Oxidative stress and change in plant metabolism of maize (*Zea mays* L.) growing in contaminated soil with elemental sulfur and toxic effect of zinc

Y. Cui[1], N. Zhao[1,2]

[1]*College of Resources and Environment, Graduate University of Chinese Academy of Sciences, Beijing, P.R. China*
[2]*College of Resources and Environmental Science, Agriculture University of Hebei, Baoding, P.R. China*

ABSTRACT

Responses of the chlorophyll, antioxidant enzymes and lipid peroxidation in maize growing in soil with zinc (Zn) and elemental sulfur (S) were studied. The results showed that sulfur alleviated the high toxicity of Zn and increased the concentration of chlorophyll. The activities of superoxide dismutase (SOD) and peroxidase (POD) increased significantly and catalase (CAT) activity significantly decreased in plants treated with 1000 mg/kg Zn alone. Compared with the control, SOD and POD activity increased from 62.3 to 77.3 U/mg protein and 28 to 41 nmol/min/mg protein, respectively. However, CAT activity decreased from 1.96 to 1.48 μmol H_2O_2/min/mg protein. The concentration of malondialdehyde (MDA) in maize leaves significantly increased as the concentration of Zn increased. Moderate concentrations of S (32 and 160 mg/kg) alleviated the increase of both SOD and POD activity and the decrease of CAT activity that were observed under conditions of Zn stress. The greatest decrease of SOD and POD activity were 17% and 21% and both were observed in the treatment of 160 mg/kg S combined with 1000 mg/kg Zn. In this treatment, the greatest increase of CAT activity (11%) was also observed. The results suggest that moderate supplementation with S may ameliorate the toxicity caused by excess Zn and plays an important role in protecting plants from oxidative stress induced by excess Zn.

Keywords: detoxification of heavy metals; senescence; antioxidant enzymes; lipid peroxidation; glutathione; reactive oxygen species; damage membrane

Zinc (Zn) is an essential micronutrient required by plants for normal growth and development. Like other heavy metals, excess Zn invariably shows marked alterations in electron transport, membrane permeability and uptake and translocation of nutrient elements (Wang et al. 2009b). In recent years, several studies (Wang et al. 2009b) have demonstrated the effects of Zn (deficiency and excess) on the activity of many antioxidative enzymes and antioxidant contents in plants. Glutathione and ascorbate detoxify reactive oxygen species (ROS) by direct scavenging or by acting as cofactors in the enzymatic reactions in the ascorbate glutathione cycle. The oxidative stress is bound up with the increased metal accumulation in plant and decrease efficiency of the ascorbate glutathione cycle under the metal stress (Wang et al. 2009a). Exposure to toxic metals also induces plant to accumulate high amounts of proline (Štefl and Vašáková 1982). Increased accumulation of proline leads to the increase of glutamate kinase activity and creates a possibility for an increase in glutamic acid content due to the synthesis of glutathione and phytochelatines in plant cells (Pavlíková et al. 2007). An increase of free proline inhibits biosynthesis of its excessive amounts in plant under heavy metal excess, and this results in the preferred utilization of glutamate for metabolic route leading to phytochelatin synthesis (Pavlíková et al. 2008).

Supported by the National High Technology Research and Development Program of China, Program No. 2008AA06Z336, and by the Main Direction Program of Knowledge Innovation of Chinese Academy of Sciences, Project No. XMXX280724.

Sulfur (S) is usually taken up by plant roots as sulfates. The reduction of sulfates to S^{2-} results in their incorporation into cysteine and methionine (Leustek and Saito 1999). Plants contain a large variety of other organic sulfur compounds, which play important roles in heavy metal detoxification and/or metabolism in plants (Kotrba et al. 1999). However, there is little information about whether the increased sulfur reduced toxic effects of zinc in relation to oxidative stress and induction of leaf senescence. The objective of the present study was therefore to investigate the variations in chlorophyll concentration, antioxidant enzyme activity and lipid peroxidation of maize to elevated concentrations of elemental S and Zn in the soil.

MATERIAL AND METHODS

Soil. The top 20 cm of soil was collected from a profile in Qinghe, Beijing, China. The soil was air-dried, ground and sieved by passing through a 2-mm sieve. The physicochemical properties of the soil were as follows: soil type, hapli-ustic argosols, pH (in 0.01 mol/dm^3 $CaCl_2$) 7.25, organic matter content 12 g/kg, total Zn 68 mg/kg, sand (> 0.05 mm) 6.4%, silt (0.002–0.05 mm), 75.1% and clay (< 0.002 mm) 18.5%.

Experimental design and treatments. The experiments were carried out using elemental S and Zn ($Zn(NO_3)_2$). The S was added to the soil at concentrations of 0 (S0), 32 mg/kg (S1), 160 mg/kg (S2) and 640 mg/kg (S3), and Zn was added in solution at concentrations of 0, 250 and 1000 mg/kg. All of the S and Zn treatments were applied prior to planting. There were 12 treatments and each was replicated three times. Basal fertilizers were applied at concentrations of 120 mg N (NH_4NO_3), 80 mg P (KH_2PO_4) and 120 mg K/kg (KCl and KH_2PO_4) and were thoroughly mixed with the soil and treatments of S and Zn. Each pot received 1 kg of soil, which was allowed to equilibrate for a period of 14 days before the plant seeds were sown. Six maize (*Zea mays* L.) seeds were sown per pot, and the plants were thinned to leave three seedlings in each pot one week after germination. The experiment was carried out in a greenhouse with a 14 h (26°C)/10 h (13°C) day/night cycle. Soil water content was adjusted regularly to maintain a weight equivalent of about 60% of the water holding capacity, and the plants were grown for seven weeks.

Soil/plant Zn analysis. Soils were digested with a mixture of concentrated HNO_3: $HClO_4$: HF (3:1:1) and total Zn was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Optima-2000, Perkin-Elmer Co. Ltd., USA). The harvested plant samples were rinsed with deionized water, oven dried at 70°C for 48 h, ground with an agate mill and digested with a mixture of concentrated HNO_3: $HClO_4$: HF (3:1:1) before the total Zn content was analyzed as mentioned above.

Chlorophyll. The chlorophyll (a + b) concentration was determined in 96% ethanol extracts of 0.5 g fresh leaf tissue as described by Cui and Wang (2006).

Activities of antioxidant enzymes. Protein content was determined according to the method of Bradford (1976). Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977). Catalase (CAT, EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 mmol/cm) at 240 nm for 3 min (Cui and Wang 2006). Peroxidase (POD, EC 1.11.1.7) activity was determined as described by Cakmak and Marschner (1992).

Lipid peroxidation. The lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) using the method of Cui and Wang (2006).

Statistics. All of the data were subjected to ANOVA and are presented here as the mean ± SE of at least three independent experiments.

RESULTS AND DISCUSSION

Plant growth and zinc accumulation. Compared to untreated controls, the shoot yield was significantly decreased in maize treated with 1000 mg/kg Zn with or without S addition. However, no significant change was observed in plants treated with any of the tested concentrations of S and the same concentration of Zn (Table 1). Zn concentration in the shoots of maize significantly increased corresponding to the concentration of the Zn treatment. The concentration of Zn in maize increased significantly in all of the plants that were also treated with S (Table 2). Growth of maize was not inhibited by addition of 250 mg/kg Zn, which suggests that moderate supplementation with Zn does not cause damage to plants. Similar results were also reported by others (Andrade et al. 2009).

Chlorophyll concentration. The concentrations of chlorophyll increased in plants treated with 250 mg/kg Zn and decreased in plants treated with 1000 mg/kg Zn at each of the tested concentrations

Table 1. Shoot dry matter yield (g/pot) of maize at different concentrations of S and Zn

Zn rate (mg/kg)	S rate (mg/kg)			
	S0	S1	S2	S3
Zn0	2.79 ± 0.17	2.89 ± 0.22	3.06 ± 0.20	2.81 ± 0.08
Zn250	2.74 ± 0.06	3.06 ± 0.29	2.96 ± 0.13	2.85 ± 0.22
Zn1000	1.73 ± 0.19	1.72 ± 0.22	1.77 ± 0.15	1.63 ± 0.24
Analysis of variance				
Zn level (Zn)	$P < 0.001$			
Elemental sulfur (S)	ns			
Zn × S	ns			

of S (Table 3). As S concentrations increased, the chlorophyll concentrations also initially increased and then decreased at same rate as in plants treated with Zn alone. The decrease in the chlorophyll concentration in plants that were not supplemented with Zn can be attributed to zinc deficiency. Moderate supplementation of Zn can increase the concentration of chlorophyll in Zn-deficient plants (Wang et al. 2009a). However, treatment with 1000 mg/kg Zn resulted in a decrease in the concentration of chlorophyll. Similar results were also reported in other studies (Wang et al. 2009b). The addition of elemental S (32–640 mg/kg) alleviated the high toxicity of Zn and increased the concentration of chlorophyll. The varying results regarding chlorophyll concentration indicate that the concentration of plant chlorophyll depends on plant species, growth conditions and the level of metal and S in the soils.

Antioxidant enzyme activity. Without S addition, treatment of maize with 1000 mg/kg Zn significantly increased SOD activity compared with the control (Figure 1, Table 4). With 32 and 160 mg/kg S, the SOD activity decreased at each concentration of Zn. The greatest decrease (17%) was observed in plants treated with 160 mg/kg S combined with 1000 mg/kg Zn. POD activity increased significantly in all Zn treatments compared with controls (28 nmol/min/mg protein). The highest value was 41 nmol/min/mg protein and occurred in the 1000 mg Zn treatment. POD activity decreased in plants treated with 32 and 160 mg/kg S compared to treatment with Zn alone (Figure 1, Table 4). The greatest decrease (21%) was observed in plants treated with 160 mg/kg S combined with 1000 mg/kg Zn. In plants treated with 250 and 1000 mg/kg Zn, catalase (CAT) activity decreased from 1.96 μmol H_2O_2/min/mg protein to 1.89 and 1.48 μmol H_2O_2/min/mg protein, respectively. A significant decrease was observed in plants treated with 1000 mg/kg Zn compared with the control (Figure 1, Table 4). CAT activity increased with the addition of 32 and 160 mg/kg S. The greatest increase (11%) was observed in plants treated with 160 mg/kg S combined with 1000 mg/kg Zn. A significant increase was observed in plants treated with 160 mg/kg S and 250 mg/kg Zn compared with the control.

Depending on the metal prosthetic group, SOD can be divided into four types: Cu/Zn-SOD, Fe-SOD, Mn-SOD and Ni-SOD. In the present study, the increased SOD activity induced by excess Zn can be attributed to an increase of Cu/Zn-SOD activity. Similarly, other plant biochemical parameters also changed under the heavy metal stress. For example, abiotic stress caused by excess Cd at concentration 1.10^{-6} mol/L in a nutrition solution increased the

Table 2. Concentration of Zn (mg/kg) in maize at different concentrations of S and Zn

Zn rate (mg/kg)	S rate (mg/kg)			
	S0	S1	S2	S3
Zn0	29.7 ± 1.8	33.8 ± 1.9	36.3 ± 2.3	38.9 ± 1.3
Zn250	166.4 ± 17.5	189.7 ± 13.8	238.2 ± 8.4	297.5 ± 17.2
Zn1000	437.6 ± 26.6	459.6 ± 29.7	507.6 ± 36.3	605.1 ± 45.5
Analysis of variance				
Zn level (Zn)	$P < 0.001$			
Elemental sulfur (S)	$P < 0.001$			
Zn × S	$P = 0.03$			

Table 3. Total chlorophyll concentration (mg/g FW) of maize at different concentrations of S and Zn

Zn rate (mg/kg)	S rate (mg/kg)			
	S0	S1	S2	S3
Zn0	2.36 ± 0.10	2.41 ± 0.03	2.48 ± 0.04	2.45 ± 0.15
Zn250	2.38 ± 0.11	2.66 ± 0.17	2.75 ± 0.06	2.49 ± 0.06
Zn1000	2.08 ± 0.30	2.37 ± 0.11	2.51 ± 0.19	2.24 ± 0.20
Analysis of variance				
Zn level (Zn)	$P = 0.05$			
Elemental sulfur (S)	ns			
Zn × S	ns			

total polyphenol and flavonoid contents in barley roots (Dudjak et al. 2004, Lachman et al. 2005). The decrease in the level of maize SOD activity resulting from treatment with S can be ascribed to the protective effects of S, which alleviated oxidative stress caused by excess Zn. In the present study, an increase in POD activity was observed in maize leaves under Zn stress. Similarly, increased POD has been observed in other plants grown under conditions of Zn stress (Prasad et al. 1999). 32 and 160 mg/kg S alleviated the increase of POD activity, suggesting that S has protective effects in plant grown under excess Zn stress. However, application of 640 mg/kg S induced a significant increase in POD activity in plants treated with 1000 mg/kg Zn. The reduction of POD activity as a result of application of S was observed in plants grown under conditions of cadmium stress (Cui and Wang 2006). Therefore, POD activity appears to be dependent on the plant species, the metal, the elemental S used for the treatment and the intensity of the stress. A decrease in CAT activity in plants grown under excess Zn stress was observed in present study. A similar decline in CAT activity was reported in other plants grown under excess Zn (Andrade et al. 2009). The decrease in CAT activity observed in plant supplemented with excess

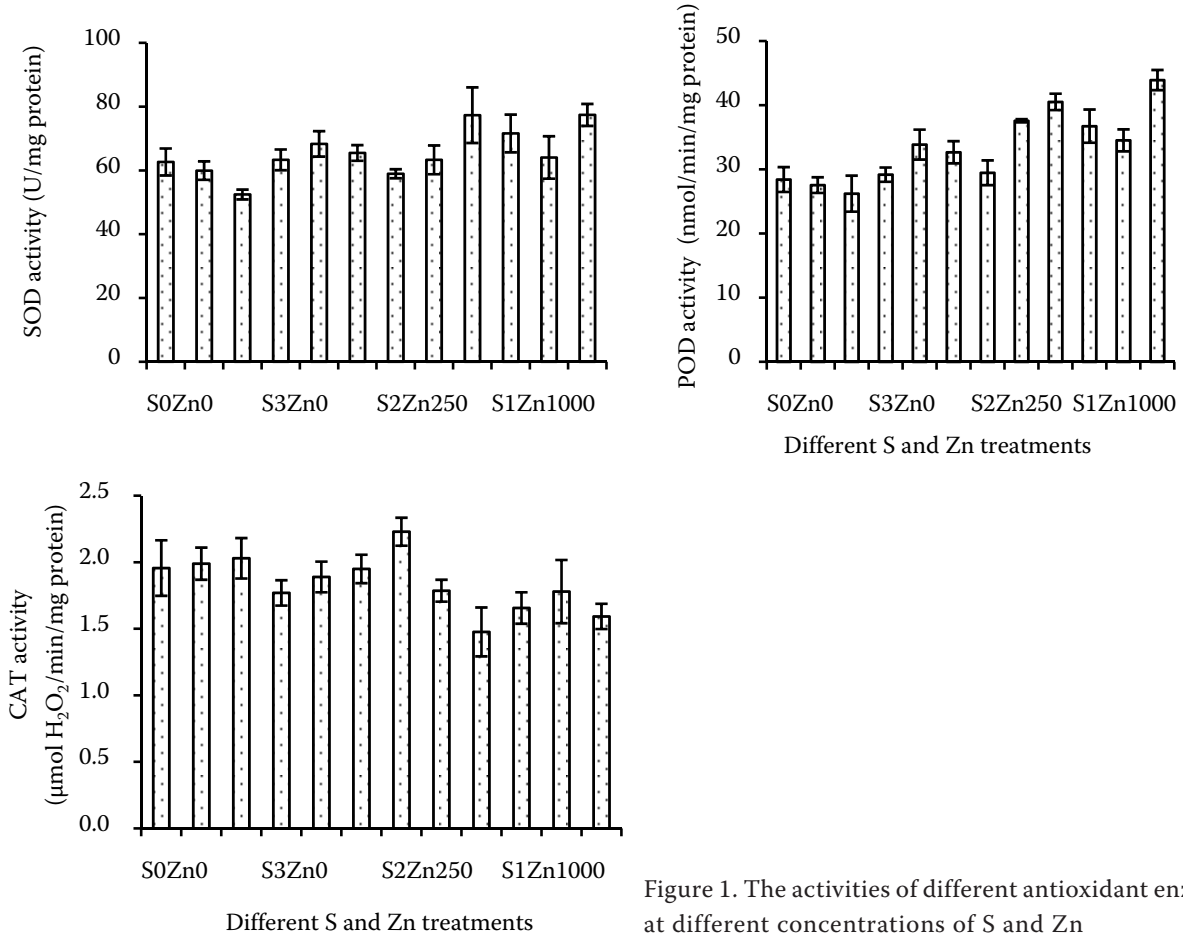

Figure 1. The activities of different antioxidant enzymes at different concentrations of S and Zn

Table 4. Analysis of variance of different antioxidant enzymes at different concentrations of S and Zn

Analysis of variance	Enzymes			
	POD	CAT	SOD	GR
Zn level (Zn)	$P < 0.001$	$P = 0.004$	$P = 0.03$	$P < 0.001$
Elemental sulfur (S)	$P = 0.001$	ns	$P = 0.002$	$P = 0.007$
Zn × S	ns	ns	ns	ns

Table 5. MDA concentration (nmol/g FW) of maize at different concentrations of S and Zn

Zn rate (mg/kg)	S rate (mg/kg)			
	S0	S1	S2	S3
Zn0	6.34 ± 0.86	5.50 ± 0.59	5.74 ± 0.77	6.78 ± 0.65
Zn250	8.60 ± 0.44	7.12 ± 0.90	5.95 ± 0.50	6.68 ± 0.53
Zn1000	11.40 ± 0.80	9.06 ± 0.89	8.36 ± 0.78	10.76 ± 0.87
Analysis of variance				
Zn level (Zn)	$P < 0.001$			
Elemental sulfur (S)	$P < 0.001$			
	$P = 0.03$			

Zn might be due to inhibition of enzyme synthesis or a change in the assembly of enzyme subunits (Radić et al. 2010). However, unlike our studies, increased CAT activity was observed in *Brassica juncea* grown under conditions of excess Zn (Prasad et al. 1999). These inconsistent results regarding CAT activity might be due to differences in the plant organs studied, the plant growth conditions, the durations and concentrations of the metals utilized and the plant species. Sulfur concentrations of 32 and 160 mg/kg prevented the decrease of CAT activity in plants treated with each of the tested concentrations of Zn. This suggests that moderate supplementation with S had a protective effect in maize.

Lipid peroxidation. The concentration of MDA in maize leaves was significantly increased as the concentration of Zn increased (Table 5). While. 32 and 160 mg/kg S were included in the treatment at each concentration of Zn, the concentration of MDA is also especially important and is characterized by a notable increase in the metabolism of ROS (Thompson et al. 1987). In the present study, the chlorophyll decrease, MDA increase and the change of antioxidant enzymes induced by excess Zn showed that the early senescence of maize may occurred.

In conclusion, even though Zn is an essential micronutrient for plants, higher Zn concentrations (1000 mg/kg) reduced maize growth and induced oxidative damage. Moderate S supplementation (32 and 160 mg/kg) played an important role in protecting plants from oxidative stress induced by excess Zn exposure.

REFERENCES

Andrade S.A., Gratão P.L., Schiavinato M.A., Silveira A.P., Azevedo R.A., Mazzafera P. (2009): Zn uptake, physiological response and stress attenuation in mycorrhizal jack bean growing in soil with increasing Zn concentrations. Chemosphere, *75*: 1363–1370.

Bradford M.A. (1976): Arapid and sensitive method for the quantification microgram quantities of protein utilizing the principle protein dye binding. Analytical Biochemistry, *72*: 248–254.

Cakmak I., Marschner H. (1992): Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiology, *98*: 1222–1227.

Cui Y.S., Wang Q.R. (2006): Physiological responses of maize to elemental sulfur and cadmium stress. Plant, Soil and Environment, *52*: 523–529.

Dudjak J., Lachman J., Miholová D., Kolihová D., Pivec V. (2004): Effect of cadmium on polyphenol content in young barley plants (*Hordeum vulgare* L.). Plant, Soil and Environment, *50*: 471–477.

Giannopolitis C.N., Ries S.K. (1977): Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology, *59*: 309–314.

Kotrba P., Macek T., Ruml T. (1999): Heavy metal-binding peptides and proteins in plants. A review. Collection of Czechoslovak Chemical Communications, *64*: 1057–1086.

Lachman J., Dudjak J., Miholová D., Kolihová D., Pivec V. (2005): Effect of cadmium on flavonoid content in young barley (*Hordeum sativum* L.) plants. Plant, Soil and Environment, *51*: 513–516.

Leustek T., Saito K. (1999): Sulfate transport and assimilation in plants. Plant Physiology, *120*: 637–643.

Pavlíková D., Pavlík M., Staszková L., Motyka V., Száková J., Tlustoš P., Balík J. (2008): Glutamate kinase as a potential biomarker of heavy metal stress in plants. Ecotoxicology and Environmental Safety, *70*: 223–230.

Pavlíková D., Pavlík M., Staszková L., Tlustoš P., Száková J., Balík J. (2007): The effect of potentially toxic elements and sewage sludge on the activity of regulatory enzyme glutamate kinase. Plant, Soil and Environment, *53*: 201–206.

Prasad K.V.S.K., Paradha S.P., Sharmila P. (1999): Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environmental and Experimental Botany, *42*: 1–10.

Radić S., Babić M., Skobić D., Roje V., Pevalek-Kozlina B. (2010): Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L. Ecotoxicology and Environmental Safety, *73*: 336–342.

Štefl M., Vašáková L. (1982): Allosteric regulation of proline-inhibitable glutamate kinase from winterwheat leaves by L-proline, adenosine diphosphate and low temperatures. Collection of Czechoslovak Chemical Communications, *47*: 360–369.

Thompson J.E., Ledge R.L., Barber R.F. (1987): The role of free radicals in senescence and wounding. New Phytologist, *105*: 317–344.

Wang C., Zhang S.H., Wang P.F., Qian J., Hou J., Li W., Zhang W.J., Lu J. (2009a): Excess Zn alters the nutrient uptake and induces the antioxidative responses in submerged plant *Hydrilla verticillata* (L.f.) Royle. Chemosphere, *76*: 938–945.

Wang H., Liu R.L., Jin J.Y. (2009b): Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. Biologia Plantarum, *53*: 191–194.

Received on July 24, 2010

Corresponding author:

Yanshan Cui, Graduate University of Chinese Academy of Sciences, College of Resources and Environment, Beijing, 100049, P.R. China
e-mail: cuiyanshan@gucas.ac.cn