

## The phytotoxic impact of lead and zinc induced changes in seed germination and seedling growth of *Dalbergia sissoo* Roxb. multipurpose wooden furniture, street avenue tree in a *vitro* study

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**Abstract:** Pollution by heavy metals in the environment results due to various types of anthropogenic, industrial, mining, automobile activities and its impact on living organisms is a worldwide problem. Among the heavy metals, lead and zinc are released in environment from automobile activities. Lead and zinc are heavy metals and in excess concentration disturb morphological, physiological function, rate of seed germination percentage, seedling growth, biomass production in different plant growth. The researchers suggested that increase in concentration of heavy metal responsible for toxic effects on plant growth. *Dalbergia sissoo* (Roxb.) is multiple purpose tree growing under stressful polluted environment along different busy roads side of Karachi, city (Pakistan). The seeds of *D. sissoo* were collected from the Karachi University campus (clan area) and highly automobile polluted site of Karachi city (Hassan Square) and treated to test the toxicity and tolerance limit to heavy metals using different level of Lead and Zinc in *in vitro* studies. In present study the treatment of two different types of heavy metals lead (Pb) and Zinc (Zn) showed variable effects on the rate of seed germination percentage and seedling growth characteristics and seedling dry weight of *D. sissoo* as compared to control. The lead and zinc treatment at all level on seed of *D. sissoo* city areas showed higher percentage of rate of seed germination percentage. Zinc is micro nutrient and in excess level produce negative effects on plant growth of *D. sissoo*. Root length of campus seed seedlings was higher in Zn treatment whereas, seeds from city area showed significantly lowest root length in Pb treatment at 90 ppm. The shoot length was found higher in campus and city area seedlings in both treatments. There was no significant difference in dry weight between city and campus seedlings but city seedlings showed higher dry weight in Pb treatment. Percent metal tolerance was higher in city seedlings treated with Zn but in Pb treatment campus seedlings showed higher percent metal tolerance.

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**Key words:** heartwood tree; heavy metals; phytotoxicity; tolerance index; toxicity; urban areas.

### 1. Introduction

Heavy metals are group of elements which have a density greater than  $5\text{g/cm}^3$  [1] and heavy metal contamination of soil, water and air has produced many environmental issues. The metal takes long time after their introduction for chemical degradation [2]. Mining activities cause an environmental impact on quality of air, water and soil [3] and worldwide environment problem [4].

Global industrial growth has contaminated the soil, water and air with many hazardous compounds, including heavy metals. These heavy metals are not only toxic to plants but also cause severe human health problems via food chain [5]. Furthermore, the addition of heavy metals in combination produced additive, antagonistic or synergistic effects on plant growth. The effects of combined heavy metals may be quite different from those of individual pollutants due to

interactions between heavy metals [6]. Plants accumulate a number of micro and macro elements from environment and some are known to be toxic even at low concentrations. Plants growing near busy roads experience abiotic stress due to different forms of pollutants available in environment and especially heavy metals are most concerns that leads to growth damage. Metals salts induced significant changes in seed germination, seedling growth, biomass production, photosynthetic pigments, and carbohydrates in plants. Heavy metal in excess concentrations produced abiotic stress in plants [7-14]. Plants evolved detoxification mechanisms to minimize the detrimental effects of heavy metal exposure and their accumulation [15]. Zhuo *et al.*, [16] reported the toxic effects of lead on growth, physiology and cellular structure in pivot seedlings.

Lead (Pb) is a potentially toxic heavy metal and has attracted considerable attention for its widespread distribution and potential risk to the environment. Pb contamination in soils resulted in soil fertility deterioration but also resulted in yield decline [17]. Lead induced abiotic stress on plant growth and development [18]. Impact of some metals (Zn, Pb, Ni, Cu, and Cd) on germination and seedling behaviors of roadside weed (*Ambrosia. artemisiifolia*) and ground cover legumes (*Coronilla varia*, *Lotus corniculatus* and *Trifolium arvense*) in southern Québec, Canada was assessed in a growth chamber experiment [19]. All metals inhibited *Trifolium arvense* germination, but the effect was least on *A. artemisiifolia*. The results have proved about the toxic effects of lead on growth and development and antioxidant capacity of naked oak (*Avena nuda* L.) seedlings under abiotic stress [20].

The zinc is required in small quantity but high levels leads to reduction in plant growth and biomass productivity. Seed germination of *Spartina alterniflora* in solutions containing zinc and lead at various salinity levels under chamber-controlled conditions were reduced [21]. *Sesbania drummondii* seedling growth was found significantly inhibited with lead and zinc treatment in the order as Pb > Zn for roots and shoots [22]. Zinc toxicity effects the germination, growth and mineral nutrients of plants [23-25]. The drastic effect of zinc (Zn) and copper (Cu) on seed germination, seedling growth, alteration in antioxidant enzymes, morphological changes and metal accumulation in various plant parts of *Cassia angustifolia* Vahl. in concentrations of 1 – 200 mgL<sup>-1</sup> was investigated [26].

The environment of Karachi city is polluting due to ever increase in industrial, auto vehicular and anthropogenic activities. The plants growing along the busy roads of the city are particularly affected. The studies of different researchers have reported about the regular increase in concentrations of different types of toxic pollutants and heavy metals in city environment of Karachi. High levels of heavy metals were investigated in soil and vegetation samples from various polluted sites of Karachi city observed [27-28].

*Dalbergia sissoo* (Roxb.) is a member of family Fabaceae. *D. sissoo* is a fast-growing large deciduous tree and vernacular name known as Shisham, Sissu, Tali. The genus *Dalbergia* with c. 250 species has a pan tropical distribution and has high economic and ecological value of the genus [29]. It has not yet been the focus of a species level phylogenetic study. *D. sissoo* is a good nitrogen fixing tree and easily grow in well-drained soil [30] and has high economic, commercial and ecological values. *D. sissoo* is very widely planted in the plains along the roadsides, canals and fields and in the forest plantations. *D. sissoo* is

widely distributed in Pakistan, India, Sikkim, Afghanistan, Persia and Iraq. The wood of *D. sissoo* which is hard, heavy and durable is commonly used for furniture, carts, boats and most desire shade tree [31-32].

Adequate strategies for the plantation of metal tolerant species in metal contaminated sites is required. Among heavy metals, lead and zinc are ecotoxicology concern due to alarming increase in environment and impact on plant growth. Less is known about the effects of heavy metals (Pb and Zn) on the germination and growth performance of an important street plant species, *D. sissoo* growing in automobile polluted environment of Karachi, city. Therefore, the present studies were carried out with the aim to evaluate the toxicity effects of lead and zinc on seed germination and seedling growth performance of *D. sissoo* widely planted on different roadside of city of Karachi.

## 2. Materials and Methods

A The healthy and uniform size seeds of *Dalbergia sissoo* Roxb were randomly collected from growing in city area (Hassan Square) and Karachi University Campus (relatively clean area) of Karachi-Pakistan.

### 2.1 Trial experiment

The seeds were brought in paper bags and stored in laboratory for experimental research work to check heavy metal (Pb and Zn) toxicity and tolerance. All the seeds were checked for their viability by putting 10 seeds in petri dish with a filter paper lined of same size and provided 10 ml of distilled water for 10 days. The seeds were surface sterilized with 0.50% of mercuric chloride for one minute to prevent any fungal growth. The seeds were selected for experiment, if they showed at least 50% germination. The rate of seed germination percentage at different time interval was checked. The experimental set up was performed in February using six different concentrations 0, 10, 30, 50, 70 and 90 ppm of Zinc nitrate and Lead nitrate which were prepared from 100 ppm stock solution as follows.

0 ppm = 0 ml solution + 100 ml distilled water  
 10 ppm = 10 ml solution + 90 ml distilled water  
 30 ppm = 30 ml solution + 70 ml distilled water  
 50 ppm = 50 ml solution + 50 ml distilled water  
 70 ppm = 70 ml solution + 30 ml distilled water  
 90 ppm = 90 ml solution + 10 ml distilled water

Five ml solutions of lead nitrate and zinc nitrate concentrations 10, 30, 50, 70 and 90 ppm were provided to respective petri dishes for ten days and the total number of seed germinated was recorded. The control set received only distilled water. The experiments were designed on the basis of three

replicates and the Petri dishes were kept at room temperature ( $32\pm 2^{\circ}\text{C}$ ) with 240 Lux light intensity and the experiment lasted for 10 days. The rate of seed germination was recorded daily. The experiment was completely randomized and seed germination, root, shoot and seedling lengths were recorded. The seedling dry biomass was determined by placing the seedling in an oven at  $80^{\circ}\text{C}$  for 24 hours and dry biomass was measured with electrical balance.

### Statistical analysis:

Mean percent seed germination, root, shoot, seedling growth and seedling dry weight data were statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the level of significance at  $p < 0.05$  on personal computer using COSTAT version 3.

Tolerance indices of seedlings were determined with the help of following formula.

Tolerance indices (T.I.). Mean root length of metal treated seedlings /Mean root length of without metal treated seedlings X 100

### 3. Results and Discussion

This is in vitro study record the variable effects of selected two different types of heavy metals Pb and Zn on seed germination and seedling growth

performances of *D. sissoo*. In the present study different (10-90 ppm) concentrations of lead and zinc were applied on seeds of *D. sissoo* collected from city and campus areas (Table 1-2; Fig. 1-2). Mean percentage germination of *D. sissoo* was much higher in both metal treatments. Rate of seed germination (%) was higher of city area as compared to other treatment. Zn treatments at 70 ppm showed higher percentage of germinations in campus seeds whereas in Pb treatments in city seeds showed highest percentage of germination as compared to campus seeds. If we compare the percentage germination of both metals, then city will appear that Pb treatment showed higher percentage of germination from both city and campus seeds. Campus seeds treatment with in showed significant different in percentage to germination and highest value (76.66%) was obtained at 70 ppm. Zn treatment when applied showed higher percent germination in campus seeds. It means that city seeds were more susceptible to Zn treatment whereas in Pb treatment city seeds showed higher germination as compared to campus seeds. This means that city seeds were more resistant to lead as compared to Zn. The different physiological responses to Pb stress by 50, 150, 300, 600, 800, 1000 mg/L for the seed germination of *Salsola passerina* Bunge and *Chenopodium album* L. were reported [32].

**Table (1): Effects of lead (TRMT=treatment) on seed germination and seedling growth of *Dalbergia sissoo***

TRMT (ppm)	Germination (%)		Root length (cm)		Shoot length (cm)		Seedling size (cm)		Seedling dry weight (g)	
	KUC	City	KUC	City	KUC	City	KUC	City	KUC	City
00	56.66 a	73.33a	1.00 a	0.67 a	3.83a	1.68a	4.83a	2.36a	0.040 a	0.066 a
10	53.33 a	70.00a	0.30b	0.77 a	1.60a	1.36a	1.91 a	2.07ab	0.020 a	0.063 a
30	63.33 a	73.33a	0.38b	0.60ab	3.30a	1.77a	3.68a	2.38a	0.020 a	0.056 a
50	60.00 a	70.00a	0.216b	0.40bc	1.60a	1.51a	1.30a	1.92ab	0.020 a	0.063 a
70	63.33 a	76.66a	0.143b	0.43bc	1.99a	1.38a	2.13a	1.81b	0.020 a	0.060 a
90	60.00 a	50.00a	0.160b	0.65c	2.16a	1.41a	2.32a	1.78b	0.017 a	0.066 a
L.S.D.	30.23	31.65	0.456	0.81	2.96	0.448	3.30	0.505	0.025	0.027

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at  $p < 0.05$  level

The two metals when compared with each other, then city seedlings showed higher root length in Zn treatment whereas, campus seedlings showed slightly higher root length in Pb treatment (Fig. 1-2). In Zn treatment campus seedlings showed slightly higher root length (2.41 cm) as compared to city seedlings, where as in Pb treatment campus seedlings showed low root length as compared to city seedlings (Table 1). Studies in response of roadside plants to metals such as lead (Pb) and zinc (Zn) stress and toxicity are very scant in literature. Pb and Zn can pose a great threat to plant growth when grown in excess polluted environment.

The exposure of heavy metals stress to plants leads to damaging effects on germination and growth characteristics. The growth of tobacco in  $\text{Cd}^{2+}$  and  $\text{Cd}^{2+} + \text{Pb}^{2+}$  polluted soil for 50, 100, and 150 d resulted in some abnormal external morphological and anatomical changes in ripe region of lateral roots [33]. City seedling when treated with Zn showed significant ( $p < 0.05$ ) difference and highest root length (0.77 cm) was obtained at 10 ppm solution, on the other hand city seedlings when treated with Pb showed significant lower root length as compared to control treatment. The campus seedlings treated Zn also showed significantly

lower root length as compared to control treatment. Shoot growth was recorded higher in campus seedlings in Zn metal treatments. Pb appeared to be more toxic for shoot lengths in both city and campus seedlings. Highest shoot length 5.86 cm was obtained in campus seedlings when treated with 10 ppm solution of Zn. The Pb treatment did not produce any significant difference in city and campus seedlings dry weight. Zn treatment

at 90 ppm showed significant difference in seedling dry weight raised from campus seed. Somova and Pechurkin [34] also showed that Zinc salt had an adverse effect on germination of wheat seeds, beginning with concentrations of 8 MPC (Maximum Permissible Concentration) and the root system of plants was more sensitive to the adverse effect of  $ZnSO_4$  than shoots of plants.

**Table 2. Effects of zinc on seed germination and seedling growth of *Dalbergia sissoo***

Treatments (ppm)	Germination (%)		Root length (cm)		Shoot length (cm)		Seedling size (cm)		Seedling dry weight (g)	
	KUC	City	KUC	City	KUC	City	KUC	City	KUC	City
00	43.3bc	60.0a	1.76 a	0.803a	9.23 a	1.76ab	11.00 a	2.58a	0.016ab	0.066a
10	43.0bc	40.0a	2.41 a	0.560abc	5.14 b	1.95a	7.55 a	2.43a	0.020ab	0.063a
30	60.0ab	53.3a	1.32 a	0.666ab	4.89 b	1.27b	6.19 a	1.94ab	0.016ab	0.033a
50	56.7abc	46.7a	1.60 a	0.560abc	5.76 ab	1.64ab	5.06 a	2.20ab	0.023a	0.036a
70	70.0 a	50.0a	1.38 a	0.540bc	6.41 ab	1.66ab	7.81 a	2.02ab	0.020ab	0.060 a
90	33.3c	43.3a	1.66 a	0.296c	4.08 b	1.32b	5.70 a	1.61b	0.013b	0.073a
L.S.D.	21.70	30.01	0.83	0.303	3.39	0.495	4.16	0.609	0.008	0.040

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at  $p < 0.05$  level

The organic material derived from plants and animals is also referred to as biomass [35]. Seedling biomass of *D. sissoo* was affected more by Pb treatments as compared to Zn. Seedling dry weight of *D. sissoo* showed higher value in control treatments as compared to Pb and Zn. In another studies, treatments of Zn (0, 2.5, 5.0, 7.5 and 10 mM) doses reduced seedling dry mass in mustard varieties [36].

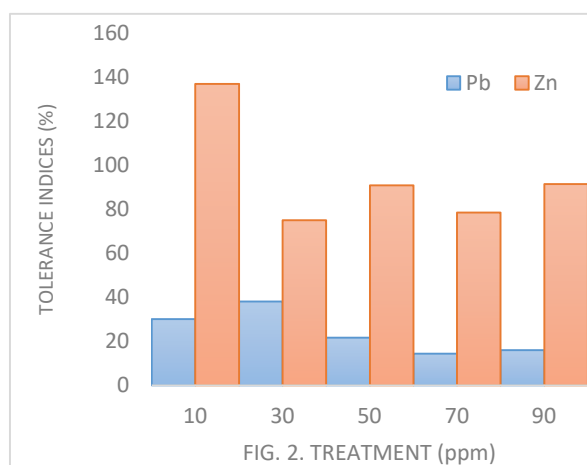


Fig. 2. Effects of lead and zinc on seedling tolerance indices (%) of *Dalbergia sissoo* from campus area.

Plants species are able to develop the capability of adaptation to grow in contaminated site and the mechanism of action of toxicity depends on the availability of chemical compounds in the surrounding environment [37]. The treatment of different concentration of (Pb and Zn) to the seedlings of *D. sissoo* can be served as a good metal pollution indicator.

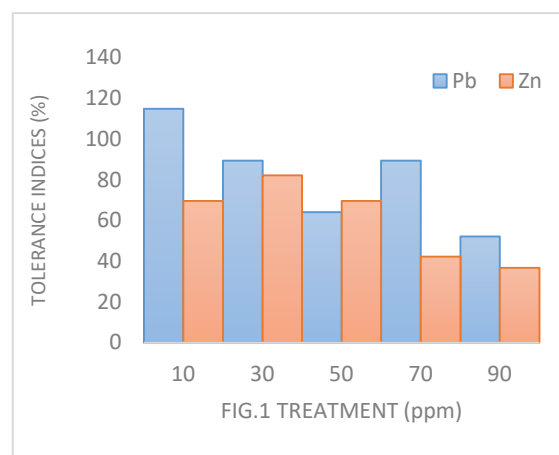


Fig. 1. Effects of lead and zinc on seedling tolerance indices (%) of *Dalbergia sissoo* from city area.

## 5. Conclusion

It was concluded that lead and cadmium treatment produced toxic effects on seed germination and seedling growth of *D. sissoo* as compared to control. The tolerance indices for *D. sissoo* seedlings was found less by both metal treatment. It is suggested that the value of difference in tolerance indices should be consider while planting *D. sissoo* in metal contaminated areas.

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## HIGHLIGHTS

The significance of heavy metal (Pb and Zn) pollution impact on seed germination and seedling growth performance of *D. sissoo* was recorded. A comparison was made between the effects of (Pb and Zn) on seedling growth performance of *D. sissoo*. The treatment of (Pb and Zn) led to decreased seedling growth and seedling dry weight of *D. sissoo*. Low seedling tolerance index was linked with the different concentration of Pb than Zn as compared to control. The treatment of different concentration of (Pb and Zn) to the seedlings of *D. sissoo* could be served as a good metal pollution indicator.

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