly, the seedling dry weight of *E. globulus* was also reduced 0.216, 0.0193 and 0.0126 g in Fe applications at 05, 10 and 15 ppm as associated to control (0.0269 g). The maximum decrease in seedling fresh weight (0.0166 g) was recorded for those seedlings which were treated with 20 ppm concentrations Fe. Seedling dry weight of *E. globulus* was also reduced (0.0126 g) in Fe applications at 15 ppm as associated to control (0.0269 g). It reduced spontaneously with increasing Fe concentration in substrate. The lowest seedling dry weight (0.0100 g) was recorded for those seedlings of *E. globulus* which were treated with 20 ppm concentrations Fe. ALIKAMANOGLU & al. (2011) reported he toxic effect of iron on growth factors, biochemical parameters, seedling fresh and dry weight and accumulation of trace elements in soybean plants (*Glycine max* L. Merrill).

A lot of evaluations have been made by scientist to access the impact of heavy metals on tolerance of plants. The tolerance of some plants to heavy metals is a precious ecological adaptation for their survival in a specific environment. The accumulation of heavy metals in soil influences on plant growth and ecosystem balance and there is a need to explore the mechanism of plant tolerance to heavy metals [GONG & al. 2019].

Table 4. Effects of Iron (Fe) on different growth parameters of *Eucalyptus globulus*

Treatment Iron (Fe) ppm	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh Weight (g)	Seedling dry Weight (g)
00	1.23 ± 0.03a	2.45 ± 0.02a	3.68 ± 0.53a	0.0348 ± 0.005a	0.0269 ± 0.003a
05	1.13 ± 0.03b	2.41 ± 0.09a	3.54 ± 0.23ab	0.0307 ± 0.009b	0.0216 ± 0.003b
10	1.02 ± 0.03c	1.90 ± 0.08b	2.92 ± 0.52b	0.0291 ± 0.003bc	0.0193 ± 0.003bc
15	0.90 ± 0.03d	1.12± 0.15c	2.02 ± 0.15bc	0.0286 ± 0.004c	0.0126 ± 0.003c
20	0.79 ± 0.03e	1.00 ± 0.10cd	1.79 ± 0.19c	0.0166± 0.005d	0.0100 ± 0.003cd
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error					

The tolerance of *E. globulus* to Pb and Fe gradually reduced with the increase in Pb and Fe concentration than control as presented in Figure 1. Lead at 05, 10, 15 and 20 ppm produced 95.08, 89.34, 79.51 and 74.59% tolerance of *E. globulus* respectively. The metal resistance is an unusual character found only in well adapted plant species. Iron treatment at similar concentrations produced 74.41, 61.06, 42.78 and 42.23% tolerance in *A. lebbeck*. It was determined that inhibitory effects of Pb were more rigorous on all growth variables of *A. lebbeck* as compared to Fe treatment. Similarly, KABIR & al. (2011) studied the tolerance of *Samanea saman* for Cu, Fe, Pb and Zn under laboratory conditions and showed that with increasing concentrations of metals reduced seed germination. Iron treatment at similar range of treatment produced 91.87, 82.93, 73.17 and 64.23% tolerance in *E. globulus* respectively. These results were similar with the results of KABIR & al. (2008).

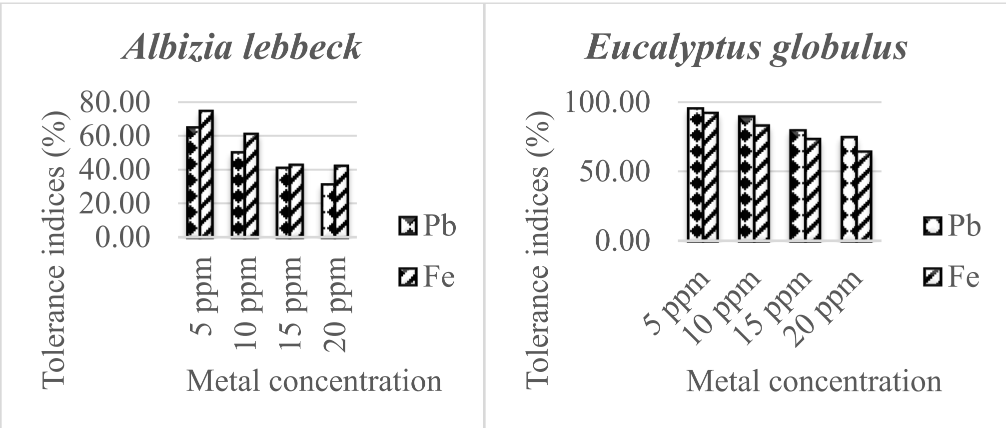

Figure 1. Tolerance indices (%) of *Eucalyptus globulus* and *Albizia lebbeck* in different concentration of Pb and Fe.

According to their results tolerance indices of *Thespesia populnea* progressively decreased with the increasing concentration of heavy metal. These results were also supported by the work of BOYD & al. (1994). It was cleared from results that Fe applications created more deadly effects on *E. globulus* seedlings than Pb applications at all concentrations. Lead is found more toxic to seedling growth of *A. lebbeck* as compared to Fe while *E. globulus* is more tolerant to Fe than Pb. The cause of low tolerance to Pb and Fe could be due to disturbance in physiological processes.

Conclusion

This study concludes that treatment of different concentration (5, 10, 15 and 20 ppm) lead and iron gradually deceased the rate of seed germination percentage, seedling growth and seedling dry weight of *A. lebbeck* and *E. globulus* as compared control (0 ppm) treatment. An increase in Pb and Fe level 5 to 20 ppm concentration also gradually decrease seedling tolerance index of *A. lebbeck* and *E. globulus* as compared to control treatment.

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