The Toxic Effects of Heavy Metal Lead (Pb) Treatment on Seed Germination and Early Seedling Growth of *Senna holosericea* Frees and *Prosopis juliflora* DC in vitro

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Abstract: Lead is highly toxic element for living organism, ecological balance and is commonly freely found in air, water and soils due to industrial, automobile and anthropogenic activities. It is evident that plants are capable of tolerating differently to toxic heavy metal, lead. The objective of this study was to investigate the influence of lead as lead acetate on the rate of seed germination percentage, early seedling growth and biomass production of *P. juliflora* and *S. holosericea.* Seeds of *P. juliflora* and *S. holosericea* were collected from polluted site (Super highway) and clean area (University Campus) and germinated in petri dish and treated with lead acetate in solution for 12 days. Five different 0, 20, 40, 60 and 80 ppm level of lead were tested in laboratory conditions. *P. juliflora* and *S. holosericea* seedlings showed a high percentage of seed germination and seedling growth in control treatment as compared to metal treatment. An increase in metal level from 20 to 60 ppm decrease seedling growth characteristics of *P. juliflora* and *S. holosericea*. Lead treatment at higher level 80 showed high potential of toxicity in *P. juliflora* seedlings. The more reduction in root growth than shoot growth might be due to rapid up take of lead from the substrate. In this study, the rate of seed germination percentage, seedling growth and seedling dry weight of two different plant species (*P. juliflora* and *S. holosericea*) under various level of lead in vitro studies were recorded. The result showed that root, shoot, leaf and seedling dry weight of both species decreased due to the complex nature of lead element available in the substrate. The lead treatment at 20-60 ppm produced negative effects on different growth such as shoot height, root length and seedling length for *S. holosericea*. The increase in lead concentration from 60-80 ppm highly decreased seed germination, root, shoot, seedling growth and total seedling dry weight of *P. juliflora* as compared to control. The availability of heavy metals includes lead (Pb) influences on plant growth and disturb the indices of tolerance. A wide difference in the rate of seed germination percentage and seedling growth between the two species was recorded by lead treatment. Lead treatment gradually decreased root, shoot and seedling height of *P. juliflora* and *S. holosericea* as compared to without lead treatment. Abiotic stress by lead treatment showed a positive influence on the growth of both plant species. The ability of *P. juliflora* and *S. holosericea* seedlings can help to lessen burden of lead pollution according to tolerance indices. The tolerance in seedling growth of *L. leucocephala* differed in their sensitivity to lead treatments. *P. juliflora* seedlings supposed to have strong Pb tolerance than *S. holosericea.*

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1. Introduction

The high level of heavy metals (Pb, Cd, Cu, Cr, Ni and Fe) in environment is a global issue for quality of life due to industrial and automobile activities which ultimately negatively affecting the environment and plant growth. Contamination of agricultural soils with trace metals present lethal consequences in terms of diverse ecological and environmental problems that entail entry of metal in food chain, soil deterioration, plant growth suppression and yield reduction (Ashraf *et al.,* 2017).

The presence of heavy metals at higher level influences on plant growth characteristics, animals, human health and properties of soils (Chander *et al*. 2001; Dribben *et al.* 2011; Yuan *et al.* 2011; Ahmad *et al*. 2012; Zhang *et al*. 2012; Gong *et al.* 2019; Rolka *et al*. 2020). Metal ions are essential for plant growth and development, but in excess, these compounds can become highly toxic. Plants have adopted numerous ways to maintain metal homeostasis while mitigating adverse effects of excess metal ions (Saeedurrehman, 2020). The heavy metal toxicity is becoming a major threat to living organisms, environment, human health, animals and lead shares 10% of pollution as compared to total pollution produced by heavy metals (Saba et al., 2019; Rigoletto et al., 2020; Usman et al., 2020; Xing et al., 2020; Abedi et al., 2022; Collin et al., 2022; Shourie et al., 2022; Yaashikaa et al., 2022).

Heavy metal toxicity role, absorbance, low tolerance causes multiple effects (Beckett *et al*. 2000; Domínguez−Solís *et al.* 2004; Dalvi & Bhalerao, 2013). Lead treatment at 10, 30, 50, 70 and 90 µmol L^{-1} affected the seed germination, seedling length and seedling dry weight of *Albazia lebbeck* and *Thespasia populnea.* (Kabir *et al*. 2008; Farooqi *et al*. 2009) and agricultural crop (Syed et al., 2023).

Lead (Pb) is the second most toxic heavy metal after arsenic and causes a range of damages to plants from germination to yield formation (Zulfiqar *et al.* 2019). The knowledge of toxicology and environmental aspects can help us protect human health and the environment (Petrová *et al.* 2017). The scientific literature is available on the effects of metallic elements on germination and plant growth. Lead (Pb^{2+}) is a wide spread dangerous heavy metal and has high toxicity to livings organism. Exotic and native plant species have been used to check the tendency of land degradation and benefic effects on soil stabilization (Sene *et al.* 2012). The distribution of *S. holosericea* and *P. juliflora* is found along the roadside in Pakistan and helps in controlling soil erosion. *Prosopis* is considered to be one of the problematic, invasive, exotic tree species. However, *P. juliflora* used for restoration of contaminated, degraded land, phytoremediation, medicinal uses, supply of fuel wood, a stabilizer of sand dunes, and to combat desertification (Prasad & Tewari, 2016).

Lead (Pb^{2+}) is a wide spread dangerous heavy metal. Therefore, in present study the object of this study was to compare and record the toxicity and tolerance of lead (Pb) on rate of seed germination percentage and seedling growth of *S. holosericea* and *P. juliflora*.

2. Material and Methods

The healthy and uniform size seeds of *S. holosericea* and *P. juliflora* DC were collected from polluted site (Super highway) and clean area (University Campus, Control) and placed in distilled water for 24 hours and then treated with 1% HCl for five minutes due to hard seed over and washed in distilled water. The tips of seeds were also cut with the help of hygienic scissors to reduce any chance of seed dormancy. All seeds sterilized with 1% of sodium hypochlorite (NaOCl) solution for two minutes to prevent fungal contamination after that the seeds were thoroughly washed with distilled water. The petri dishes and filter papers were sterilized in autoclaved to prevent any type of fungal contaminations. The desired concentrations (0, 20, 40, 60 and 80 ppm) of lead (Pb) in lead acetate [(Pb) $CH_3COO)_2.3H_2O$] were prepared in distilled water. Afterward ten seeds of each species were placed on the filter paper (Whatman No. 42) in medium size petri dishes of 90 mm diameter. Initially, 5 ml of distilled water was added to control petri dish and 5 ml of each concentration of metal solutions were given to each set of respective treatment. Both treated and control petri dishes were kept continuously moist by distilled water whenever needed necessary. The petri dishes were kept in dark at room temperature (30 ± 4 °C) and atmospheric relativity humidity (74%). The experiment was lasted for 10 days. The experiment was completely randomized with six replicates. The germination was scored as protrusion of the radical through the testa. The seed germination percentage, root, shoot and seedling length were measured. Seedling dry weight was obtained after drying the samples in an oven at 80 °C for 24 hours. All statistical analysis was performed using SPSS 10.0 (SPSS) inc., U.S.A.) for windows.

Tolerance indices (T.I.) was determined using the following formula given by Iqbal and Rahmati (1992):

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

3. Results

Plants are necessary part of ecosystems (Movafeghi et al. 2018). The impacts of lead as lead acetate on seed germination and seedling growth of *P. juliflora* and *S. holosericea* are little available. The toxicity of heavy metal pollution is an important issue due to rapid accumulation in the environment (Yan et al. 2020). In the present study the increase in concentration of lead led to decrease in all of the growth parameters includes root, shoot, seedling length and seedling dry weight of *P. juliflora* and *S. holosericea* (Table 1-2; Fig. 1). Different treatment of lead showed highest reduction in seed germination of *P. juliflora* except 40 ppm lead concentration. The seeds of polluted areas showed decrease in germination as compared to control. As the concentration of lead increased, there was a slight decrease in germination. The inhibition in seedling growth of *Vigna radiata* (L.) Wilczek in the presence of 1.0 mM lead acetate recorded (Singh et al. 2003). Results of this experiment showed that with increasing concentrations of Pb reduced seedling germination of both plant species.

Both species showed reduction in root, shoot and seedling length as compared to control this might be due to the uptake of lead from substrate. Plant metabolism might have been severely affected which ultimately led to reduce plant growth as a whole (Greeman et al. 2001). Effects of different level of lead treatment showed high reduction in rate of seed germination parentage of *S. holosericea.* In control seed germination percentage of *S. holosericea* and *P. juiflora* was recorded high. Similarly, Jaja and Odoemena (2004) recorded the toxic effect of metals, Pb, Cu and Fe compounds on the germination and early seedling growth of tomato varieties. Lead effects the shoot and root growth of *S. holosericea* as concentrations increased. The shoot growth of polluted area seed was decreased at 20 and 40 ppm lead concentrations as compared to shoot growth of campus. Results of root growth on both sites seedlings also showed decreased growth as concentration of lead increased but here was slight difference in root growth. Metals are extremely toxic contaminants that produce ecotoxicological effect on the environment (Gong et al. 2001; Chaturvedi, 2004; Wolska et al. 2007). Overall, control set has good seedling growth. Like other growth parameters, seeding dry weight of *S. holosericea* seedling showed decreased in weight when lead concentration were increased in the medium. This reduction in seedling dry weight was more prominent at higher concentrations of applied metals comparative to control. The treatment of lead showed reduction in root length of *P. juliflora* as the increase in lead concentration. The treatment of lead also showed reduction in shoot length of *P. juliflora* as the increase in lead concentration. The treatment of lead showed slight decrease in root growth of control seeding while polluted seed showed better root growth of *P. juliflora*. Whereas, an increase in concentration showed decrease in root growth of both sites. Overall effect of lead on seedling height was similar as the effect on root and shoot growth. No such difference in growth was found among the concentrations of 20 to 60 ppm of lead except 80 ppm, which showed much reduction in seedling of *P. juliflora* as compare to control. There was not much difference in total seedling dry weight of control and polluted areas the weight was slightly increase to concentration of 60 ppm.

Table 1. Effects of Lead (Pb) on seed germination and different growth parameters of *Senna holosericea* Frees

Treatment lead (Pb) ppm	Seed germination (%)		Shoot length (cm)		Root length (cm)		Seedling length (cm)		Seedling dry weight (mg)	
	Contro	Pollute	Contro	Pollute	Contro	Pollute	Contro	Pollute	Contro	Pollute
00	100.00 ± 0.578	93.00 ± 0.577	5.99 ± 0.743	5.266 ± 0.667	2.05 ± 0.563	1.760 ± 0.03	8.043 ± 0.577	7.206 ± 0.002	8.30 ± 0.002	7.60 ± 0.006
20	50.00 ±0.578	90.00 ± 0.577	6.37 ± 0.467	5.85 ± 0.686	1.76 ± 0.577	1.820 ± 0.02	8.130 ± .450	7.170 ± 0.002	7.00 ± 0.002	5.30 ± 0.002
40	50.00 ±0.578	56.00 ± 0.611	4.78 ± 0.185	3.56 ± 0.577	1.18 ± 0.577	1.810 ± 0.03	5.960 ±0.470	5.373 ± 0.002	3.50 ±0.002	4.00 ± 0.003
60	20.00 ± 0.577	30.00 ± 0.577	4.43 ± 0.557	3.29 ± 0.578	0.53 ±0.005	0.840 ±0.02	4.960 ± 0.010	4.130 ± 0.002	3.60 ±0.002	3.00 ± 0.001
± Standard Error										

Lead treatment reduced growth of plants and this inhibitory effect of heavy metals was also noted by some other workers (Morzeck & Funicelli, 1982; Shafiq & Iqbal, 2006). The length (root, shoot and seedling) of *P. juliflora* showed reduction at different concentrations of Pb treatment as related to control as shown in Table 2.

Table 2. Effects of Lead (Pb) on seed germination and different growth parameters of *Prosopis juliflora* DC

Treatment lead (Pb) ppm	Seed germination (%)		Shoot length (cm)		Root length (cm)		Seedling length (cm)		Seedling dry Weight (mg)	
	Contro	Pollute	Contro	Pollute	Contro	Pollute	Contro	Pollute	Contro	Pollute
00	76.00 ± 0.400	56.60 ± 0.731	5.82 ±	5.31 ± 0.722	4.94 ±	5.440 ±	10.76 ±	10.75 ± 0.650	7.00 ±	5.00 ±
20	40.00 ±0.574	43.30 ±	4.30 ±	5.18 ±	1.90 ±	5.780 ±	6.20 ±	10.90 ±	4.00 ±	11.00 ±
40	33.30 ±	33.30 ±	2.42 ±	3.94 ± 0.344	1.23 ±	2.880 ±	5.30 ±	6.82 ±	5.00	7.00 ±
60	20.00 ± 0.577	30.00 ± 0.577	2.77 ±	4.65 ±	0.720	2.580 ±0.609	3.49 ±	7.23 ± 0.002	4.00	6.00 ± 0.580
80	06.00 ± 0.010	20.00 ± 0.574	1.50 ±	1.75 ±	0.200	0.630 ±0.176	1.70 ±	2.38 ± 0.005	1.00	6.00 ± 0.058

± Standard Error

The toxicity and tolerance of lead depends on the concentration and plant species. The effects were found more pronounced at higher concentrations and durations. The major processes such as seed germination, seedling growth, photosynthesis, plant water status, mineral nutrition, and enzymatic activities are affected (Patra *et al.* 2004).

Toxicity of metals depends on plant species (USO, 2007). The tolerance in seedling growth of *P. juliflora* and *S. holosericea* differed in their sensitivity to lead treatments (Fig. 1).

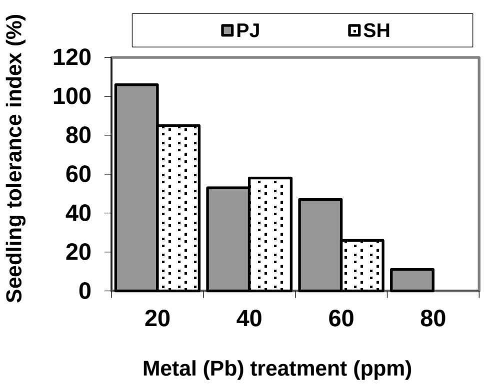

Figure. 1. Seedling tolerance index of PJ (*P. juliflora*) and SH (*S. holoericia*) to different concentrations (20, 40, 60 and 80 ppm) of Pb.

An increase in lead concentration from 20 ppm decreased tolerance indices for *P. juliflora* and *S. holosericea* as compared to control. Low concentration of lead showed high tolerance indices percentage of *S. holosericea* and *P. juliflora* seedlings as compared to control. The importance to explore the mechanism of plant tolerance to heavy metals studied by few researchers. The cell ultrastructure of spinach seedlings was substantially changed with the increases of $CuSO_4$ treated concentrations (Gong *et al.* 2019). The increase in the concentrations of lead to 60 ppm produced highest reduction in tolerance indices (26%) for *S. holosericea*. The seedlings of *P. juliflora* indicating high tolerance indices (106%) at 20 ppm of lead and lowest tolerance (11.11%). The tolerance of plants to heavy metals stress is an ecological character for adaptation and survival in a specific environment. The metal resistance and tolerance are adapted by plant species according to prevailing environmental conditions available in the area. The inhibitory effects of two lead compounds on rates of seed germination, root and stem elongation, and seedling fresh weight for six different plants species, viz., *Amaorpha fruticosa* L., *Robinia psedoacacia* L., *Pinus tabuliformis* Carr., *Platycladusorientalis* L., *Koelreu eria paniculata* Laxm., *Hippophae rhamnoides* L.) recorded. The response of these trees to lead nitrate and lead acetate toxicity varied significantly, and the order of tolerance to lead pollution was calculated in order of *Amaorpha fruticosa* L. *Platycladus orientalis* L. *Koelreuteria paniculata* Laxm. *Robinia psedoacacia* L. *Pinus tabuliformis* Carr. *Hippophae rhamnoides* L (Yang *et al.*, 2016).

Conclusion

It is concluded from the present study that rate of seed germination percentage, root, shoot and seedling length and seedling dry weight of *P. juliflora* and *S. holosericea* reduced with the increasing concentrations of Pb treatment. The lead treatment at 20 ppm presented less toxicity in seedlings of *P. juliflora* and *S. holosericea*. Seedling tolerance index of *P. juliflora* and *S. holosericea* progressively decreased with increase in lead concentration. Low concentration of lead showed less toxic effect on the growth of both seedlings. Thus, indicated that *S. holosericea* seedlings had lowest lead resistance. Certain plant species have ability to adapt in the harsh environmental metal polluted conditions. In the present study, the toxicity and tolerance limit to lead in the seedlings of *P. juliflora* and *S. holosericea*. *P. juliflora* showed the potential of some tolerance to lead at 60 ppm and suggested for plantation in lead polluted areas to overcome the burden of heavy metal pollution. Seedlings, of *S. holosericea* as compared to *P. juliflora* indicated low lead resistance and tolerance.

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