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RESEARCH PAPER

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Effect of zinc and lead toxicity on some physiological parameters of *Glycine max* L.

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Key words: Toxicity, Zn, Pb, *Glycine max* L.

Abstract

The presence of heavy metals is counted as one of the most important environmental stresses. In this research, the effects of different treatments of two heavy metals, Lead and Zinc on the growth and rate of Chlorophyll, sugar and protein are studied. The effects of different concentrations of $ZnCl_2$ (5, 10, 15, 20 mM) and $PbCl_2$ (0.5, 2.5, 4.5, 6.5, mM) on Soybean were examined. For this purpose, the seeds were transferred to flowerpots containing Loam – Clay soil after surface sterile. In each flowerpot were cultivated 5 seeds. For each treatment are considered 4 repeat. The duration of treatment period has continued for 14 days. The results showed that the stress of Pb caused the decrease in biomass weight. The stress of Zn terminated in decrease of biomass weight in all concentrations. The rate of Chlorophyll a, b and total Chlorophyll in comparison with control plant showed significant decrease in both treatments. The increase in sugar content except for the concentration of 0.5 mM Pb, was significant in comparison with control plant in other concentrations but, in the stress of Zn, this increase had significant effect in all concentrations. The containing of protein in plants under treatment with Zn and Pb had significant increase in all concentrations. The 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.

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Introduction

Soybean (*Glycine max* L.) is one of the important sources for herbal yarns. It is consisted of ingredients known as Isoflawons which are structurally similar to the natural estrogens called phyto-strogens. These compositions have anti-cancer effects and are considered important in this regard. Consuming diets and eating habits rich in Soybean is effective in digestion and nutrient absorption, and the fibers are beneficial in preventing from constipation and reduction of indigestion's side effects (Kemin, 2007).

Soybean oil is rich in non-saturating and cholesterol-free oily acids. It is also a good resource for calcium, iron, zinc, phosphate, manganese, vitamin B and folate, and due to numerous availability has no problem in accessing. All the essential 8 acid amines which are vital for human's nutrition and are not naturally made in the body are found in soya bean (Kemin, 2007).

Significant increase in lead content of agricultural soils especially near industrial areas is quite evident. The accumulation of lead is more critical on the surface of soil, and its density reduces by increase in soil's depth, therefore being absorbed very easily by the plant, assembles in its organs. Lead is known to be a protoplasmic toxicant (Sharma, 2005).

Inhibition of cell growth in the elongation phase, and irreversible inhibition of proton pump due to lead's toxicity reduce plant's growth (John, 2009). Reduced shoot and root growth in aerial part is also evident in Seyyedi's research (Seyyedi, 1999). Increased plant biomass as a result of metal toxicity has been rarely reported in some studies; however these reports have been the results of studies on very low concentration of lead and other heavy metals (John, 2009).

Zinc is an essential element for all living organisms. Some of its unpleasant effects are fever, stomachache, vomit and diarrhea following the consumption of acidic drinks or food that is conserved in galvanized containers (Laura et al, 2010).

Zinc in toxic concentrations will cause decrease on the nitrogen content of the leaf by affecting the main enzymes responsible for ammonium assimilation and nitrate reductase enzyme, and since chlorophyll synthesis is dependent on nitrogen sources, by decreasing in nitrogen assimilation caused by zinc toxicity, the chlorophyll's content will also decrease (el-ghamery, 2003).

Lead will affect the electron donor and receptor in phytosystem II, b/f citochrome complex and phytosystem I. Phytosystem I has less sensitivity to lead comparing phytosystem II (Islam et al, 2008). Lead also causes collapse in external polypeptides of oxygen-evolving complex (OEC) in phytosystem II and movement of calcium, chloride and manganese ions from the OEC complex. Scientists believe that reduce in photosynthesis caused by lead due to the Stomatal closure rather than its direct influence on the photosynthesis process (Sharma et al, 2004). It's possible that heavy metals induce the lipids' proxidation and the biodegradation of proteins by reactive oxygen radicals and cause a decrease in protein's content (John, 2009).

According to the mentioned topics and also considering that Soybean is cultivated in vast scales in Iran, and due to having rich sources of protein, lipid, carbohydrate and minerals, this study investigated the effect of heavy metals such as zinc and lead on some of the physiological and biochemical parameters of Soybean plant.

Materials and methods

Plant materials

In this study, the seeds of *Glycine max* L were prepared from the Seeds and Plants Research Institute in karaj. $ZnCl_2$ was used in 4 levels (5, 10, 15, 20 mM). $PbCl_2$ was also used in 4 levels (0.5, 2.5, 4.5 and 6.5 mM). 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.

The sterilized seeds were cultivated in pots filled with Loam - Clay soil (pH 6.8). The cultures were incubated at 25±2 °C with 16/8 h D/N at 40 µmol m s photon flux density (cool white fluorescent light).The approximate humidity was about 60 to 70 percent. Plants were fed by Hogland solution. After 45 days morphological parameters were recorded for each treatment and the plant materials were frozen in liquid nitrogen and were stored at -80 °C for subsequent analysis.

Growth Parameters: Shoot length (cm) of plants from each treatment was measured. For dry weight determination samples were oven dried at 70°C for 48 h and then weighed.

Chlorophylls measurement

Photosynthetic pigments were determined according to the method of Lichtenthaler. Leaves were grinded well in 80% acetone. After straitening, the absorptions were measured in 663.2 and 646.8 nm and 80% acetone was used as control solution for setting the spectrophotometer's light absorption. Chlorophyll a, chlorophyll b and total chlorophyll, were calculated using the following formulas:

$$\text{Chl a} = 12.25\ A(663.2) - 2.79\ A(646.8)$$

$$\text{Chl b} = 21.51\ A(646.8) - 5.1\ A(663.2)$$

$$\text{(Total chlorophyll) chl T} = \text{chl a} + \text{chl b}$$

Content of photosynthetic pigments was expressed as (mg g^{-1} F.W.)

Protein content

For measuring the total protein density, Bradford method was used (Bradford, 1976).

In order to provide protein extraction, 0.5 g fresh leaf was homogenized in 5 ml of 500 ml potassium phosphate buffer (ph=7.5) containing 1% poly vinyl pyrodedyn (pvp) and 1 ml EDTA. All the extraction stages were performed in ice. The extractions were then centrifuged for 20 minutes in 2000 g and 4 centigrade temperature. In order for measuring the total protein density, 100 μl of the extracted solution was poured into a laboratory tube and 5 ml Bradford or Biorre was added to it, so the solution changed to blue. After at least 25 minutes of the beginning of the experiment, light absorption of each tube in 595 nanometer wave length was measured in contrast to the controlled sample. The controlled sample in this experiment was made by adding 100 ml extraction buffer (potassium phosphate buffer) to 5 ml-liter Bradford. The extracting proteins' concentrations were measured by standard absorption curve.

Sugars content

In the current experiment the presence of reducing sugars in the solution causes Cu $^{2+}$ reduced to Cu $_2$O. Cu $_2$O then reduces the existing phosphomolybdic acid in the experimental environment, which produces a blue color. The intensity of the produced color in the solution is measurable by spectrophotometer. 0.01 g dry leaf and root was homogenized with distilled water. Then a mixture of the prepared extractions with 2 ml solphate copper solution in experimental tube was heated for 8 minutes in a warm bath in 10 °C. After cooling, 2 ml phosphomolybdic acid was added. Absorption was determined in 600 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).

Results

Shoot fresh weight in plants treated with znCl2at all concentrations significantly reduced compared to the control plants (P= 0.05) (Fig. 1). The fresh weight of aerial parts in plants under lead treatment was also decreased, which was significant in all concentration comparing to the control. (P= 0.05) (Fig. 2).

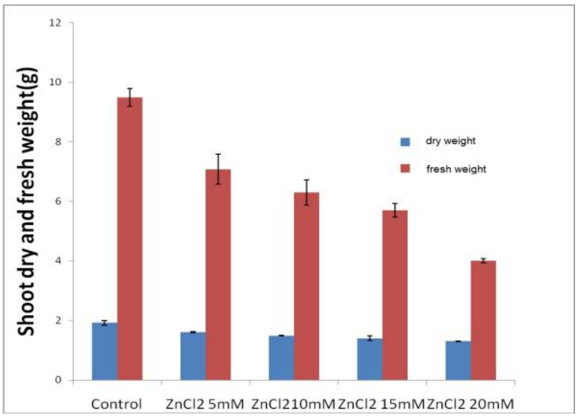

Fig 1. Effect of different concentrations of $ZnCl_2$ on the fresh and dry weight of soybean.

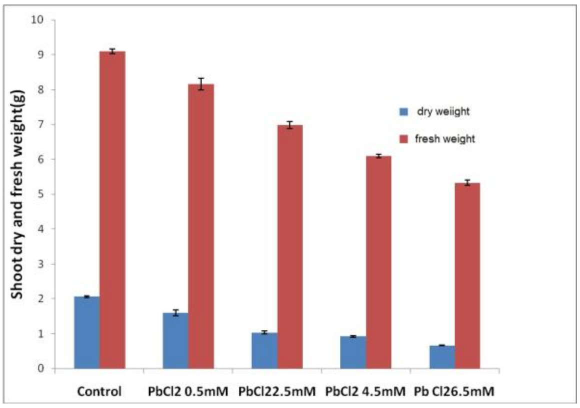

Fig 2. Effect of different concentrations of $PbCl_2$ on the fresh and dry weight of Soybean.

As it is shown in Fig. 3. The fresh root weight in plants under zinc treatment was reduced significantly (P=0.05) in all concentrations comparing to the control plant. The dry root weight in plants under zinc treatment was also decreased, however, the decrease was not significant (P=0.05) in 0.5 mM. But in other concentrations the decrease was significant (Fig. 3).

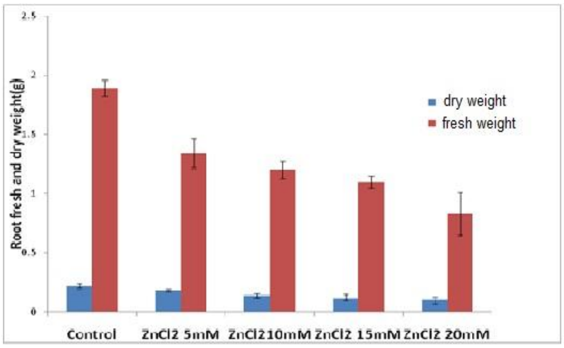

Fig 3. Effect of different concentrations of $znCl_2$on the fresh and dry weight of the root in Soybean plant

The fresh root weight in plants under lead treatment was also reduced, however it was not significant in 0.5 mM, comparing with the control (P=0.05). But the decrease was significant in other concentrations. The dry root weight was also decreased in all concentrations in plants under lead treatment comparing the control. (Fig. 4).

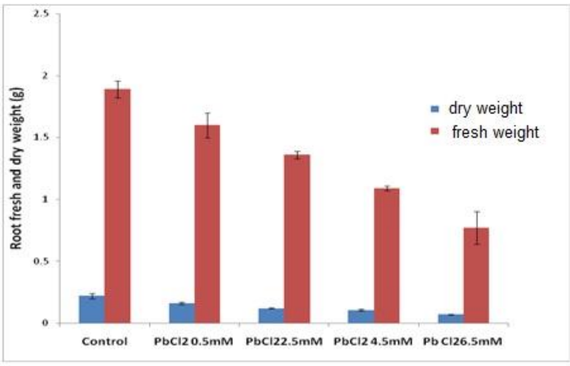

Fig 4. Effect of different concentrations of $PbCl_2$ on the fresh and dry weight of the root in Soybean plant

Results indicated that different concentrations of zinc significantly reduced seeding dry weight (Fig. 5).

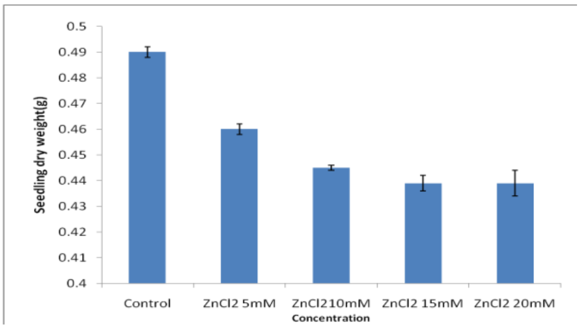

Fig 5. Effect of different concentrations of $ZnCl_2$on seedling dry weight in Soybean plant

The seedling dry weight under lead treatment was also reduced, however the reduction was not significant in 0.5 mM, comparing with the control (P=0.05) (Fig. 6).

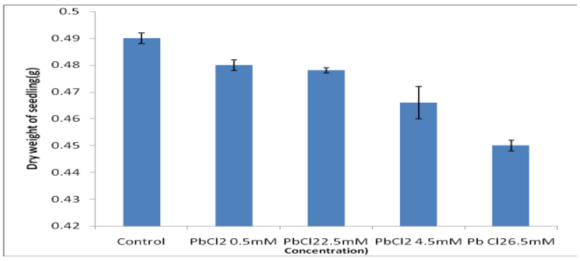

Fig 6. Effect of different concentrations of $PbCl_2$on seedling dry weight in Soybean plant

The chlorophyll a, b content was significant reduced under zinc treatment. The total chlorophyll content was reduced also (Fig. 7). lead treatment significantly decreased chlorophyll content in all concentrations. (Fig. 8). The total chlorophyll content in control plants was 3.13 ml g^{-1} Fw. After application of lead and zinc chlorites total chlorophyll content reduced to 0.51 and 0.89 respectively.

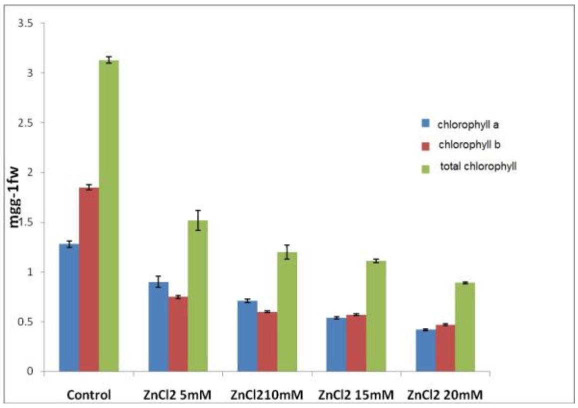

Fig 7. Effect of different concentrations of znCl$_2$on contents of chlorophyll a, b and total, in Soybean plant

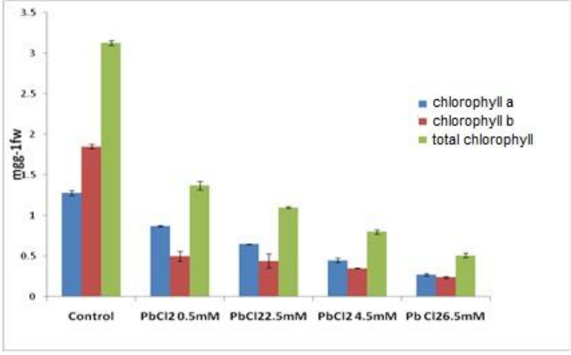

Fig 8. Effect of different concentrations of PbCl$_2$on contents of chlorophyll a, b and total, in Soybean plant

Results obtained from the effect of various concentrations of zinc and lead on sugar content was shown in Fig. s 9 and 10. The sugar content significantly increased under zinc treatment in all concentrations. The sugar content increased in plants under lead treatment also, however was not statistically significant in 0.5 mM comparing the control plants (alpha=0.05).

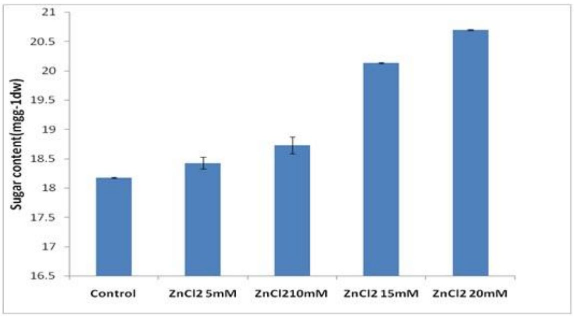

Fig 9. Effect of different concentrations of znCl$_2$on the sugar content in Soybean plant

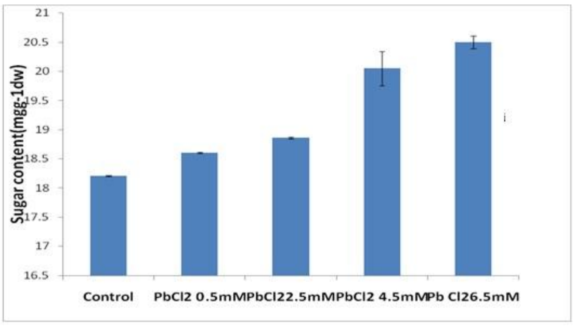

Fig 10. Effect of different concentrations of PbCl$_2$ on the sugar content in Soybean plant

Results of the effect of different concentrations of Zn and Pb on the protein content were shown in Fig. 11 and 12. The protein content significantly increased in plants under zinc and lead treatment. The increase under Zn treatment was higher than the Pb. The protein content in controlled plants was changed from 316.9 mg^{-1} Fw to 186.3 and 186.8 mg^{-1} Fw in lead and zinc treatments respectively.

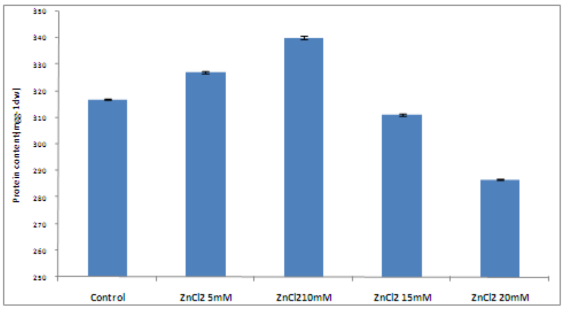

Fig 11. Effect of different concentrations of znCl2on the protein content in Soybean plant

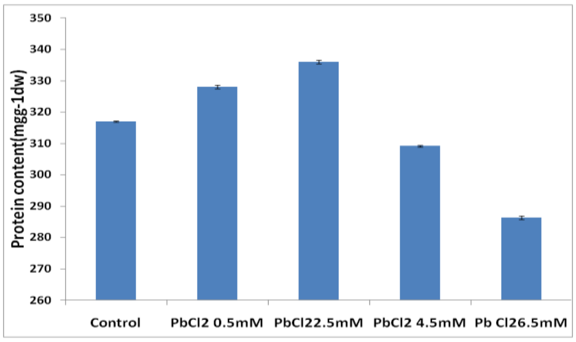

Fig 12. Effect of different concentrations of lead chloride on the protein content in Soybean plant

Discussion

The results found in John's et al (2009) experiment on canola confirmed the decrease in the plant's growth due to the tension of lead intoxication. The decrease in the plant's growth was caused by reduction of water potential, prevention of nutrient absorption and secondary stress such as oxidatives stress (eun, 2000). Studies have shown that in less thicker parts of the cell wall such as the plsmodesmata, large amounts of lead accumulate. the accumulated lead on the cell wall cause considerable reduction in cell wall elasticity, and this fact might inhibit cell growth and consequently Suppression of plant growth (ruley, 2006). The reduced growth is due to the effects of heavy metals on the metabolism and transport of auxin (israr, 2008).

In the current study, the fresh and dry weight decreased as a result of lead toxicity and inhibition of growth. The toxicity effects of lead on the biomass in other plants have been reported. However, in corn, the increase of dry weight as a result of increase in cell wall polysaccharide synthesis due to lead toxicity has been reported in contrast to this study result.

Inhibition of cell growth in the elongation phase and irreversible inhibition of proton pump's activity due to lead toxicity, are the factors responsible for reduction of plant's growth. (john, 2009). The decrease of root and shoot growth has also been mentioned in seyyedi's experiment. In some rare cases the increase of plant biomass due to metal contamination is reported in some studies. But these reports have been the results of very low concentration of lead and other heavy metals (john, 2009).

The results found by Jiang (2010) regarding the roast seeds of the *luffa cylindrical* L. showed that up to 100 mM lead, no significant change in the seedling fresh weight was seen compared with the control. However in upper concentrations (200 mM & 300 mM) the growth of root and shoot was decreased.

Studies done by Stoyanova & Doncheva on pea plant showed that low concentrations of zinc increase the nitrogen amount in the root and shoot, and thereby increased shoot growth (Stoyanova, 2002). Despite the important role of zinc in the structure and operation of the plant metabolic processes, the high accumulation of this element in soil and plants, just like any other heavy metals would cause incidence of stress symptoms in plants. According to many reports, high rates of zinc metal can hinder many metabolic activities in plants (Rout, 2003).

Murakami et al (2009) findings on corn and Soybean plant under zinc and lead treatments confirmed the decrease of plant's root and stem's fresh weights. In this study the decrease of growth in corn was more evident than Soybean plant. The decrease of all growth parameters found by Stoyanova (2002) on the pea plant was also consistent with the results of this study. Chlorosis and growth inhibition in the leaves as a result of lead toxicity have also been reported for Daturea (Vaillant, 2005), Canola (Parasad, 1999) and mustard plants (Wang, 2009).

In our study, increased concentrations of lead and zinc, resulting in decreased chlorophyll content in soybean which is consistent to (John et al, 2009), (Jiang, 2010), (Prassad, 1999) and (Wang et al, 2009) results, however standing in contrary to Zare et al (2007) 's findings. Chlorophyll content is a sensitive parameter to heavy metal toxicity. In Soybean plant, the chlorophyll content was reduced considerably under the stress induced by lead and zinc. It is proven that intensive effects of heavy metals on photosynthesis are in multiple stages, one of which is defection in chlorophyll's biosynthesis. Based on john et al reports the decrease of chlorophyll content in plants under lead stress is resulted by the inactivities of essential enzymes such as aminolevolenic dehydrates acid and protochlorofiled reductase which are important for chlorophyll biosynthesis. The decrease of chlorophyll content in aquatic plants under lead stress has also been reported by Jiang (2010) *luffa cylindrical* L. The significant decrease in chlorophyll up to 36% is reported, which is consistent to this study's findings.

As reported by Prassad (1999) key enzymes responsible for chlorophyll biosynthesis such as aminolevolenic dehydrates inactivate under lead stress.

Lead inhibits chlorophyll synthesis by destructing plant's absorption of essential elements like Mg and Fe. Lead can destroy the photo synthesis apparatus by binding to N and S ligands of protein. The increase of chlorophyll decomposition as a result of increased chlorophyllas activity due to lead toxicity is another reason of reduced chlorophyll contents under lead treatments (Sharma, 2005).

Zare et al (2007) results showed that 5 and 10 mM lead can cause a slightly increased on chlorophyll and carotenoid content. Increase of chlorophyll content in the presence of Zn can be due to the functional role of this metal on activation of protein synthetase of the chlorophyll biosynthesis pathway, and also some anti oxidant enzymes like Ascrobat peroxide and Glutathione reductase that protect chlorophyll from destruction by active oxygen radicals.(zare, 2007).

Findings of wang et al (2009) indicated extra zinc (1.1 mM) can significantly decrease the chlorophyll content. Recent reports have indicated that Zn through disarrangement of chloroplast , reducing the number of granas and tylakoids and substituting of magnesium by zinc in tylakoid membrane may decrease the chlorophyll content (jiang, 2010). Findings of artetxe et al (2002) also confirm these results.

Zinc in toxic concentrations may reduce leaf nitrogen content by influencing the main enzymes involved in ammonium assimilation, nitrate reductas enzyme, and since chlorophyll synthesis is dependent on nitrogen resources, by nitrogen assimilation decrease due to zinc toxicity, chlorophyll content would also decrease (el-ghamery, 2003).

According to our study, zinc and lead had a significant effect on increasing the sugar content in Soybean plant. Lead by reducing water transport to leaves and the disruption of leaf transpiration rate, makes ultra structural changes to cell and also changes in key enzymes activation in several metabolic pathways such as sugar metabolism. By decreased water transfer to the leaves and as a result of the lead accumulation in cell, the sugar content will decrease (Dwived, 2005).

The significant increase of the soluble sugar in the root might be due to the accumulation of sugars as a result of starch degradation. In the early stages of growth of seedlings, starch degradation due to amylase activity led to the consumption of the starch of the grain in the root and shoot. As the current study shows, increase in soluble sugar is a tolerance mechanism against stress and actually adjust water potential in the cytosol to deal with a high concentration of absorbed and accumulated ions in the vacuole (Dwived, 2005). The increase of invertase and sucrose synthase enzymes activity (which are the two enzymes involved in breaking sucrose into reducing sugars in the cytosol and vacuole) causes to decrease the non-reducing sugar and sucrose extent available for cells. Sucrose synthase is an enzyme which catalyzes sucrose breaking within the cell wall and vacuole. The increase of theses enzymes' activity and the accumulation of reducing sugars in stress conditions are probably important in Intracellular osmotic adjustment and protection of biological membranes and molecules (Dwived, 2005). It has been observed that increase of soluble sugar in shoot is not as much as the root. Less variation in shoot soluble sugars changes also indicate that lead toxicity in roots is greater than shoots because of the less accumulation of lead in shoot. The carbohydrates content in plants treated with $ZnCl_2$ increased. This increase is likely due to induced stomatal closure, damage to chloroplast structure, decrease of pigment concentration, enzyme disorders, imbalance in water relations and severe damage to the photosynthetic apparatus (Parassad, 1999). The change in carbohydrates content is one of plant's reactions to environmental stress like zinc metal. It is made clear that following reduced water transport to the leaves and after zinc's assembly on the cell, such rates would increase in the leaves. This is, in fact an adaptive mechanism for the maintenance and protection of the osmotic potential under zinc stress (Dwived, 2005). In this study, by increase in lead concentration in the nutrient solution, the protein content increased significantly up to 2.5 mM lead comparing with the control plant, but higher than that, up to 6.5 mM, protein content decreased.

The increase of total protein in heavy metal stress has been previously reported by john et al., (2009) that is consistent to current study results. In the first stage, stress induces various proteins including anti oxidant enzymes, but in later stages or in higher concentrations of lead, protein content decreases due to proteins degradation in stress condition. On the other hand, some other proteins such as membrane and cell wall proteins would decrease as a result of lead's leakage on the cell wall and damage to ionic channels of the membranes. It is possible that heavy metals induced lipid proxidation and breaking of proteins into pieces as a result of active oxygen radicals. toxicity causes a decrease in the protein content (John et al, 2009). The reduced protein content in Singh and Sinha's study on the *B.Juncea* has been reported, which is in contrary to this study's results (Singh & Sinha, 2005).

Some researchers believe that reducing the amount of leaf protein in high concentrations of lead is a result of the inhibitory activity of nitrate reductase (Singh et al, 1997). Another reason for reducing the amount of leaf protein could be due to the decrease of zinc and magnesium absorption in the presence of lead.

In this study, up to 10 mM Zn, the protein content first goes high significantly and after that goes down significantly. Scientists believe that in response to the Zn stress, stress proteins from the chaperon family and small hsp proteins go synthesized and increase the total protein content. Heat shock proteins are induced in order to compensate for the proteins degredations by lead stress (Piechalak, 2002). It is probable that phytochelatin synthesis be also one of the other reasons for increase of total protein content under lead and zinc stress (Piechalak, 2002).

As a co-factor of RNA polymerase enzyme, zinc has an essential role in protein synthesis. The main effect of Zn is increase in metabolism of proteins and the function and stability of the genetic molecules. The exposure of the plant to heat, salinity, drought and heavy metal stress induce synthesis Heat shock proteins (HSPS), which are called Stress proteins (Rion, 2004). The study of root cells exposed to zinc stress has been shown destruction of the cytoplasm structure and most organelles, this led to the synthesis of some proteins are more resistant to heavy metals in plant (Stresty, 1999). The increase of the total protein of the plant as a reaction against zinc might play an important protective role for the plant by preventing damage to the cell membrane proteins. The tiny decrease of protein amounts in higher concentrations of zinc ion can be due to decrease in some proteins' synthesis or due to the increased activity of some proteolytic enzymes induced by the oxidative stress in the plants. The active radicals induced by biotic and abiotic stress may also invade the proteins and cause some changes in amino acids or might cause fragmentation of a peptide chain and assembly and cross-connections between protein components, and alters the electrical charge and increase the sensitivity to proteolysis enzymes (Zare, 2007).

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