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Original Article

EFFECT OF SELECTED HEAVY METALS (LEAD AND ZINC) ON SEEDLING GROWTH OF SOYBEAN *GLYCINE MAX* (L.) MERR

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ABSTRACT

Objective: This study was designed to evaluate the effects of different concentrations of both zinc (250, 500, 750, 1000 and 1250 mg/kg) and lead (200, 400, 600, 800, 1000 mg/kg) as zinc sulphate and lead acetate respectively on 7days seedling of soybean (*Glycine max* (L.) Merr.)

Methods: To investigate morphological growth parameters seedlings were cut at the root-shoot junction and the length of their root and shoot was measured with a metric scale and expressed in centimetre's. The fresh weight of seedling samples was recorded on an analytical balance and expressed in gram per seedling. Later, seedlings were dried in an oven at 80^{o} C for 24 h to get constant dry weight. After 24 h the dry weight was recorded.

Results: The study revealed that elevated dose of lead concentrations reduces the growth parameter as compared to control. Lead concentrations of 1000 mg/kg significantly decreased the percentage of germination and root length. However, at low levels of zinc (250 and 500 mg/kg) showed increased germination percentage and also increase root length shoot length. But at high levels (750–1250 mg/kg) showed a detrimental effect on the growth parameter and germination.

Conclusion: Consequently, higher concentrations of heavy metals had an increased inhibitory effect on seed germination percentage, root length, shoot length, tolerance index, fresh weight and dry weight of soybean seedlings, but the low concentration of zinc can be applied for increasing the growth and yield of soybean plants.

Keywords: Lead, Zinc, Seed germination, *Glycine max* (L.) Merr

INTRODUCTION

Heavy metal pollution of the environment is increasing day by day due to the discharge of untreated chemical by different industries and anthropogenic sources, receives a high amount of heavy metals which, when taken up by crop plants in higher concentrations, creates not only problems in plant body and reduced crop production but also enter into the food chain [1] potentially causing human liver and brain disorders. Moreover, it also decreases soil fertility, affects soil microbes and yield losses [2]. The noxious heavy metals in different valence states include zinc (Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni) and lead (Pb), of these zinc are an essential element for several biochemical processes, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation and membrane integrity [3]. But the hyper accumulation of zinc in the planet's limits shoots and root growth and also inhibit the uptake of iron that leads to iron deficiency i.e. characterized by pale yellow to white interveinal chlorosis on the younger leaves may eventually lead to necrosis of the leaf blades and growing points [4]. Whereas lead is non-essential element, the high concentration of lead inhibits plant growth, alters the transcriptional process, denatures the proteins [5] and disturbs photosynthesis [6]. Soybean is an important crop in the world, offering high-quality protein and increasing the input of combined nitrogen into the soil, but its yield and nutritional quality adversely affected by heavy metals. It is fact that germinating seeds and developing seedlings are more sensitive to metal elements than mature plants, as their defense mechanisms are not yet fully developed [7]. Hence, the present study was carried out with an objective to analyze the effect of different concentrations of both zinc and lead on 7days seedling growth of soybean.

MATERIALS AND METHODS

Plant material and chemicals

The certified seeds of soybean (*Glycine max* (L.) Merr.) Produced by vigor Biotech Pvt. Ltd., variety JS-95-60 was procured from Agriculture station, Kota, Rajasthan. The seeds with uniform size, color and weight were chosen for the experimental purpose. Chemicals were zinc sulphate ($ZnSO_4.7H_2O$) and lead acetate ($(CH_3COO)_2$ $Pb.3H_2O$, with various concentrations (250, 500, 750, 1000, 1250 mg/kg and 200, 400, 600, 800, 1000 mg/kg respectively) used for the treatment purpose.

Soil collection and physiochemical properties

The agricultural soil was collected from the experimental farm (depth 0-15 cm) in Rajasthan University campus, Jaipur. The soil was air dried at room temperature and then ground through 2-mm mesh. Some basic physiochemical properties were measured at Durgapura, Jaipur. Four (4) kg soil was weighed and loaded into each pot. Physicochemical properties of soil are given in table 1.

Table 1: Soil analysis of the experimental site

Soil properties	Values
pH	8.6±0.4
Electrical Conductivity DS/m	0.11±0.01
Biological carbon %	0.16±.02
Phosphate (Kg/hac)	24±1.1
Potash (Kg/hac)	290±5.5
Available Zinc (Zn) ppm	0.60±0.06
Available, Iron (Fe) ppm	7.52±0.44
Available Copper (Cu) ppm	0.68±0.04
Available Maganese (Mn) ppm	6.56±0.70

Number of experiments (N) = 3, Values were expressed as mean±SEM

Experimental setup

The experiments were set up in green house by Botany Department, University of Rajasthan, Jaipur during the month of April in natural outdoor conditions where the photoperiod was 12 h and the average temperature was 30 °C. Four kg of soil was filled in pots 30 cm high and 25 cm in diameter. Five concentrations of lead (200, 400, 600, 800, 1000 mg/kg) and zinc (250, 500, 750, 1000, 1250 mg/kg) were applied as lead acetate and zinc sulphate forms respectively. No other supplement nutrients were applied. Pots without added heavy metals constituted were taken as controls. Soybean seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for two minutes and thoroughly washed with DW water. Ten sterilized seeds of soybean were sown equidistantly at 2 cm deep in each pot. Three replicates were used for each concentration. Watering was done on alternate days.

Germination and growth measurements

The number of seeds germinated in each treatment was counted on the seventh day and germination percentage (%G) was calculated by following formula [8]:

%G = (number of germinated seeds/total number of plant seeds) × 100

The emergence of radical was taken as a criterion for germination. Seedlings from each replica was selected for recording the morphological parameters such as root and shoot length, dry and fresh weight. Seedlings were cut at the root-shoot junction and the length of their root and shoot was measured with a metric scale and expressed in centimetres. The fresh weight of seedling samples was recorded on an analytical balance and expressed in gram per seedling. Later, seedling were dried in an oven at 80° C for 24 h to get constant dry weight. After 24 h the dry weight was recorded.

Stress tolerance index

Stress tolerance index is a useful tool for determining the high yield and stress tolerance potential of genotypes. Stress tolerance indices for different growth parameters were calculated using following formulae [9]:

RLSTI = (Root length of stress plant/Root length of control plant) × 100

SLSTI = (Shoot length of stress plant/Shoot length of control plant) × 100

SFSTI = (Seedling fresh weight of stress plant/Seedling fresh weight of control plant) × 100

SDSTI = (Seedling dry weight of stress plant/Seedling dry weight of control plant) × 100

Statistical analysis

Each treatment was analyzed with at least three replicates and the standard error (SE) was calculated. The data were expressed in mean data±SEM (Standard error of mean).

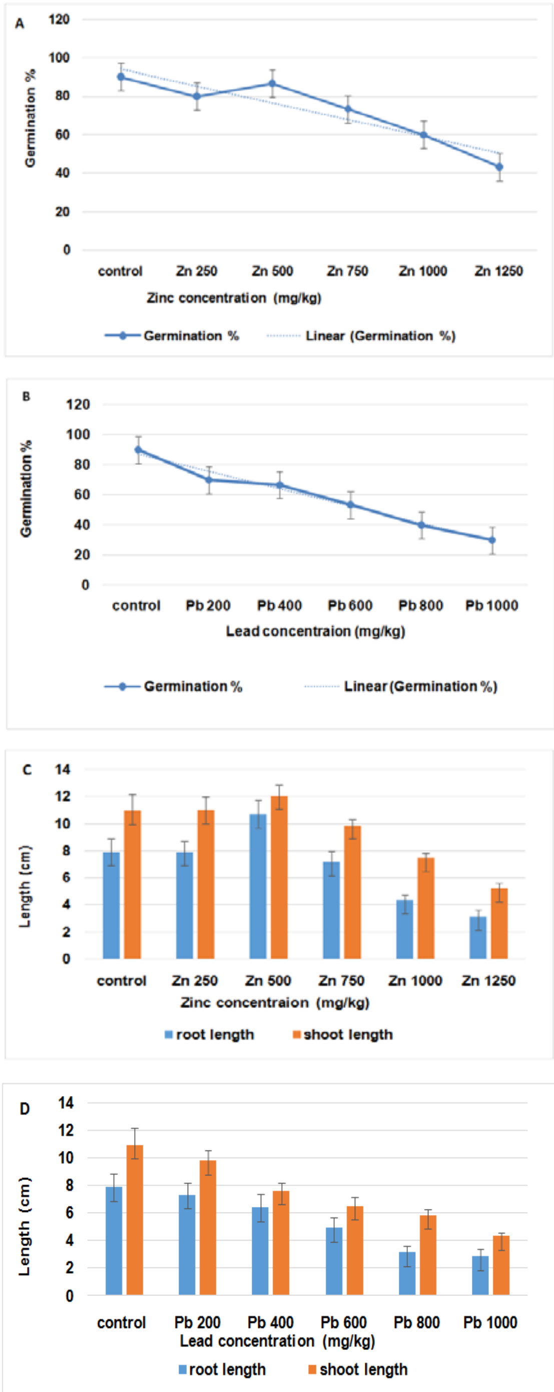
RESULTS AND DISCUSSION

Seed germination

To evaluate the effects of the different concentrations of both lead and zinc on seed germination of soybean (*Glycine max* (L.) Merr.) grown in pots. Generally, increasing concentrations of heavy metal solutions significantly reduced the strength of germination of soybean seeds. Higher seed germination (90%) was observed in control seedlings. In this study, at low levels of zinc (250 and 500 mg/kg) showed increase germination percentage, but the growth process beyond these levels (750–1250 mg/kg) are impacted adversely. Similar findings were observed in earlier reports [11] and in Cluster Bean (*Cyamopsistetra gonoloba* (L.) Taub [10]. This value indicates that zinc at a lower level had a significant stimulatory, beneficiary and nutritional effect. However, lead exhibit gradually decrease the germination percentage. The 800 mg/kg dose of Pb^{+2} significantly reduced the seed germination. At a concentration of 1000 mg/kg, Pb^{+2} inhibited seed germination significantly by 30%. Similar findings were observed in earlier reports such as *Spartiana alterniflora* [12], *Pinus helipensis* [13], *Hordeum vulgare*, *Elsholtzia argyi*, *Oryza sativa*, and *Z. mays* [14, 15].

Root growth

When the lead concentration was increased, the amount of root length was significantly declined. The lower root length 2.87 cm/seedling was observed at 1000 mg/kg lead concentration. Similar findings were observed in an earlier report [16] that root length of *Leucaena leucocephala* significantly (p<0.05) decreased at 100 ppm of lead treatment. In the case of zinc treatment, root length of soybean increased at 250 mg/kg (7.91 cm/seedling) and 500 mg/kg (10.7 cm/seedling) and decreased further with an increase of the zinc level (750–1250 mg/kg) into the soil. At Zn1250 mg/kg, the lower root length was observed 3.13 cm/seedling. Similar findings were observed in earlier studies of Cluster Bean (*Cyamopsistetra gonoloba* (L.) Taub. Treated with low level of zinc (10 and 25 mg/l) showed a significant increase in germination, seedling growth over the control and then decline beyond these levels (50–200 mg/l) [10]. Zinc at high levels may inhibit the root growth directly by inhibition of cell division or cell elongation or combination of both, resulting in the limited exploration of the soil volume for uptake and translocation of nutrients and water and induced mineral deficiency [17].



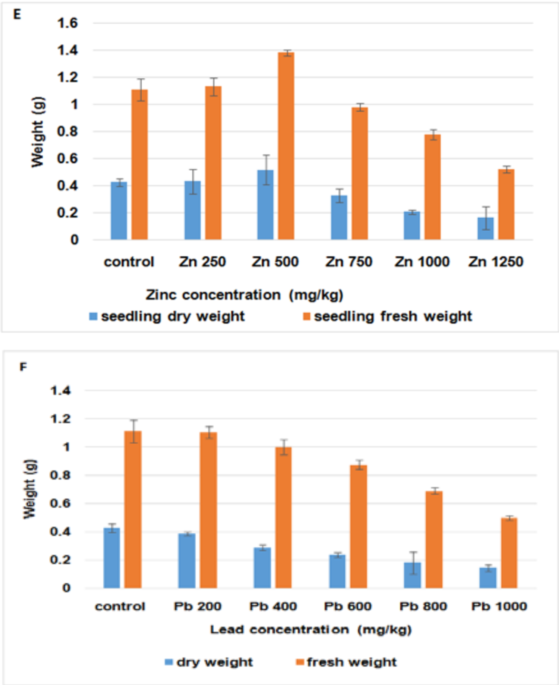


Fig. 1: A: Seedling germination percentage under influence of Zn; B: Seedling germination percentage under influence of Pb; C: Effect of Zn on root length and shoot length of seedling; D: Effect of Pb on root length and shoot length of seedling; E: Effect of Zn on dry weight and fresh weight; F: Effect of Pb on dry weight and fresh weight

A number of experiments (N) = 3; Sample size (n) =30; Values were expressed as mean±SEM

Shoot growth

The highest decrease in mean shoot length (5.25 cm/seedling and 4.34 cm/seedling) of soybean seedling was found at 1250 mg/kg zinc and 1000 mg/kg lead concentrations, respectively, whereas maximum shoot length (12.08 cm/seedling), were observed at zinc 500 mg/kg. When the lead concentration was increased, the amount of shoot length was significantly declined. The similar finding, the results of [18] showed that soil lead can cause phytotoxicity to *V. faba* seedlings, which was evidenced by the significant decrease in shoot heights at higher concentrations of 1,000–2,000 mg kg^{-1}. But at low levels of zinc (250 and 500 mg/kg) showed a significant increase in shoot length, but growth gradually decrease when concentration increased (750–1250 mg/kg). Similar findings were observed in earlier studies of cluster bean (*Cyamopsistetra gonoloba* (L.) Taub. Treated with a low level of zinc (10 and 25 mg/l) showed a significant increase in germination, seedling growth over the control and then decline beyond these levels (50–200 mg/l) [10].

Fresh and dry weight

Average fresh and dry weight of seedling under different lead treatments were significantly decreased. Lead concentrations of 1000 mg/kg showed lower fresh weight (0.496 g/seedling) and dry weight (0.143 g/seedlings). Variable effects of lead on plant growth have been observed by various workers. Inhibition in fresh weight, dry weight and length of shoot and root of *Sesamum indicum* CV. HT-I [19], maize [20] has been observed. The results showed in graph E that the maximum fresh and dry weight value occurred at 500 mg/kg (1.384 g/seedling, 0.516 g/seedling) respectively, and Minimum fresh and dry weight of shoot was observed at 1250 mg/kg (0.523 g/seedling, 0.163 g/seedling) respectively. The low levels of zinc (250 and 500 mg/kg) showed a significant increase in fresh and dry weight, but growth gradually decrease when concentration increased (750–1250 mg/kg). [21] observed similar effects using zinc in bean (*Phaseolus vulgaris* L.).

Tolerance index

Table 2 shows the higher lead and zinc treatment lowers the percentage of tolerance in *Glycine max*. In zinc stress condition, Tolerance index higher at 250 and 500 mg/kg but lower at 750-1250 mg/kg. The highest value of RLSTI, SLSTI, SFSTI and SDSTI was recorded 135%, 110.31%, 124.46% and 121.70% respectively at zinc 500 mg/kg. Minimum value at zinc 1250 mg/kg, RLSTI, SLSTI, SFSTI and SDSTI was recorded 39.67%, 47.94%, 47.03% and 38.44% respectively. In lead stress condition, when lead concentration was increased, the amount of tolerance index was significantly declined. The lowest value of RLSTI, SLSTI, SFSTI and SDSTI was recorded 36.33%, 39.63%, 44.6% and 33.73% respectively. Similar effects were observed in earlier studies of (*Glycine max* L.) Exposed to chromium stress [9], in rice (*Oryza sativa*) under chromium Stress [22].

Table 2: Stress tolerance index (%) of *Glycine max* (L.) Merr. Growing in Lead and Zinc contaminated soil

Groups	Control	RLSTI (%) 100±00	SLSTI (%) 100±00	SFWSTI (%) 100±00	SDWSTI (%) 100±00
Zinc sulphate (mg/l)	250	100.25±0.79	100.45±0.78	101.97±0.82	101.65±0.52
	500	135±1.06	110.31±0.64	124.46±0.25	121.70±0.34
	750	90.70±0.78	90.04±0.35	88.21±0.37	76.42±0.66
	1000	55±0.37	68.40±0.26	70.05±0.48	48.11±0.5
	1250	39.67±0.47	47.94±0.29	47.03±0.32	38.44±0.8
Lead Acetate (mg/l)	200	93.28±0.83	89.68±0.62	99.28±0.53	91.27±0.46
	400	81.49±0.97	69.86±0.44	89.74±0.63	67.22±0.7
	600	62.73±0.75	59.72±0.53	78.68±0.4	55.19±0.56
	800	40.17±0.46	53.51±0.35	61.96±0.27	41.98±0.73
	1000	36.33±0.53	39.63±0.21	44.60±0.2	33.73±0.53

Number of experiments (N) = 3; Sample size (n) = 30; Values were expressed as mean±SEM; RLSTI: Root length stress tolerance index; SLSTI: Soot length stress tolerance index; SFSTI: Seedling fresh weight, stress tolerance index; SDWSTI: Seedling dry weight stress tolerance index.

CONCLUSION

Zinc and lead have gained considerable attention as a potent heavy metal pollutant due to the growing anthropogenic pressure on the environment. It shows a sharp decline in crop productivity. The data reported in the present study suggest that increasing concentrations of lead had an increased inhibitory effect on seed germination percentage, root length, shoot length, tolerance index, fresh weight and dry weight of soybean seedlings. However, at low levels of zinc showed a significant increase in morphological growth parameters, but growth process on beyond these levels are impacted adversely. The highest concentrations of heavy metal solution had the most negative influence on all the parameters which are considered for examination. The results indicated that the low concentration of zinc can be applied for increasing the growth and yield of soybean plants. In future two general strategies for engineering tolerance to oxidative stresses can be applied, firstly increase the level of the enzyme that remove reactive oxygen species (ROS), and secondly to increase the level of antioxidant compound that react with ROS. Such strategies may help ameliorate the pollution of on lands and water by heavy metals.

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CONFLICT OF INTERESTS

Declared none

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