Effects of lead and zinc on seed germination and seedling growth of soybean (Glycine max L)

Fatemeh Naghavi*

*Young Researchers and Elite Club, Kerman Branch, Islamic Azad University, Kerman, Iran, Employee of education and training of Kerman

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Abstract

In this research, the effects of different concentrations of ZnCl₂ (5, 10, 15, 20 µM) and PbCl₂ (0.5, 2.5, 4.5, 6.5 µM) on Soybean were examined. The effects of ZnCl₂ and PbCl₂ on the percentage of germination and germination rate of seeds, longitudinal growth of roots and shoots of seedlings of Soybean and the activity of antioxidant enzymes were studied. The seeds were transferred to petril dishes containing filter paper after surface sterile. There were 4 repeat from each treatment and there were 6 seeds in each repeat. The results showed that Pb stress had no significant effect in the percentage of germination of seedlings, and increase in both Zn and Pb treatment caused increasing in germination rate, but, it caused significant decrease in the length of roots, shoots and seedlings. Zn stress caused significant decrease in the percentage of germination in the higher concentrations and decrease in the length of roots and shoots in all of concentrations. The activities of Catalase and Peroxidase (POX) enzymes had significant increase in the plants under treatment with Zn and Pb in all concentrations. Obtained results of this research indicated that in comparison with Zn, Soybean has more Tolerance to Pb. It seems that in short term stress of Zn and Pb, the plant decreases stress intensity with induction of fast response, but, in spite of definitions that exist for the tolerable plants of Zn and Pb, it does not seem to include Soybean from tolerable plants to these heavy metals.

*Corresponding Author: Fatemeh Naghavi
**Introduction**

The first effects of metal pollution on an ecosystem is the presence of heavy metals in contaminated biomass that could endanger human health. Accumulation of heavy metals in water, air and soil, is an important environmental problem (Ernest, 1998). Phytoplankton functional impairment as an important source of oxygen in the oceans and thus the global balance of aquatic organisms in aquatic ecosystems is important adverse effects of lead (Chao, 2003).

Visual symptoms of lead toxicity include root growth and plant growth inhibition and chlorosis. Plant uptake of trace amounts of lead can have a major negative impact on plant physiological processes. Lead toxicity leads to cessation of enzyme activities, disturbances in mineral nutrition, plant water imbalance, hormonal changes and changes in membrane permeability. At the cellular level activity of the enzymes that contain (-SH) groups are inhibited. Lead toxicity in maize seedlings, causing leakage of potassium ions from the cell root (Malkovski, 2002).

Ceratophyllum demersum plants grown in media containing PbNO₃ show certain changes in chloroplast structure, including reduced Grana and stroma. Lead also destroys the lipid structure of the thylacoid membrane (Islam et al., 2008). Lead affects the electron donor and receptor sites in photosysteme II, complex cytochrome b / f and photosysteme I. Photosysteme I compared with photosysteme II Show less sensitivity to lead toxicity (Islam et al., 2008). Lead also breakdown the external polypeptides in Oxygen evolving complex (OEC) in PSII and displacement of calcium, chlorine and manganese ions from OEC.

Scientists believe the photosynthesis decline due to use of lead is more as a result of stomatal closure to its direct effect on photosynthesis (Sharma et al., 2004).

Pb stress-induced ROS production depends on factors such as stress intensity, stress duration, plant species and age. Lipid peroxidation, which is considered as an indicator of oxidative damage involved in the degradation of acyl group residue in unsaturated lipids membrane (Yadav, 2010).

Inhibitory activity of PSII is due to substitution of Zn instead of Mn on the thylacoid membrane. In normal conditions there are 16 Mn and Zn atoms per 400 chlorophyll molecules in the membranes. However in stress condition caused by high concentrations of zinc, the ratio changes to 12 Mn and 30 Zn atoms (Ghorbanli and Babalar, 1993). In response to reactive oxygen species, activity of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase and glutathione cycle enzymes - such as ascorbate peroxidase, ascorbate and reductase increase (Prasad, 1999). In rice (Murakami et al., 2009) and canola as well as an increase in antioxidant enzyme activity has been reported (Wang, 2009).

According to the above and given that soy is grown in a very wide area in Iran and due to presence of proteins, lipids, carbohydrates and minerals, it have high nutritional value, So that In the Ministry of Agriculture a project in the country's oil resources has been carried out in 1384-1390 to increase the production of oilseeds in the country and self-sufficiency percentage in this production (Noori and Jahan Nama, 2008) so this study investigate the effect of Zn and Pb heavy metals on Soybean germination and some physiological and biochemical parameters.

**Material and method**

**Plant material and culture conditions**

In this study, soybean (Glycine max) was prepared from Seeds and Plants Research Institute in Karaj. ZnCl₂ was used in 4 levels (5, 10, 15, 20 mM) and PbCl₂ was also used in 4 levels (0.5, 2.5, 4.5 and 6.5 mM). 60 Seeds were sterilized in 5% hypochlorite solution and after rinsing were transferred the Petri Dish containing filter paper. Different levels of lead and zinc were added to Petri (4 replicates). Sterilized distilled water was used as a control. In order to
achieve the desired purposes in this study, the experiments were divided into 2 groups, which generally include:

-Effect on seed germination and some growth and developmental processes in plants that were cultured for 7 days in the Petri Dish.

-Check out some growth processes and biochemical measurements in pot cultivation.

Petri Dishes were transferred into Anko Bator for germination under 16 h light and 8 h dark at 25 °C. Repeat this procedure for 7 days. During this period, the number of germinated seeds was determined in order to set the germination percentage and germination rate. Exit from the root bark is considered as the onset of germination (Shakirova, 2003). Percentage of seed germination (GP) was obtained from the following equation:

\[
(\%\text{ PG}) = 100\left(\frac{N'}{N}\right)
\]

\(N'\) : The number of germinated seeds, \(N\) : Total number of seeds.

The time required for germination of 50% of seeds considered as the germination rate.

The experiment was conducted in a completely randomized design. After 7 days, the seedlings were investigated. In order to pot growth, the pots containing clay-loam soils with a pH of 8/6 were used. The pots were placed in the light of fluorescent lamps and tungsten (2000-2500 Deluxe), during the period 8-16 hours of light and darkness and a relative humidity of 60 to 70 percent and were fed with 1/2 Hoagland solution. 4 replicates for each treatment were considered then the treatment period began and lasted for 14 days. Later than 45 days plants were harvested for biochemical and physiological measurements.

-Plant Length: Using a scale ruler, the whole plant, roots and shoot length were measured.

The different parameters such as root and shoot length per centimeter (hypocotyls and Epicotyls) was calculated and recorded.

Enzyme extractions and assays
One gram of frozen leaves was homogenized in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA, 1 mM PMSF and 1% soluble PVP. The homogenate was centrifuged at 14000 g for 20 min at 4°C and the supernatant used for assay of the activity of enzymes. The protein content in the supernatant was measured according to the method of Bradford (1976). Bovine serum albumin was used as standard. The activity of CAT was estimated by monitoring the decrease in absorbance of \(\text{H}_2\text{O}_2\) within 30 s at 240 nm. The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM \(\text{H}_2\text{O}_2\) and 100μl enzyme extract. Unit of activity was taken as the amount of enzyme, which decomposes one μmol of \(\text{H}_2\text{O}_2\) in one minute (Dhindsa and Motowe, 1981).

The activity of POD was measured according to the method of Biles and Abeles, 1991. The 4 ml reaction mixture contained 0.2 M (pH 5) Acetate buffer, 400 μl of 3% \(\text{H}_2\text{O}_2\), 200 μl of 0.02 M benzydin, 50 ml of 50% methanol and 100 μl of enzymes extract. The change in absorbance was determined within 1 min at 590 nm.

Statistical Analysis
The data were subjected to an analysis of variance (two ways) and the means were separated using LSD Multiple Range test \((p = 0.05)\) and graphs were plotted using Excel software.

Results
In this study, results of survey of germination percentage within 4 days showed that germination percentage increases with higher concentrations of lead chloride.

In the case of Zinc, germination percentage decreased with increasing Zn concentration. On the fourth day, germination was reduced in the different Zinc chloride treatments. The decrease in both the 15 and
20 mM concentrations than the control plants were significant. Germination percentage increased in different lead chloride treatments while this increase was not statistically significant (Fig 1 and 2).

**Fig. 1.** The effect of various concentrations of zinc chloride on germination of Soybean.

**Fig. 2.** The effect of different concentrations of lead chloride on germination rate of soybean.

Results of soybean seed germination rate in different treatments of zinc chloride and lead chloride is shown in Fig 3 and 4. The data show that increasing concentrations of zinc chloride and lead chloride increases the rate of germination. This increase is statistically significant at the level of 0.05.

**Fig. 3.** The effect of various concentrations of zinc chloride on germination rate of soybean.

**Fig. 4.** The effect of different concentrations of lead chloride on germination rate of soybean plants.

The data show that the roots length reduced in plants treated with zinc. The decrease in the concentration of 5 mM compared to control plants statistically (at 0.05) was not significant but in the other concentrations was significant. Root length was significantly reduced in plants treated with high concentrations of lead (4.5 and 6.5 mM). Shoot length was significantly reduced in plants treated with high concentrations of zinc (15 and 20 mM). Shoot length was significantly reduced in plants treated with high concentrations of lead (4.5 and 6.5 mM).

**Fig. 5.** The effect of various concentrations of zinc chloride on the roots and shoots length of soybean.

Results to the seedling in pot cultivation in different concentrations of lead and zinc are shown in Fig 7 and 8. The data show seedling length reduced in plants treated with the zinc. The decrease was significant in concentrations of 0.05. Seedling length reduced significantly in plants treated with lead in all concentrations.
The results of measuring the effect of different concentrations of Zn and Pb on catalase activity are shown in Fig 9 and 10.

**Fig. 6.** The effect of different concentrations of lead chloride on roots and shoots length in soybean.

Catalase activity increased in plants treated with Zn and Pb that this increase was significant at all concentrations. Catalase activity was reported in control plants as much as 1.9 UnItg-1 fw. The amount increased in plants treated with Pb and Zn, 2.8 UnItg-1 fw and 2.3 respectively.

**Fig. 7.** The effect of various concentrations of zinc chloride on the length of soybean seedlings.

The results of measuring the effect of different concentrations of Zn and Pb on the peroxidase enzyme activity are shown in Fig 11 and 12. The data show that the root peroxidase activity increased from 1.4 in control plants to 2.9 in plants treated with zinc and to 1.94 in plants treated with lead. This increase was significant at all concentration. The amount of peroxidase activity in the leaves of control plants was reported 1.29 UnItg-1 fw.

**Fig. 8.** The effect of various concentrations of lead chloride on the length of soybean seedlings.

In this study, 50 μM Pb had no significant effect on germination. But 100μM concentration reduced germination and shoot and root growth significantly (Islam, 2007).

**Fig. 9.** The effect of various concentrations of zinc chloride on catalase activity in soybean.

The results of An (2006) identified various concentrations of lead have different effects on germination of various plants. For example, germination percentage in sorghum (Sorghum bicolor) decreased in 80-640 MgPb Kg-1 in the soil, but germination increased gradually at higher concentrations (from 640 to 1280 MgPb Kg-1). Germination (%) of Cucumis sativus and Zea mays showed no significant change in 80-1280 mg Pb Kg-1. wheat (Triticum aestivum) germination increased, but this increase was not significant compared to the control plant (An, 2006).

Discussion

The results showed that Lead toxicity increased soybean seed germination rate and percentage. Zinc toxicity at high concentrations decreased significantly seed germination (Fig 1,2). In experiments conducted by Islam et al (2007) showed that different concentrations of lead have different effects on germination percentage.
Lead has no effect on the absorption of water by the seed because at the beginning of germination, the seed coat is impermeable to lead but in the final stage seed coat will be permeable to lead. Lead penetrates to the embryo in the final stage of water absorption - delays germination (Cherati and khanlorian, 2007).

In this study due to lack of penetration of lead, soybean seed germination was not significantly decreased compared to control plants. Zinc toxicity deceased the germination by disrupting the membrane integrity and permeability (Rout, 2003). According to the results, the ability of soybean germination in different concentration of lead was higher than zinc. So that high concentrations of lead increased the germination (%). However, this increase was not significant. Inhibitory effects of zinc and lead on the root elongation exposed to the metals are the most common way to detect the inhibitory effects of this heavy metals on the growth (Zu, 2005). Results of this study showed that lead toxicity is primarily inhibiting root growth, due to the high concentration of lead in the root and it is toxic effects. Lead retention in roots is depends on pb binding to ion exchangeable sites on the cell wall and deposition of lead carbonate in the cell wall. Studies have shown that in some parts of the wall that thickness is less like plasmodesmata, a greater volume of a lead accumulates. It can be argued that lead accumulated on the wall causing cracks in the cell walls and greatly reduces the elasticity of it. This phenomenon restrict cell growth and subsequent organ growth inhibition (Ruley, 2006). The results of the John et al (2009) confirmed the reduction of plant growth as a result of lead heavy metals. Reduction of plant growth is due to reduced water potential, preventing the absorption of nutrients and secondary stresses such as oxidative stress. In addition, lead break the arrangement of microtubules in meristematic cells which is a barrier to growth (Eun, 2000). According to Yang, lead can damage the microtubules involved in mitosis and therefore causes cell cessation at the metaphase stage (Yang, 2000). Much of lead absorbed by root cells deposited on the walls caused gaps in the wall, and thus inhibits the growth of roots (Elloumi, 2007). Ma (2004) experiments on wheat indicate that heavy metals reduce viscosity and elasticity of root the cell wall and cause a reduction in root growth. The amount of Pb and Zn in roots and shoots are connected together. So that the amount of lead that reaches into shoot, depends on the capacity and capability of roots to keep the metal ions as well as the ability of shoot to use sulfur for binding to the metal. As a result, two different parts of plant have
different responses to heavy metal accumulation (Yanqun, 2005). It has been reported that the reduction of lead transport from root to shoot is because of lead retention in form of heavy metal – thiol complexes in root cells (Yanqun, 2005). Inhibition of cell growth in the elongation phase and the irreversible inhibition of proton pump activity as a result of lead toxicity, are effective factor for growth reduction (John, 2009). Stoyanova & Doncheva studies (2002) showed that low concentrations of zinc on chickpea increased roots and shoots growth. Zinc toxicity can limit the growth of plant and induce chlorosis in young leaves (Murakami et al., 2009). Zinc affects the process of cell division by blocking interphase, prolonging prophase and G2 and also inhibiting of the synthesis of nucleic acids and proteins required for cell cycle (El-Ghamery, 2003). Zinc toxicity is disrupted coherence, integrity and permeability of membrane (Wang, 2009). An important response to stress is ROS productions that one example of which is hydrogen peroxide (Lin, 2000). Catalase activity increases under heavy metals stress. Catalase and peroxidase regulate the optimum concentrations of hydrogen peroxide in cell. However, catalase plays a more important role (Lin, 2000). According to the Verma et al., (2003) study, 1,000 µM Pb reduced CAT activity in rice roots, which is in contrast with the results. Increase in CAT activity in bean plants has been reported by Wane et al., which confirms the present results (Schutzendubl & Polle, 2002). Four isozyme of peroxidase was detected by John et al., (2009). In this study peroxidase activity increased under lead toxicity in soybean. Increase of peroxidase activity is closely associated with changes in physiological processes such as respiration, photosynthesis. Increased in peroxidase activity is because of release of the enzyme from its position in cell wall in response to Pb toxicity (Sharma, 2005). Increase of peroxidase activity has been reported by Wang (2009) and Tomas (2004). CAT activity increased in Mustard leaves under Zn toxicity (Wang, 2009). With increasing of zinc concentration, CAT activity decreased. CAT activity in roots initially increased and then decreased. Increase peroxidase activity been reported in roots and leaves of bean under zinc toxicity (Cuypers, 2002).

In general it can be concluded that the induction of oxidative stress caused by zinc toxicity lead to reduced growth in the plants. On the other hand, lead stress caused biochemical changes in the plant. But according to the comparison of the two factors (Zn and Pb concentrations) affected soybean, it can be concluded that the plant is more sensitive to zinc than lead and this metal has more negative effects on the Physiological and biochemical parameters in soybean. Another point to be noted that the above experiment has been done under greenhouse condition and further studies (Ph, Soil texture) should be considered to investigate the effects of these metals on plants. It seems, in the short-term stress of Pb and Zn, plant reduces the stress intensity with the rapid induction. But according to the definitions for Pb-bearing plants, it does not seem to be called the soybean as plant bearing heavy elements.

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