INT. J. BIOL. BIOTECH., 3 (2): 411-417, 2006.

EFFECT OF ZINC ON GROWTH, NUTRIENT COMPOSITION AND ANTIOXIDATIVE ENZYME ACTIVITIES OF MAIZE AS INFLUENCED BY PHOSPHORUS

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M. Aydin*, M. A. Demiral and M. A. Kaptan

Faculty of Agriculture, University of Adnan Menderes, 09100, Aydın-Turkey; E-mail:maydin@adu.edu.tr

ABSTRACT

Effect of zinc on growth, chemical composition and antioxidative enzyme activities of maize as influenced by different phosphorus rates were investigated. For this purpose, control and 3 rates of Zn (0.4, 2.0 and 10 mg Zn kg-1 soil) were applied to cv. Pioner-32K61 planted in pots by using Zn deficient soil under different P rates (50, 100 and 150 mg P kg-1). Phosphorus rates significantly increased P, K, Mg and protein contents and superoxide dismutase activity (SOD), but they decreased number of leaf per plant and Zn content. In contrast, increasing Zn doses significantly enhanced all growth parameters (plant height, number of leaf, dry matter yield, chlorophyll a and b, protein content of leaves), SOD activity and plant Zn content, but decreased other nutrient contents of plant and also catalase (CA) and peroxidase activities (PO). The correlation coefficients of SOD activity was affected significantly by either dry matter yield or plant Zn content. The level of plant Zn content plays an important role on growth, nutrient composition and antioxidative enzyme activities of maize. Besides, increasing PO and CA activities were not sufficient enough to increase dry matter yield under Zn deficient conditions.

Key-words: Zinc deficiency, dry matter, superoxide dismutase, peroxidase, catalase

INTRODUCTION

Zinc deficiency in soils has been reported worldwide. The major reason for the widespread occurrence of Zn deficiency in soils is low availability of Zn to plant roots rather than low Zn content in soils. High pH and high levels of $CaCO_3$, and low levels of organic matter, soil moisture, high level P applications, and soil factors predominantly responsible for low availability of Zn to plant roots (Cakmak and Marschner, 1987; Marschner, 1993; Marschner, 1995).

Zinc uptake drops when the P content of the nutritive medium increases. This is particularly critical if levels of available Zn are moderate to low. Phosphorus induced Zn deficiency occurs mainly due to the immobilization of Zn in the root owing to high P uptake (Bergman, 1992). Zinc plays an important role in several plant metabolic processes, it activates enzymes and involved in protein synthesis and in carbohydrate, nucleic acid and lipid metabolism. It forms complexes with DNA and RNA and affects stability of these compounds (Collins, 1981). These functional roles also affected by other macronutrients and micronutrients. Zinc deficiency may also affect uptake of other nutrients (Rahimi and Bussler, 1979, Bonnet *et al*., 2000, Cakmak, 2000). Accumulation of photosynthates leads to enhanced photoreduction of O_2 to reactive O_2 species (ROS) under P, K and Mg deficiency (Marschner, 1995; Tewari *et al*., 2004).

Reactive oxygen species (ROS) are produced in both unstressed and stressed cells. Under unstressed condition, the formation and removal of O2 are in balance. However, the defense system, when presented with increased ROS (O_2-, HO, O^1_2, H_2O_2) under formation in stress condition, can be overwhelmed. Reactive oxygen species can seriously disrupt normal metabolism through oxidative damage to cellular components. Within a cell, SOD constitutes the first line of defense against ROS. (Elstner, 1991; Alscher *et al.*, 2002). The decrease in SOD activity which takes place under Zn deficiency is particularly critical, as a simultaneous increase in rate of ROS generation occurs. One of the most damaging effects of ROS and their products in cells is the peroxidation of the membrane lipids. This process results in an increase of plasma membrane permeability, which lead to leakage of solutes and finally cause cell death (Chaoui *et al*., 1997). For their protection, plants possess a range of defense systems for oxygen radical detoxifying enzymes, such as CA and PO (Foyer *et al*., 1994; Arora *et al*., 2002).

The purpose of this study was to investigate the consequences of Zn application and P in pots filled with Zn deficient soil. The act of Zn and other nutrient uptakes was compared through a study of growth, nutrient composition and antioxidant enzyme activities**.**

*Corresponding author: Faculty of Agriculture, University of Adnan Menderes, 09100, Aydın-Turkey; E-mail:maydin@adu.edu.tr

MATERIALS AND METHODS

In the research, the most widespread second crop maize (*Zea mays* L.) cultivar in the region, Pioner-32K61, was used as test plant. Seeds were sown in January 6, 2004 in 12 liter pots filled with Zn deficient soil taken from top layer of local fields in Aydin, Turkey. Some soil characteristics of the soil follows: texture; loam, $CaCO_3$ 1.6%, pH (1:2.5 water) 7.97 and organic matter 1.15%DTPA-extractable Zn 0.32 mg kg-1, NHCO3-available P 7.65 mg kg-1. Three levels of P (20, 100 and 150 mg P kg-1) and 3 levels of Zn (0.4, 2.0 and 10.0 mg kg-1 soil as $ZnSO_4.7.H_2O$) besides control were applied together with a basal treatment of 200 mg N kg-1, 100 mg P kg-1, 100 mg kg-1 K and 100 mg kg-1 Ca as NH_4NO_3, CaH_4 (PO_4) $_2$, K_2SO_2 and $Ca(NO_3)_2$. All nutrients were mixed thoroughly with the soil before sowing. The pots were placed in greenhouse. Experimental design was randomized block with 2 factors and 4 replications, 3 plants per pot making a total 12 plants per replicates.

For nutrient element analyses, leaf samples were placed in paper bags and dried in a forced-air oven at 70°C for 72 hours. The dried leaf samples were then ground in a stainless steel Wiley mill. For the determination of P, K, Ca, Mg, Fe, Zn and Mn, the dried leaf samples were wet digested in a mixture of 4:1 nitric acid:perchloric acid. The concentrations of Ca, Mg, Fe, Zn and Mn in the digest were determined by atomic absorption spectrophotometry (Varian SpectrAA 220FS), K by flame photometry (Jenway PFP7) and P by spectrophotometry (Shimadzu UV-160A) (Kacar, 1972).

Third leaves from the top were taken at 26 DAS for chlorophyll and enzyme analyses. All operations (until enzyme determinations) were carried out at 0-4 °C. Concentrations of chlorophylls were determined in 80% acetone extract of the third leaf using the method of Lichtenthaler and Wellburn (1983). Color intensity of clear supernatant was measured at 663.2 and 646.8 nm for chlorophyll a and chlorophyll b respectively. Results have been expressed as mg chlorophyll g-1 fresh weight.

Superoxide dismutase (SOD;EC 1.15.1.1) activity determined by following method. Fresh leaf tissue (1 g) was homogenized with 5 ml 0.05 M Na phosphate buffer (pH 7.8) containing 1 mM EDTA Na_2 in presence of 0.2 g Dowex 1 x 8. After 20 min the extract was centrifuged at 13000 g for 40 min. Clear supernatant is used for measurement of both protein and enzyme activity. An aliquaot of 1 ml of enzyme extract was used for determination of protein content by using the method of Lowry *et al*. (1951). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium, adapting the method of Beauchamp and Fridovich (1971). One enzyme unit (IU) SOD is defined as that the amount of protein (in mg) causing a 50% inhibition of the photoreduction. Peroxidase (PO;EC 1.11.1.7) activity was determined following method described by Herzog and Fahimi (1973). One g of each sample was homogenized with 3 ml 0.5 m trisglycine buffer (pH 8.3) containing 17% saccharose in the presence of 0.2 g Dowex 1 x 8.

After 20 min the extract was centrifuged at 13000 g for 40 min. Clear supernatant is used for measurement of both protein and enzyme activity. Rate of formation of the oxidized 3.3'- diaminobenzidine tetrahydrochloride (DAB) was followed up 465 nm against a blanc using a self-recording spectrophometer (Shimeadzu 1601). The specific enzyme activity was expressed as enzyme units, IU, (extinction (E) per 1 minute and per mg protein).

Catalase (CA;EC 1.11.1.6) activity was determined by following method described by Bergmayer *et al.* (1974). Clear supernatant, held in SOD activity determination, is used. Decrease of the content of H2O2 resulting in decline of the extinction at the absorption maximum of H2O2, 240 nm was expressed in the enzyme unit (µM H2O2 destroyed per min per mg protein).

Plant growth was determined by measuring plant height at weekly. Another approach to determine plant growth was to measure accumulation of weight in plant organs. After sampling each sample weighted and dried at 65oC for 72 hours and weighted again. In addition leaf number per plant was counted weekly during the growing period. The obtained data are presented with the least significant difference (LSD 0.05) between treatments, derived from an analysis of variance (Littel and Hills, 1978).

RESULTS

Plant did not show any P deficiency symptoms during the experiment. However Zn deficiency symptoms and visual differences in growth were apparent within 30 days after seedling emergence. Then, all plant leaves were dried up in control pots while others having various necrosis. Deficiency symptoms were more apparent in higher P treatments. Plant height, dry matter and number of leaf per plant decreased (only significant, $P<0.01$) by P applications. On the other hand, Zn rates increased significantly ($P<0.01$) plant height, number of leaf per plant and dry matter yield (Table 1). Phosphorus-Zn interaction was significant ($P<0.05$) only in dry matter. Effect of Zn on the growth parameters was not significant over 2 mg Zn kg-1. Plant height, number of leaf per plant and dry matter yield was increased by Zn doses as 238.7%, 65.89% and 1190% respectively when compared to control treatment

(Table 1). Phosphorus, K, Ca, Mg, Fe, Zn, Mn and Cu contents of shoot at 30 DAS is influenced significantly (exception of Ca, Fe, Mn and Cu in P rates) by addition of P and Zn to the soil (Table 2). Phosphorus application enhanced all nutrient contents with the only exception of Zn. On the other hand, Zn application increased plant Zn content significantly ($P<0.01$). Zinc accumulation in shoot leads reduction in ion contents. Depressions of nutrient contents were in the order of P> Fe>Cu>Mn. At the starting Zn rate, Ca, Mg and Mn contents decreased, then they increased or leveled out (Table 2).

Table 1. Effect of Zn treatment on the plant height, the number of leaf per plant and dry matter yield per maize plant at 30 DAS.

Treatments	Plant height (cm)	Number of leaf	Dry matter (g plant-1)
Phosphorus level (mg P kg-1)			
50	38.56	5.88 a	12.31
100	39.31	5.19 b	11.03
150	42.31	5.07 b	11.02
Zinc level mg P kg-1)			
Control	15.67 c	3.83 c	1.30 c
0.4	40.33 b	5.50 b	13.89 b
2.0	51.17 a	5.83 ab	16.77 a
10	53.08 a	6.33 a	14.13 b
Phosphorus x zinc			
50 x 0	18.75 c	4.75 cd	1.82 d
50 x 0.4	43.00 b	6.50 a	17.47 a
50 x 2.0	48.25 ab	6.00 abc	17.77 a
50 x 10	44.25 b	6.25 ab	12.19 bc
100 x 0	15.50 c	3.75 de	1.16 d
100 x 0.4	37.25 b	5.00 bcd	10.78 c
100 x 2.0	47.00 ab	5.75 abc	15.17 abc
100 x 10	57.50 a	6.25 ab	17.00 ab
150 x 0	12.75 c	3.83 e	0.93 d
150 x 0.4	40.75 b	5.50 bcd	13.41 abc
150 x 2.0	58.25 a	5.83 abc	17.38 a
150 x 10	57.50 a	6.33 a	13.19 abc

Effect of increasing level of P and Zn rates on chlorophyll, protein contents and antioxidant enzyme activities (SOD, PO and CA) in 3rd leaf of plants at 30 DAS is shown in Table 3. These components were generally enhanced by P applications. However, only effect of P on SOD activity was statistically significant ($P<0.05$). On the other hand, P-Zn interaction was not significant ($P<0.05$) in any of these components. Increasing Zn rates raised chlorophyll a, chlorophyll b and protein content of maize leaf as 260%, 104% and 74%, respectively. This result suggests that chlorophyll b was more severely affected than chlorophyll a by Zn deficiency. Both total chlorophyll and protein content were increased sharply and then slowed down by Zn rates (Table 3). Activities of antioxidative enzymes were affected significantly by increasing P and Zn rates. Superoxide dismutase activity was enhanced by Zn rates while PO and CA activities decreased (Table 3). Compared to the control, SOD activity increased 78.2%, PO and CA activities decreased 47.5% and 67.4%, respectively.

Relationship between antioxidative enzyme activities and plant nutrient contents or dry matter yield of 30 day old maize in response to different P and Zn doses were explained by correlation coefficients in Table 4. Increasing Zn applications affects positively plant Zn content and plant dry matter yield. Hence, Among the correlation coefficients of nutrient contents and SOD activity were positive. While correlation coefficients of Zn and P, K, Fe, Mn and Cu were negative (Table 4).

Table 2. Effect of Zn treatment on P, K, Ca, Mg, Fe, Zn, Mn and Cu contents of shoot at 30 DAS in maize plant.

Treatments	Macro elements (%)		Trace elements (mg kg-1)					
	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Phosphorus level (mg P kg-1)								
50	0.318 b	4.68 b	0,917	0.572 b	293	70.9	25.33 a	9.3
100	0.378 ab	5.25 a	0,874	0.705 a	350	69.4	22.17 b	11.1
150	0.459 a	4.90 ab	0.985	0.647 a	334	71.8	20.67 b	11.3
Zinc level mg P kg-1)								
Control	0.826 a	6.27 a	1.32 a	0.72 a	579 a	108.3 a	6.3 d	17.6 a
0,4	0.303 b	4.73 b	0.70 b	0.58 b	242 b	54.0 b	10.1 c	8.5b
2	0.201 b	4.28 b	0.86 b	0.61 b	221 b	52.2 b	14.1 b	5.6 c
10	0.210 b	4.50 b	0.82 b	0.66 ab	154 b	52.0 b	60.4 a	6.3 bc
Phosphorus x zinc								
50 x 0	0.637 b	5.75 ab	1.405 a	0.740 a	558	107.3	7.37 ef	14.7
50 x 0.4	0.216 c	4.39 cd	0.599 d	0.438 c	216	57.4	10.51 def	7.8
50 x 2.0	0.201 c	4.28 cd	0.588 cd	0.466 bc	199	55.7	15.78 d	5.0
50 x 10	0.218 c	4.30 cd	0.781 cd	0.644 a	173	62.7	67.70 a	7.1
100 x 0	0.775 b	6.60 a	1.357 a	0.723 a	589	113	5.83 f	20.1
100 x 0.4	0.307 c	4.66 cd	0.599 d	0.694 a	208	47.2	9.11 ef	8.4
100 x 2.0	0.220 c	4.79 c	0.703 cd	0.685 a	299	52.4	14.57 d	5.6
100 x 10	0.210 c	4.95 bc	0.833 cd	0.719 a	160	42.3	39.20 b	5.9
150 x 0	1.067 a	6.45 a	1.200 ab	0.707 a	590	105	5.50 f	18.0
150 x 0.4	0.384 c	5.15 bc	0.912 bcd	0.598 ab	301	57.5	10.79 def	9.2
150 x 2.0	0.181 c	3.76 d	0.989 bc	0.673 a	165	48.6	12.02 de	6.1
150 x 10	0.203 c	4.24 cd	0.839 cd	0.610 a	129	51	54.39 c	6.0

Table 3. The effect of Zn doses on chlorophyll, protein concentrations and activities of SOD, PO and CA in 3rd leaf of maize at 30 DAS.

Treatments	Chlorophyll a (mg g-1)	Chlorophyll b (mg g-1)	Protein (mg ml-1)	SOD (IU)	PO (IU)	CA (IU)
Phosphorus level (mg P kg-1)						
50	1.191	0.408	9.633 a	77.1 c	10.86	15.80
100	1.115	0.466	7.708 b	104.5 b	10.32	13.10
150	1.459	0.471	8.792 ab	123.3 a	10.97	20.90
Zinc level mg P kg-1)						
Control	0.462 b	0.278 b	6.96 b	73.1 a	14.12 a	24.94 a
0.4	1.339 a	0.436 ab	8.30 b	93.4 b	10.41 b	16.41 b
2.0	1.527 a	0.483 ab	8.66 b	108.3 c	8.28 bc	9.97 b
10	1.692 a	0.569 a	10.90 a	130.3 d	7.40 c	8.06 b
Phosphorus x zinc						
50 x 0	0.593 bc	0.277	7.90 bc	56.2 f	13.94 ab	29.4 a
50 x 0.4	1.227 ab	0.397	9.00 abc	76.0 de	9.79 cd	9.9 bc
50 x 2.0	1.330 ab	0.423	10.00 ab	75.1 de	7.60 d	5.7 c
50 x 10	1.563 a	0.533	11.63 a	101.0 bc	8.13 d	9.9 bc
100 x 0	0.370 c	0.410	6.57 c	72.3 ef	12.95 abc	21.5 abc
100 x 0.4	1.090 abc	0.377	7.40 bc	91.0 cd	10.59 bcd	9.7 bc
100 x 2.0	1.377 ab	0.447	6.97 bc	113.8 b	9.00 cd	11.5 bc
100 x 10	1.623 a	0.550	9.90 ab	140.7. a	7.03 d	5.7 c
150 x 0	0.423 c	0.147	6.40 c	90.7 cd	15.46 a	23.9 ab
150 x 0.4	1.650 a	0.533	8.53 abc	113.3 b	10.86 bcd	29.6 a
150 x 2.0	1.873 a	0.580	9.00 abc	136.0 a	8.23 d	12.7 bc
150 x 10	1.890 a	0.623	11.23 a	149.2 a	7.08 d	8.6 bc

Table 4. Correlation coefficients between antioxidative enzyme activities and plant nutrient contents or dry matter yield of 30 day old maize in response to different P and Zn doses.

	SOD	PO	CA
P	-0.474	0.926**	0.714**
K	-0.473	0.850**	0.693**
Ca	-0.372	0.0668*	0.709**
Mg	0.136	0.395	0.370
Zn	0.570*	-0.650*	-0.526+
Mn	-0.649*	0.864**	0.714**
Fe	-0.617*	0.931**	0.783**
Cu	-0.554+	0.907**	0.723**
Dry matter	0.516+	-0.883**	-0.708**

+, * and ** = Significant at P<0.10, P<0.05 and P<0.01 levels, respectively.

DISCUSSION

Zinc deficiency caused to chlorosis on leaves, also reduced leaf size and number of leaf per plant, stunned and thin stems. Under severe Zn deficiency (control) older leaves show wilting and curling with extensive chlorosis and stunned growth. This visible effect described in details by some researchers (Bergmann, 1992; Hacisaliho_lu and Kochain, 2003). Severity of Zn deficiency may increase with P accumulation in soil (Bergman, 1992). Suppression in plant growth and reduced biomass production in plants may be subjected to Zn deficiency is attributed to decrease in photosynthesis, increased oxidative damage to cell compounds (Cakmak, 2000). The results obtained from the experiment reveals that chlorophyll and protein contents increased with Zn application rates. Reducing chlorophyll content might be explained by the role of Zn in protein synthesis and also ROS to be expected in Zn stressed plants. These might be evaluated as possible reasons of increasing plant height, number of leaf per plant and DM yield on maize under increasing Zn rates.

Phosphorus:Zn ratio of the plant has a major role influence on both Zn status and the absolute Zn content. Optimal ratio varied from 50 to 200, depending on species and variety (Rahimi and Bussler, 1975). In the present experiment P:Zn ratio ranges between 32 and 1940. Phosphorus:Zn ratio increased by P applications under same Zn rates. It was higher than 200 mg Zn kg-1 when the soil Zn applications were lower than 2 mg Zn kg-1. Zinc deficiency resulted in a high accumulation of P, K, Ca, Mg, Mn, Fe and Cu in shoot. It was maximum in high P accumulation. The higher P content in the leaf, the higher is the physiological requirement of Zn (Cakmak and Marschner, 1987). Excessive accumulation of Fe in Zn deficient plant can be responsible for the initiation of severe oxidative stress because they produce ROS by various cellular reactions (Halliwell and Gutteridge, 1984). On the other hand, activity of heme enzymes, CA and PO (ascorbate- dependent peroxidase or guaiacol dependent peroxidase), declines under Fe deficiency (Marschner, 1995).

The relationship between Zn rates and some growth components (plant height, number of leaf per plant, DM yield, chlorophyll and protein contents) may reflect deficiencies in growth and how severe the oxidative damage under Zn deficiency conditions (Kuldeep and Banerjee, 1986; Hacisalihoglu and Kochian, 2003). All these components were increased in a gradually decreasing rate by Zn applications.

Superoxide dismutase, PO and CA have protective roles in scavenging free radicals. However their activities may change with not only nutrient deficiencies but also plant age. The nutrient uptake is also affected by intercepted radiation and soil water conditions. Thus, it is really difficult to realize the behaviour of antioxidative enzyme activities. In this research SOD activities increased by Zn application. Similar findings were reported by several researchers (Wenzel and Mehlhorn, 1995; Chaoui *et al*., 1997; Candan and Tarhan, 2003). Because, by catalysing detoxification of ROS in driven cell damage, SODs are major component of the antioxidative defence system of plant cell (Bowler *et al*., 1994). Increases in SOD activity with Zn rates might not be sufficient enough to detoxify reactive oxygen species in the control and low level Zn rates.

Catalase and PO activities, H_2O_2 scavenging enzymes, decreased with Zn rates. In a long term stress exposure, similar to our experimental conditions, Zn inhibits CA activity in bean (Weckx *et al*., 1993). On the contrary, CA can take part in an efficient defense mechanism in the detoxification of H2O2 in chloroplasts. General decline in antioxidative enzyme activity in increased oxidative stress (H_2O_2 accumulation and lipid peroxidation) could be one of the reasons declining chlorophyll content in maize leaves (Prochazkova *et al*., 2001). This result might be supported by increasing leaf Fe content under Zn deficiency. Because of, CA, PO and heme enzymes, activities

increases with leaf Fe content (Machold, 1968). Peroxidase activity also increases with Cu content (Davies *et al*., 1978).

As a result, there were positive correlations ($r<0.01$) between dry matter yield and PO, CA activities. However, it does not mean that dry matter yield decreased due to high PO and CA activities. Under Zn deficient conditions, oxidative stress were partly owerwhelmed by increasing PO and CA activities. Due to lower Zn contents of plant tissues, P, Fe, Cu and Mn contents increased.. However increasing PO and CA activities were not sufficient enough to increase dry matter yield under Zn deficient conditions.

The results showed that excess P application has an adverse effect on Zn uptake. The level of plant Zn content, in deficient and sufficient ranges, plays an important role on growth, nutrient composition and antioxidative enzyme activities of maize. Besides, under Zn deficient conditions, antioxidative enzyme activities also depends on other nutrients content of shoot with different extents.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Scientific and Technical Research Council of Turkey and Research Fund of University of Adnan Menderes, Aydin, Turkey.

REFERENCES

Alscher, R.G., N. Erturk and L.S. Heat (2002). Role of superoxide dismutases (SODs) in controlling oxidative stres in plants. *J. Experimental Botany*, 53: 1331-1341.

Arora, A., R.K. Sairam and G.C. Srivastava (2002). Oxidative stress and antioxidative system in plants. *Current Science*, 82: 1227-1238.

Beauchamp C. and I. Fridovich (1971). Superoxide dismutese; Improved assays and applicable to acrylamide gels. *Anal. Biochem*, 44: 276-287.

Bergmann W. (1992). *Nutritional disorders of plants*. Gustav Fischer Verlag Jena.

Bergmayer, H. U., K. Gawch, and M. Grassl (1974). Enzymes as biochemical reagents. In: *Methods in enzyme analysis* (H.U. Bergmayer ed), pp 425-522. Acad. Press. New York.

Bonnet, M., O. Cameras and P. Veisseire (2000). Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. Cv Apollo). *J. Experimental Botany*, 51: 945-953.

Bowler, C. and M. Van Montagu, D. Inz (1994). superoxide dismutase in plants. *Critical Reviews in Plant Science*, 13 : 199-218.

Cakmak I. (2000). Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytologist*, 146: 185-205.

Cakmak, I. and H. Marschner (1987). Mechanism of phosphorus induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. *Physiologia Plantarum*, 70: 13-20.

Candan, N and L. Tarhan (2003). Changes in chlorophyll-carotenoid contents, antioxidant enzyme activities and lipid peroxidation levels in Zn-stressed *Menta pulegium*. *Turkish J. Chemistry*, 27: 21-30.

Chaoui, A., S. Mazhoudi, M. H. Ghorbal and E.E. Ferjani (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Science*, 127: 139-147.

Collins J.C. (1981). Zinc. In: *Effect of heavy metal pollution on plants* (N.W. Lepp ed). Vol 1. London: Applied Science publishers, 145-169.

Davies, K.L., P. Adams and G.W. Winsor (1978). Bud development and flowering of *Chrysanthemum morifolium* in relation to some enzyme activities and to the copper, iron and manganese status. *Commun. Soil. Sci. Plant Anal*., 9: 249-264.

Elstner E.F. (1991). Mechanisms of oxygen activation in different compartments of plant cells. *In*: *Active oxygen/oxidative stress and plant metabolism* (Pell. Ed. K.L. Steffen. Eds). Rockville, MD: American Society of Plant Physologists, 13-25.

Foyer, C.H., M.L. Lelandais and K.J. Kunert (1994). Photooxidative stress in plants. *Physiol. Plant*. 92: 696-717.

Hacisalihoglu G. and L. Kochian (2003). How do some plants tolerate low lewels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytologist*, 159: 341-350.

Halliwell, B and J.M.C. Gutteridge (1984). Oxigen toxicity, oxigen radicals, transition metals and disease. *Biochmical J*., 219: 1-14.

Herzog, V and H. Fahimi (1973). A new sensitive colorimetric assay for peroxidase using 3-3'- diaminobenz-idine as hydrogen donor. *Analytical Biochemistry*, 55: 554-562.

Kacar, B. (1972). Bitki ve Topragin Kimyasal Analizleri, II. Bitki Analizleri. (Analyses of Plant and Soil, II. Plant Anaysis). *Ankara Universitesi Ziraat Fakültesi Yayınları*. No. 453, Ankara Turkey.

Kuldeep, S. and N.K. Banerjee (1986). Growth and zinc content of maize (*Zea mays* L.) as related to soil-applied zinc. *Field Crops Research*, 13: 55-61.

Litchtenthaler, H. K. and A. R. Wellburn (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Transac*., 11: 591-592.

Littel, T.M. and F. J. Hills (1978). *Agricultural Experimentation Design and Analysis*. John Wiley and Sons Inc., New York.

Lowry, O.H., N.J.Rosebrough, A.L. Farr and R.L. Randal (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Machold, O., W. Meisel and H. Schnorr (1968). Bestimmung der bindungsformen des eisens in bl_ttern durch Mössbauer-spektrometric. *Naturwissenschaften*, 55: 499-500.

Marschner, H. (1993). Zinc uptake from soils. *In: Zinc soils and plants* (A.D. Robson ed), pp. 59-77. Kluwer Acedemic Publishers. Dordrecht.

Marschner, H. (1995). *Mineral nutrition of higher plants*, 2nd edn. London, UK. Acedemic Pres.

Pahlsson, A.M.B. (1989). Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Literature Review. Water, air and soil pollution*. 47: 287-319.

Prochazkova, D., R.K. Sairam, G.C. Srivastava and D.V. Singh (2001). Oxidative stress and antioxdant activity as the basis of senesence in maize leaves. *Plant Science*, 161: 765-771.

Rahimi, A. and W. Bussler (1975). Der Einflu__ Unterschiedlicher Zn-Gaben auf die Entwicklung von mais. *Lanw. Forsch*., 31/1:138-150.

Rahimi, A. and W. Bussler (1979). Die entwicklung und der Zn-, Fe- und P- Hehalt höherer pflanzen in abh_ngigkeit vom zinkangebot. *Zeitschrift für pflanzenernachrung und Bodenkunde*, 142: 15-27.

Tewari, R.K., P. Kumar, N. Tewari, S. Srivastava and P. N. Sharma (2004). Macronutrient deficiencies and differential antioxidant responses-influence on the activity and expression of superoxide dismutase in maize. *Plant Science*, 166: 687-694.

Weckx, J.and J. Vangronsveld, H. Clijsters (1993). Heavy metal induction of ethylene production and stress enzymes: I. Kinetix of the responses, In: *Cellular and Molecular aspects of the plant hormone ethylene* (J.C. Pech, A. Latach, C. Balagu Eds.). Kluwer, Dordrecht, pp. 238-239.

Wenzel, A.A and H. Mehlhorn (1995). Zinc deficiency enhances ozone toxicity in bush beans (Phaseolus vulgaris L. Cv. Saxa). *J. Experimental Botany*, 46: 867-872.

(*Accepted for publication April 2006*)