# The Effect of Zinc excess on antioxidant enzymes, proline and soluble carbohydrates in *Plantago major* L.

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## Abstract

The increase of environmental pollutants especially heavy metals derives from human industrial communities. Zinc is one of the natural elements that exist in biotic environment, plants body, and animals in different forms. Zinc has toxic and lethal effects in high concentrations on plants. Also some plants are introduced as accumulators of this element. Therefore, an investigation was carried out to consider different concentrations of Zn on *Plantago major* in completely random form and with three replications. The $ZnSO_4.7H_2O$ was included at 0, 50, 100, 250, 500, and 700 µmolar concentrations that were applied to the plants during 8 days. The results indicated that catalase enzyme activity increased significantly in comparison with control in root and shoot except at 50 µmolar concentration. Ascorbate peroxidase enzyme activity increased significantly only at 50 and 500 µmolar concentrations in comparison with control in shoots but it increased significantly in root with increasing metal concentration. Peroxidase enzyme activity increased significantly only at 50 and 700 µmolar concentration in shoot; however, this enzyme increased significantly in root with increasing metal concentration except at 500 µmolar concentration. The amount of proline increased significantly with increasing of zinc concentration. Soluble carbohydrates decreased significantly in comparison with control in root and aerial organs except at 700 µmolar concentration. These results derive from plant resistance against high concentrations of Zn.

*Keywords:* Ascorbate peroxidase; carbohydrate; catalase, peroxidase; *Plantago major*

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## Introduction

Medicinal plants are important economical plants that are used in traditional and modern therapy. Heavy metal stress is one of the environmental stress factors that causes plant physiological injuries, nutrition problems, and metabolic process destruction (Levitt, 1980). The whole parts of plants growing in the media containing high concentrations of heavy metals are negatively affected. So it is urgent to take advantages of accurate planning, selecting or reforming resistant plants against metals stress.

Zinc is one of the necessary elements that are important for protecting membranes and structures and it is important for natural plant growth, protecting them against viruses (Buksh et al., 2007). Zinc deficiency causes severe reduction in plants products (Kosesakal and Unal, 2009).

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Some investigations indicated that the amount of zinc metal tolerance is different in different plants and maize physiological response to sulphur elements caused reduction in catalase enzyme activity and increase in peroxidase enzyme (Cui and Wang, 2006). Studies on the effects of cadmium and zinc on plants indicated that cadmium increases antioxidant enzymes but zinc alleviates these changes (Erdei et al., 2007). Odjego and Fasidi (2007) reported that heavy metals activity increases catalase and peroxidase enzyme activity in both *Eichhorniacrassipes* and *Pista stratiotes*. The effect of different concentrations of Cu indicated that Cu at high concentrations increases catalase and peroxidase enzyme activity in *Allium sativum* (Meng et al., 2007). Lin and Kao (2005) showed that NiSO4 increased ascorbate peroxidase but it did not show any effect on catalase and superoxide dismutase activity. Saxena and Arfeen (2009) indicated that the amount of carbohydrates decreased with increasing metal concentration in mosses plant. Studies by Amal and Saleh (2002) indicated that root and shoot carbohydrates reduced with increasing Ni concentration. Recent studies showed that heavy metals stress by Cd and Pb increase proline at low concentrations of Cd and Pb but decrease proline at high Cd and Pb concentrations (John et al., 2009). Gradual increase of proline in heavy metal stress was reported in bean plants, wheat seedling, and rice plants (**Berg** et al., 2013). The effect of heavy metal stress like Cu, Pb, Cd, and Hg on bean indicated that proline decreased with increasing heavy metal concentration (Zengin and Munzurogl , 2005).

The study is aimed at evaluating the effect of zinc stress on plant growth and physiology and also an analysis of the activity of antioxidant enzymes, proline, and soluble carbohydrates in *Plantago major*.

## Material and Method

Plantago *major* seeds were provided from Isfahan Research Centre 3 times more than it was needed and cultured in perlite puts 2 centimetres deep at equal distances. These seeds were irrigated by distilled water for 10 days. After germination, the seeds were nourished with Hoagland feeding solution for 15 days and the treatments were commenced after culturing for 25 days. Hoagland feeding solution at different Zn concentrations (0, 50, 100, 250, 500, and 700 µmolar) was then provided as sulphate.

### Protein Extraction

Protein was extracted by Benavidis et al., (2000) method. First, proteins were extracted from fresh leaf tissue in mortar and 5 ml Tris buffer HCl 0.05M added at 5.7 pH for 30 minutes and the tissue was ground with pestle in ice bath. Then the solution was transferred to centrifuge tube at 13000 rpm at 4° C. Protein extraction was used for studying peroxidase and catalase enzymes activity. Ascorbate peroxidase is vulnerable to the absence of ascorbate. So 0.2 ml ascorbate 0.5 µmolar was added to this extract.

### Ascorbate peroxidase enzyme activity assay

Ascorbate peroxidase was determined by Nakano and Asada (1981) method. After preparing protein extract for measuring peroxidase enzyme activity, these indicators were used: 2ml Acetate buffer 0.05M, 0.2 mlhydrogen peroxide 3% v/v, 0.2 ml ascorbate 50 µM, these materials were combined together in ice bath and 0.025 µL enzyme extract was added to this solution. The absorbance was measured spectrophotometrically at 290 nm. Enzyme activity was estimated based on $OD\min^{-1}g^{-1}FW$ .

### Catalase enzyme activity assay

Catalase was estimated by Kar and Mishra (1976) method. After preparing protein extraction for measuring catalase enzyme activity, these indicators were used: 2.5 ml Tris buffer 50 mM, 0.3 ml hydrogen peroxide 3% v/v, these materials were combined together in ice bath and 60 µL enzyme extract was added to the solution. The absorbance was measured spectrophotometrically at 240 nm. Catalase enzyme activity was calculated base on $OD\min^{-1}g^{-1}FW$ .

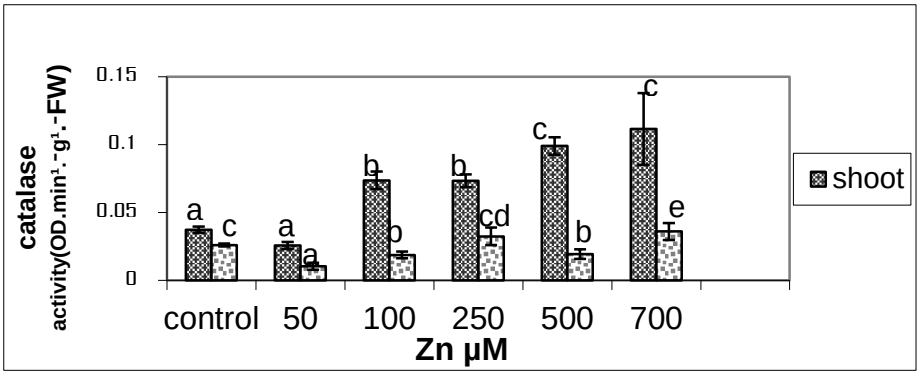


Fig. I. The catalase enzyme activity at different Zn concentrations in roots and aerial organs of *Plantago major*

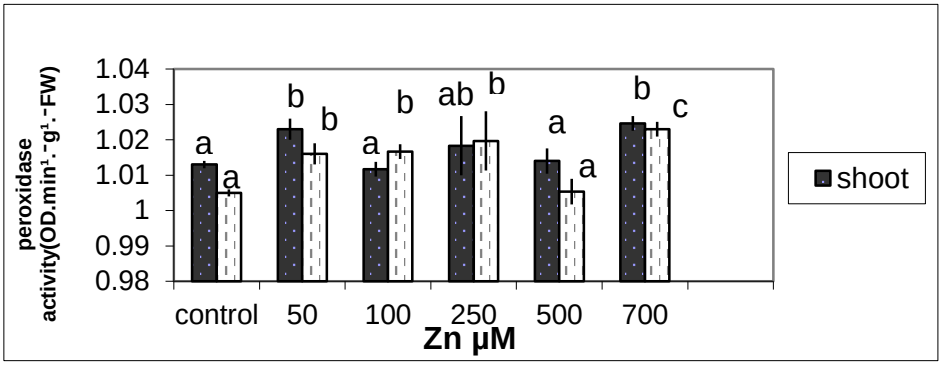


Fig. II. The peroxidase enzyme activity at different Zn concentrations in roots and aerial organs of *Plantago major*

## Peroxidase enzyme activity assay

Peroxidase enzyme activity was determined by Kar and Mishra (1976) methods. After preparing protein extract for measuring peroxidase enzyme activity, these indicators were used: 2 ml acetate buffer 0.2 M, 0.2 ml hydrogen peroxide 3% v/v, 0.2 ml banzydin 0.04 M in methanol solution 50%, then these substances were combined together in ice bath and 0.1 µL enzyme extraction was added to the solution. The absorbance was measured by VARIAN spectrophotometry device (CARY 100 model) at 530 nm against blank samples. Enzyme activity was estimated base on $OD\min^{-1}g^{-1}FW$.

## Soluble carbohydrates assay

The 0.1 g tissues were extracted from dry weight and 10 ml hot 70% ethanol was added. Then the extracts containing sugars were put in refrigerator for one week. The total soluble and reducing sugars were determined colorimetrically using the reactions with 1 ml phenol 0.5. Then 5 ml sulphuric acid was added to this solution (Kochert, 1987). The absorbance was measured spectrophotometrically at 485.

## Proline measurement

Proline was determined by Bates et al. (1973) method. 0.5 g fresh plant material was added to 10 ml sulfusalicylic acid solution 3%. The homogenate was filtered using Watman filter paper2. Then 2 ml of each was poured into test tube and 2 ml ninhydrin, reagent, and 2 ml ascetic acid was added. Then, all tubes were put in water bath at 100° C. Afterwards, 4 ml toluene was added. The top layer contained toluene and proline and the absorbance was measured spectrophotometrically at 520 nm. Proline content was calculated based on $mg.g.^{-1}FW$.

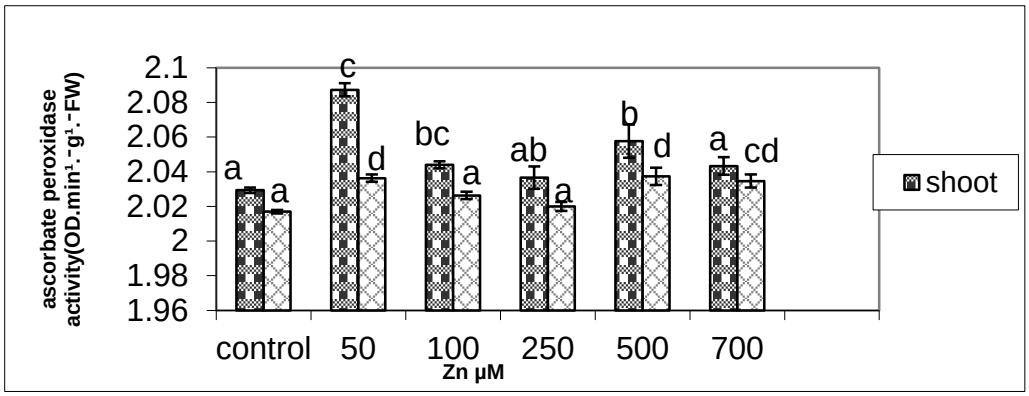


Fig. III. The ascorbate peroxidase enzyme activity at different Zn concentrations in roots and aerial organs of *Plantago major*

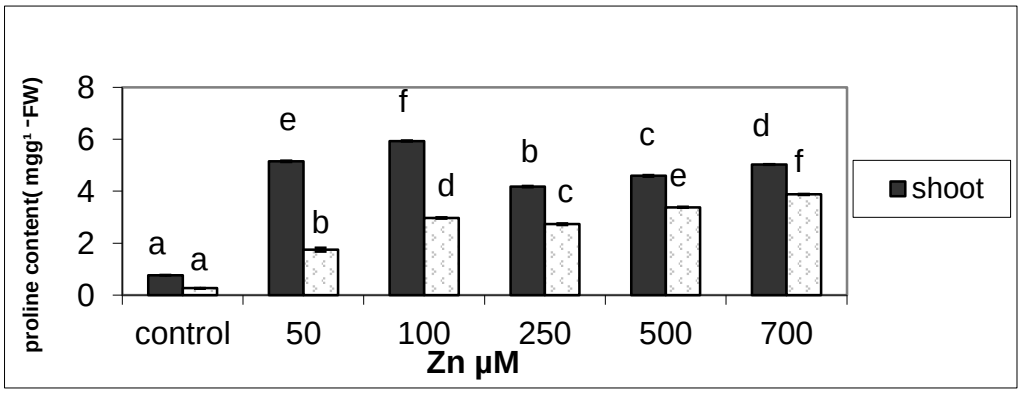


Fig. IV. The amount of proline at different Zn concentrations in roots and aerial organs of *Plantago major*

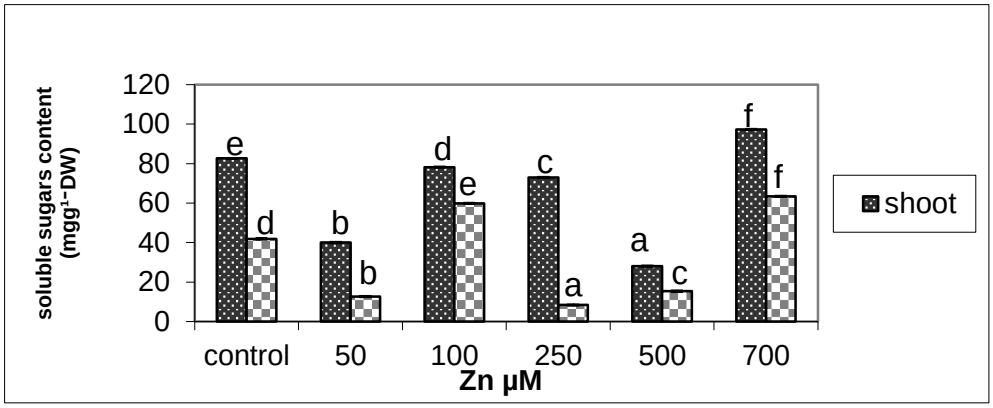


Fig. V. The amount of soluble sugar at different Zn concentrations in roots and aerial organs of *Plantago major*

## Results

The results indicated that in *Plantago major* different treatments of Zn result in significant difference in antioxidant enzymes activity in comparison with control. Catalase activity increased significantly with increasing Zn in plant nutrient environment in shoots and roots except at 50 µmolar concentrations in comparison with control and the highest level of activity was seen at 700 µmolar concentrations in roots and shoots (Fig. I). Peroxidase enzyme activity at 50 and 700 µmolar concentrations increased significantly in comparison with control but at other concentrations no difference in shoots was observed in comparison with control. The amount of peroxidase enzyme at 100 and 250 µmolar concentrations in roots increased in comparison with shoots (Fig II).

Ascorbate peroxidase enzyme did not show any difference in different treatments in comparison with control hoots except at 50 and 500 µmolar concentrations. The highest amount of ascorbate peroxidase was seen at 50 and 500 µmolar concentrations in roots (Fig. III). The amount of proline increased significantly with increasing Zn concentration in roots and shoots in different treatments. The highest amount of proline was seen at 50 and 100 µmolar in aerial organs and at 700 and 500 µmolar Zn concentrations in roots (Fig. IV). The amount of carbohydrates soluble decreased significantly in roots and aerial shoots in different treatments except at 700 µmolar Zn concentration. The highest amount of carbohydrates soluble was seen at 700 µmolar Zn concentration and the lowest was seen at 250 µmolar Zn concentration in shots (Fig. V).Totally the amount of carbohydrates increased in shoots in comparison with roots.

## Discussion

Totally, antioxidant enzymes activity increased with increasing of Zn concentrations although this increase was not significant at some concentrations and did not have any difference with control. At some concentrations some reduction is observed in catalase enzyme activity in roots. Meng et al. (2007) observed that catalase enzyme activity decreased with increasing metal concentrations in *Allium sativum* L. but peroxidase enzyme activity increased at beginning and then stopped. The study of Jaleel et al. (2009) showed that catalase enzyme activity decreased with increasing metal concentrations while peroxidase enzyme activity increased with increasing metal concentrations. For resisting against oxidative stress condition like high or low temperature degree, water deficiency, salinity, etc the plants should have antioxidant immune system (Jaleel et al., 2009b, 2008a). Plants have antioxidant enzymes like ascorbate peroxidase, catalase, metabolites, ascorbic acid, and carotenoids (Jaleel et al., 2008b). This antioxidant increased in environmental pressure (Jaleel et al., 2008c). Generally, heavy metals have less influence on catalase enzyme activity in comparison with other antioxidant enzymes activity (Lin and Kao, 2005). The increase of antioxidant enzymes under copper and cadmium stress in moss indicated that this increase is related to oxidative antioxidant in correspondence with peroxidase and free radicals increase in cells (Saxena and Arfeen, 2009). This increase could be a relative protection against abundant production of peroxide radicals at low heavy metals concentrations (Munne-Bosh and Alegre, 2002). Canadan and Tarhan (2003) explain that Zn is a wide spread pollutant that occupies plants and could be phototoxic. The amount of proline in *P. major* increased with increasing of Zn concentration in comparison with control in root and shoot. The proline accumulation could regulate cell level secretion and prevent enzyme in molecular structure fixation (John et al., 2009; Emamverdian et al., 2015). The reason for proline increase is *De novo* or injuries reduction or both of them (Dhinl et al., 2009). Odjegba and Fasidiin (2006) proved that the proline content increases quickly with increasing of each metal concentration. The increase of leaflet proline level probably act as an antioxidant in cells under metal stress condition and decreases free radicals and protects the environment (Siripornadulsil, 2002).

Soluble carbohydrate investigation in *P. major* indicated that soluble carbohydrate increased significantly at 700 µmolar concentration in aerial organs in comparison with other concentrations. Soluble carbohydrates are another group of organic solutions. A

carbohydrate content change is one of the important characteristics in stress time and has direct relation with physiological processes like photosynthesis, transduction and respiration. Carbohydrates metabolism is different in plants' stress responses and probably metals decrease carbohydrates synthesis (Subbaiah and Sachs, 2003). In spite of carbohydrates role in regulating osmotic pressure it is assumed that the increase of plant soluble carbohydrates supply could protect basic cell metabolism in the environment conditions (Verma and Dubey, 2001).

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