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Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solanum lycopersicum*)

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Abstract

The interaction between zinc and cadmium was investigated in tomato plants (*Solanum lycopersicum*). Ten-day-old seedlings were treated with 10 μmol/L $CdCl_2$ associated to different concentrations of $ZnCl_2$ (10, 50, 100, and 150 μmol/L). Zn supply clearly reduced Cd accumulation in leaves and simultaneously increased Zn concentration. Cd induced oxidative stress in leaves as indicated by an increase in thiobarbituric acid-reactive substances (TBARS) level and chlorophyll breakdown. Furthermore, compared with control, Cd-treated plants had significantly higher activities of superoxide dismutase (SOD, EC 1.15.1.1), whereas, catalase (CAT, EC 1.111.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and glutathione reductase (GR, EC 1.6.4.2) activities were significantly suppressed by Cd addition. Zn supplementation, at low level, restored and enhanced the functional activity of these enzymes (SOD, CAT, APX and GR) as compared to Cd-alone-treated plants. The beneficial effect of adequate Zn level on Cd toxicity was confirmed by a significant decrease in TBARS level and restoration of chlorophyll content. However, when Zn was added at high level in combination with Cd there was an accumulation of oxidative stress, which was higher than that for Cd or excess Zn alone treatments. These results suggested that higher Zn concentrations and Cd are synergistic in their effect on plant growth parameters and oxidative stress.

Key words: *Solanum lycopersicum*; cadmium; zinc; oxidative stress; antagonism; synergism.

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Introduction

Cadmium (Cd) is a non-essential metal, which enters the environment mainly through Zn mining and smelting, industrial processes and the application of phosphate fertilizers (Waisberg et al., 2003). It can be transferred to the food chain by plant uptake. Studies carried out in different plant species have revealed that Cd can interfere with a number of metabolic processes. It diminishes water and nutrient uptake (Li et al., 2008) results in visible symptoms of injury in plants such as chlorosis and necrosis of leaves and reduced length and browning of roots. The photosynthetic apparatus is one of the target sites of Cd action in plants. In fact, Cd can directly or indirectly interacts with different components of the photosynthetic apparatus and can decrease electron transport efficiency, inhibit chlorophyll biosynthesis and reduce the photosynthetic carbon assimilation (Maksymiec et al., 2007).

Cadmium has also been found to cause oxidative stress. Cd exposure induces overproduction of reactive oxygen species (ROS) and increases lipid peroxidation of plant leaves and roots (Smeets et al., 2005). It has been reported that Cd causes a series of three waves of ROS generation, first with the NADPH oxidase-dependent accumulation of hydrogen peroxide (H_2O_2), followed by the accumulation of superoxide anions ($O_2^{\bullet-}$) in mitochondria, and finally, fatty acid hydroperoxide, as detected in tobacco cells (Garnier et al., 2006). High ROS levels can damage lipids, proteins and DNA. Thus, plant cell needs to thoroughly control ROS overall levels by the coordinated action of several antioxidant enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Foyer and Noctor, 2003). Together with low molecular weight antioxidant metabolites like ascorbic acid (Asc) and reduced glutathione (GSH), these enzymes provide cells with efficient machinery for detoxifying $O_2^{\bullet-}$ and H_2O_2, through the ascorbat-glutathione cycle. The cellular pools of Asc and GSH are maintained in their reduced state by the NAD(P)H-dependent enzymes, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Foyer and Noctor, 2003). The response of antioxidant enzymes to Cd stress, can vary among species and among different tissues (Hassan et al., 2005a). Depending on its concentration, Cd can either inhibit or

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stimulate the activity of several antioxidative enzymes before any visible symptoms of toxicity appear (Aravind and Prasad, 2003; Smeets et al., 2005).

On the other hand, it is well known that zinc (Zn) is an important component of a large number of enzymes, it is associated with the carbohydrate metabolism, proteins synthesis, gene expression and regulation, ribosome's structural integrity and phosphate metabolisms. Zinc is an essential component of key enzymes such as Cu-Zn superoxide dismutase, alcohol dehydrogenase, RNA polymerase and DNA-binding proteins (Broadley et al., 2007). Moreover, Zn plays critical roles in the defence system of cells against ROS, and thus represents an excellent protective agent against the oxidation of several vital cells component such as membrane lipid, chlorophyll and –SH groups of protein (Cakmak, 2000). However, excess Zn can have negative effects on plants. Zinc accumulation at supra-optimal concentrations may delay or diminish the growth and root development and causes leaf chlorosis (Wang et al., 2009) of Zn-exposed plants. Similarly to Cd, Zn excess could cause the formation in the plant cell of ROS, which results in cellular oxidative damage and membrane lipid peroxidation in plant cells (Jin et al., 2008). Recently, Wang et al. (2009) have demonstrated the effects of Zn stress on the activity of many antioxidative enzymes (APX, SOD, POD and CAT) and antioxidant contents (ascorbate and GSH) in plants.

Cadmium is often associated with Zn as a contaminant up to 5% in the processed Zn-ores of Zn mines and smelters (Sterckman et al., 2000). Cd and Zn have many physical and chemical similarities. Biologically, however, these two elements have different properties. The fact that Cd is a toxic heavy metal and Zn is an essential element makes this association interesting as it raises the possibilities that the toxic effect of Cd may be preventable by Zn. The co-existence of these two heavy metals in the ecosystem can lead to various synergistic and antagonistic interactions in their uptake and tissue content. Nan et al. (2002) observed that no antagonistic Cd-Zn interaction at uptake level, while Hassen et al. (2005b) showed that the increase in Zn level in the culture medium leads to a reduction in the Cd uptake and accumulation in roots associated to an increase in its content in shoot of rice cultivar. Hart et al. (2002) showed that in both durum and bread wheat decreases in Cd uptake by roots with increasing Zn treatment is possibly due to a competition between Zn and Cd for uptake. Thus, several studies have been conducted to investigate the Cd-Zn interaction on Cd and Zn uptake and accumulation in some plants species. However the influence of Cd-Zn interaction on free radical generation and antioxidant enzymes is little known especially in higher plants. In the present work, the influence of Zn on Cd uptake and toxicity was studied in *Solanum lycopersicum* by analyzing several parameters of stress such as growth inhibition, lipid peroxidation and antioxidant enzymatic activities.

1 Material and methods

1.1 Plant material and growth conditions

The seeds of tomato (*Solanum lycopersicum* cv. Chebli) were sterilized in 10% hydrogen peroxide for 20 min. The seeds were then thoroughly washed with distilled water and germinated on moistened filter paper at 25°C in the dark. The uniform seedlings were then transferred to continuously aerated nutrient solutions containing KH_2PO_4, 0.5 mmol/L; $Ca(NO_3)_2$, 1.25 mmol/L; KNO_3, 1 mmol/L; $MgSO_4$, 0.5 mmol/L; Fe-K-EDTA, 50 μmol/L; $MnSO_4{\cdot}4H_2O$, 5 μmol/L; $ZnSO_4{\cdot}7H_2O$, 1 μmol/L; $CuSO_4{\cdot}5H_2O$, 1 μmol/L; H_3BO_3, 30 μmol/L; and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1 μmol/L. The pH of the medium was checked and adjusted daily to 5.3–5.6. After an initial growth period of 10 days, 10 μmol/L $CdCl_2$ was added to the medium. Cd concentration of 10 μmol/L was selected for the analysis based on its intermediate level of growth inhibition. Zn supplementations (10, 50, 100, and 150 μmol/L) was given to the plant as $ZnCl_2$ along with the Cd concentration. Treatment with Zn only (10, 50, 100, and 150 μmol/L) was also given to the plant. Plants were grown in a growth chamber (26°C/70% relative humidity during the day, 20°C /90% during the night). A 16 hr photoperiod was used with a light irradiance of 150 μmol/(m^2·sec) at the canopy level. After 7 days of metal exposure, leaves were harvested and used for chemical analyses.

1.2 Determination of metal contents

Cadmium and Zn contents in various plant tissues were analyzed by digestion of dried samples with an acid mixture (HNO_3/$HClO_4$, 4/1, *V*/*V*). Metal concentrations were determined by atomic absorption spectrophotometry (AAnalyst 300, flame spectrometer, Perkin-Elmer, USA).

1.3 Estimation of photosynthetic pigments contents

Photosynthetic pigments were extracted in 80% acetone for 24 hr in darkness, at 4°C. The resulting suspension was centrifuged for 5 min at 3000 ×*g*, then the absorbance of the supernatant was measured at 460, 645 and 663 nm with an UV/Vis spectrometer (Lambda 25, Perkin-Elmer, USA). The pigment concentrations were calculated by equations allowing a simultaneous determination of chlorophyll *a* (Chl-*a*) and *b* (Chl-*b*) and carotenoids in the same supernatant, as in the work of Arnon (1949).

1.4 Lipid peroxide determination

Lipid peroxide was determined by measuring the concentration of thiobarbituric acid-reacting substances (TBARS), as described by Alia et al. (1995). The leaves were homogenized in 5% (*W*/*V*) trichloroacetic acid (TCA). After centrifugation, a sample of the supernatant was added to 20% TCA containing 0.5% (*W*/*V*) thiobarbituric acid (TBA). The mixture was incubated at 95°C for 30 min. The concentration of thiobarbituric acid reacting substances was calculated using an extinction coefficient of 155 mL/(mol·cm).

1.5 Extraction, protein determination

Enzyme extractions were carried out at 4°C. The plant tissue was reduced to powder in liquid nitrogen and extracted at a ratio 1:3 (*W*/*V*) fresh weight in 50 mmol/L potassium phosphate buffer (pH 7) containing 1 mmol/L EDTA, 3 mmol/L dithiothereitol (DTT) and 5% (*W*/*V*) insoluble polyvinylpoly-pyrrolidone (PVPP). For the APX assay, 5 mmol/L ascorbate was added to the extracted buffer. The homogenate was centrifuged at 14,000 ×*g* for 30 min and the supernatant was used for enzyme assays.

Protein content was determined spectrophotometrically at 595 nm as described by Bradford (1976) using bovine serum albumin (BSA) as standard.

1.6 Enzyme assay

Total CAT (EC 1.11.1.6) activity was assayed in presence of H_2O_2, according to Chaparro-Giraldo et al. (2000), by monitoring the decline in absorbance at 240 nm, as H_2O_2 was consumed. Enzyme activity was calculated using the extinction coefficient of 40 mL/(mol·cm) for H_2O_2.

Total SOD (EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm according to Beyer and Fridovich (1987), based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT). One unit of enzyme activity was defined as the quantity of SOD required for 50% inhibition of NBT reduction.

Total APX (EC 1.11.1.11) activity was assayed in the presence of ascorbate by following the decline in absorbance of the oxidized ascorbate at 290 nm, according to Chen and Asada (1989). Enzyme activity was calculated using the extinction coefficient of 2.8 mL/(mol·cm) for ascorbate.

Total GR (EC 1.6.4.2) activity was determined by following the rate of NADPH oxidation, as measured by the decrease in the absorbance at 340 nm (Rao et al., 1996).

1.7 Statistics

The data are presented in the text as the average of at least six replicates per treatment. The mean values ± standard error (SE) are reported. Pair-wise analyses of variance (ANOVA) were used to detect differences between treatments, taking p-values < 0.05 as significant.

2 Results

2.1 Metals accumulation and growth

Tomato plants exposed to 10 μmol/L $CdCl_2$ in the nutrient solution accumulated 31.6 μg Cd/g dw in their leaves and reduced Zn content compared to the control plants, whereas, the Cd concentration decreased up to 11.8 μg Cd/g dw in treatments with supplemented Zn (especially at 150 μmol/L). Simultaneously, Zn accumulation increased from 10.44 μg/g dw in control to 43.57 μg/g dw in Cd-treated plants with supplemented 150 μmol/L Zn (Fig. 1a). Treatments with only Zn enhanced Zn concentration in leaves (Table 1), which is higher than in Cd-treated plants with supplemented Zn, indicating a competition between Cd and Zn at uptake level.

The presence of 10 μmol/L Cd in the nutrient medium inhibited growth of tomato plants (Fig. 1b). Chlorosis of leaves and reduced length and browning of roots are the main visual toxicity symptoms. On the other hand, Zn at low level (10 and 50 μmol/L), stimulated growth and alleviated Cd-toxicity. Conversely, significant reduction in biomass production associated to appearance of chlorosis and necrosis were observed when the plants were exposed to higher Zn levels (100 and 150 μmol/L) alone or in combination with Cd (Table 1 and Fig. 1b).

2.2 Photosynthetic pigments contents

The photosynthetic pigments showed a drastic reduction (50%, 28% and 45% of Chl-*a*, Chl-*b* and carotenoids,

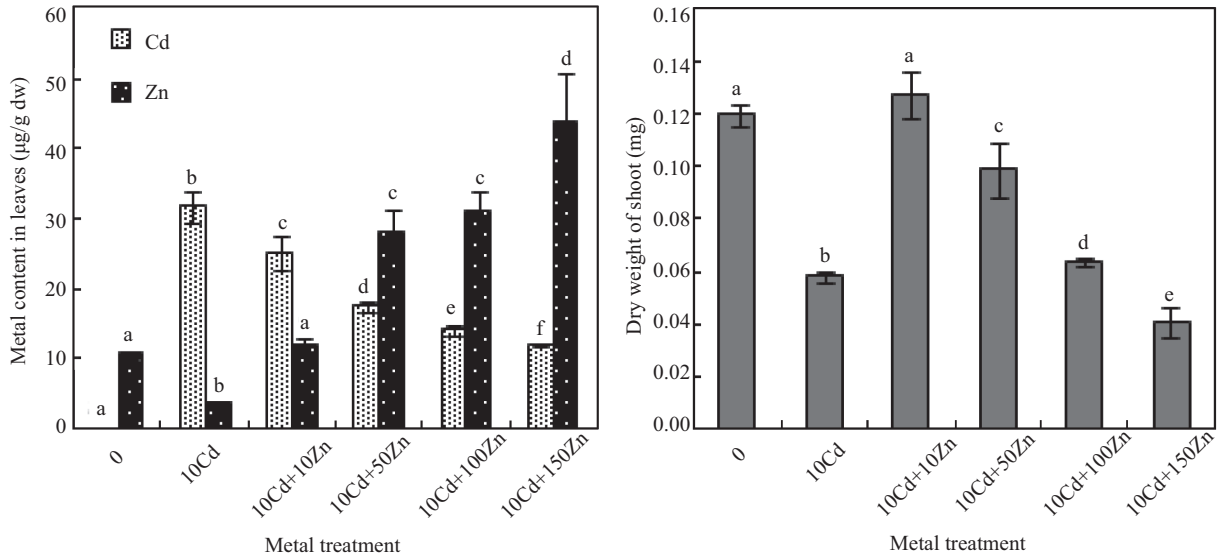

Fig. 1 Metal accumulation in leaves (a) and dry weights (dw) of shoots (b) of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

Table 1 Zn content, dry weights, photosynthetic pigments and lipid peroxidation levels in *Solanum lycopersicum* treated with $ZnCl_2$ for 7 days

Zn (μmol/L)	Zinc content in leave (μg/g dw)	Dry weight (mg)	Photosynthetic pigments (mg/g fw)			TBARS (nmol/g fw)
			Chlorophyll *a*	Chlorophyll *b*	Carotenoides	
0	10.44 ± 0.43 a	0.120 ± 0.002 a	1.34 ± 0.04 a	0.58 ± 0.09 a	0.52 ± 0.01 a	17.28 ± 0.35 a
10	25.73 ± 2.07 b	0.129 ± 0.010 ab	1.50 ± 0.03 b	0.65 ± 0.01 b	0.52 ± 0.01 a	16.90 ± 1.44 a
50	65.43 ± 6.86 c	0.109 ± 0.008 b	1.22 ± 0.09 ac	0.56 ± 0.06 a	0.49 ± 0.04 a	18.90 ± 1.02 b
100	75.05 ± 2.65 d	0.095 ± 0.006 c	1.00 ± 0.09 c	0.50 ± 0.02 c	0.47 ± 0.04 b	22.41 ± 0.31 c
150	92.36 ± 0.57 e	0.063 ± 0.001 d	0.80 ± 0.05 e	0.45 ± 0.02 d	0.45 ± 0.02 b	26.01 ± 0.64 d

TBARS: thiobarbituric acid-reactive substances.
Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

respectively) in Cd-10 μmol/L treated plants in comparison to the control (Fig. 2). The addition of Zn (especially 10 and 50 μmol/L) to the medium with Cd restored the photosynthetic pigments levels. Zn-alone treatments (10–100 μmol/L) did not show much variation in photosynthetic pigments level (Table 1). Whereas, the highest Zn concentration (150 μmol/L), when addition alone or in combination with Cd, induced a severe reduction in the Chl-*a* and Chl-*b* levels compared to the control. This results suggest that excess of Zn becomes toxic to plants.

2.3 Lipid peroxydation level

The effect of Cd toxicity on lipid peroxydation was determined by evaluating the TBARS levels in the Cd treated tissues. Compared to the control, plants exposed to Cd showed an increase in the TBARS level (Fig. 3). This was decreased up to 63% from the increased TBARS value at Cd-10 μmol/L in Cd-treated plants with supplemented Zn (especially 10 μmol/L Zn). This indicates the antagonistic effect of low Zn concentrations against Cd at the membrane level. However, Zn became synergistic with Cd at high concentration (150 μmol/L) and showed an increase in TBARS by approximately 20% compared to Cd alone (Fig. 3). In Zn treated plants the amount of TBARS was significantly increased from 100 μmol/L Zn (Table 1).

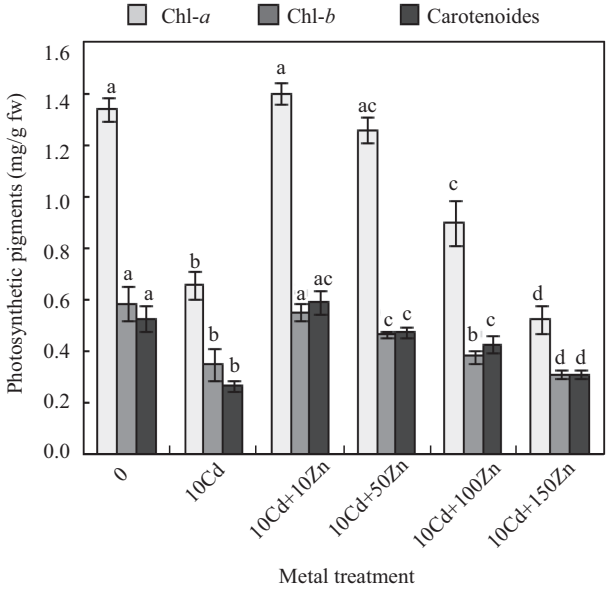

Fig. 2 Level of chlorophyll and carotenoids in *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

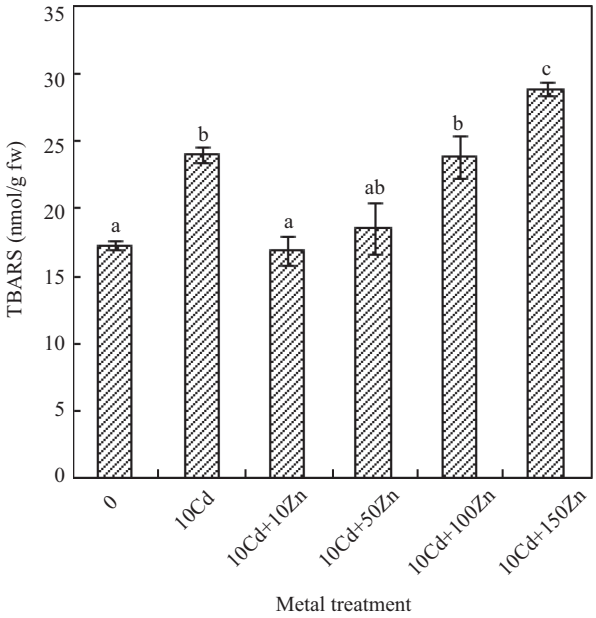

Fig. 3 Level of lipid peroxidation as a measure of TBARS in leaves of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

2.4 Antioxidant enzymes activity

2.4.1 SOD activity

Superoxide dismutase (SOD) activity showed 120% increase in 10Cd treated plants compared to the control (Fig. 4). Adding Zn (10–100 μmol/L) to the nutrient medium with Cd led to a very high increase in the SOD activity, indicating the efficiency of this antioxidant enzyme in the presence of Zn (10–100 μmol/L) than without any Zn supplementation. A decrease in the SOD activity was observed at the highest level of Zn supplementation (150 μmol/L). However, in Zn-treated plants, the SOD activity increased with increasing Zn concentration (Table 2).

2.4.2 CAT activity

Cd treatment led to a significant decrease in CAT activity (Fig. 5). However, after low Zn level (10 and 50 μmol/L) supply to Cd, the CAT activity was significantly increased compared to the control. When Zn added to Cd at high level (150 μmol/L), there was synergistic effect to Cd toxicity on the CAT activity, which was reduced by approximately 27% compared to Cd alone. In Zn-treated plants the CAT activity was significantly increased and

Table 2 Influence of Zn on the activities of antioxidant enzymes in *Solanum lycopersicum*

Zn (μmol/L)	SOD (U/mg protein)	CAT (μmol H_2O_2 /(mg protein·min))	APX (nmol H_2O_2 /(mg protein·min))	GR (nmol H_2O_2 /(mg protein·min))
0	133.45 ± 10.00 a	26.68 ± 0.61 a	24.84 ± 2.15 a	30.67 ± 0.97 a
10	162.91 ± 14.23 b	28.09 ± 1.80 a	25.78 ± 1.40 a	32.04 ± 4.87 a
50	222.60 ± 24.76 c	50.37 ± 3.25 b	46.09 ± 7.75 b	38.76 ± 1.54 b
100	255.31 ± 13.56 d	46.94 ± 4.59 b	52.97 ± 3.96 c	40.71 ± 1.61 b
150	285.97 ± 11.43 e	38.08 ± 5.40 c	43.77 ± 2.55 b	24.95 ± 0.97 c

Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

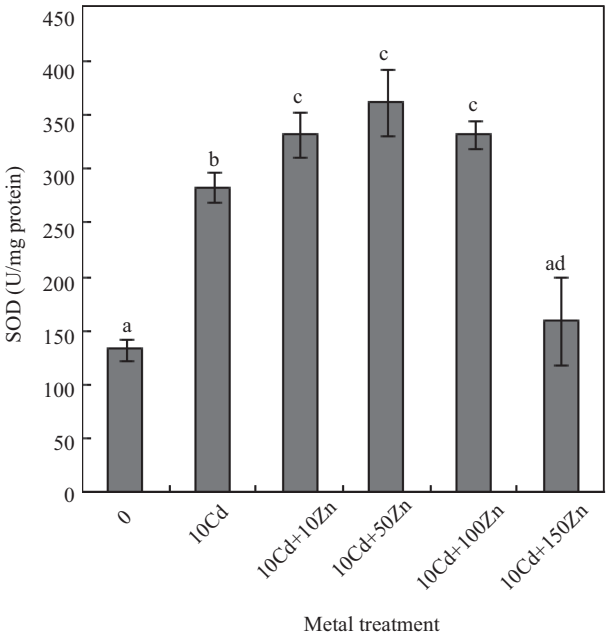

Fig. 4 Activity of SOD in leaves of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

showed a maximum activity at 50 μmol/L (Table 2).

2.4.3 APX activity

The APX activity showed a little decrease in Cd-treated plants by approximately 13% compared to the control. Supplementation of Zn at low concentration ⩽ 50 μmol/L to Cd treatment caused a significant enhance in APX activity, which was increased by approximately 83% at 50 μmol/L Zn addition compared to Cd-alone (Fig. 6), indicating that addition of Zn, at low levels, improved APX functioning. However, Zn addition at high level reduced APX activity. Treatments with only Zn induced an increase in the APX activity especially at 100 μmol/L $ZnCl_2$ (Table 2).

2.4.4 GR activity

Cadmium addition induced a decrease in GR activity up to 20% compared to the control (Fig. 7). However, Zn (10 and 50 μmol/L) supplementation to Cd-treatment efficiently restored and enhanced the GR activity. When Zn was added at high level (150 μmol/L) in combination with Cd there was a reduction of the GR activity by 29% compared to plants treated with Cd alone. GR activity was increased in Zn-treated plants and showed a maximum activity at 100 μmol/L, conversely, at 150 μmol/L Zn there

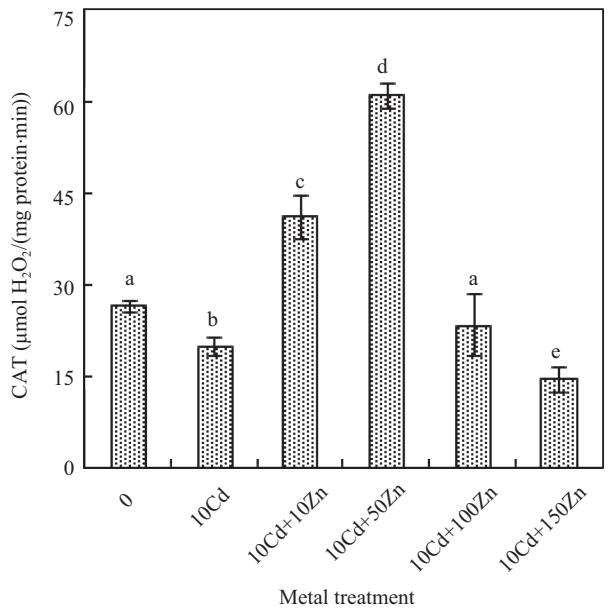

Fig. 5 Activity of CAT in leaves of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with same letter are not significantly different at $p < 0.05$.

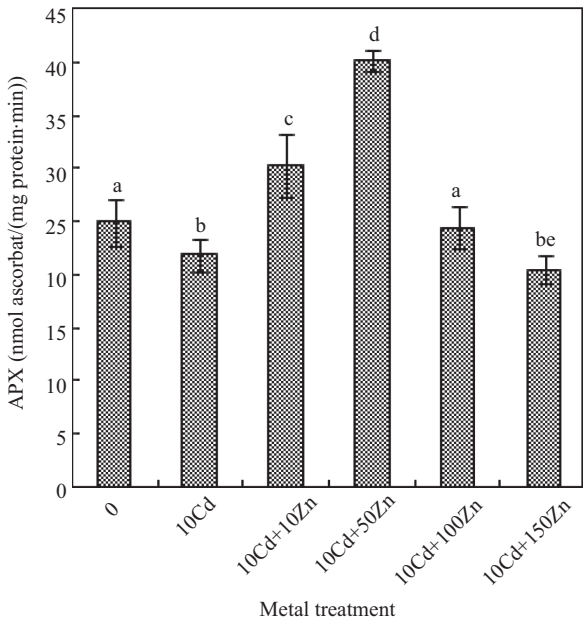

Fig. 6 Activity of APX in leaves of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with the same letter are not significantly different at $p < 0.05$.

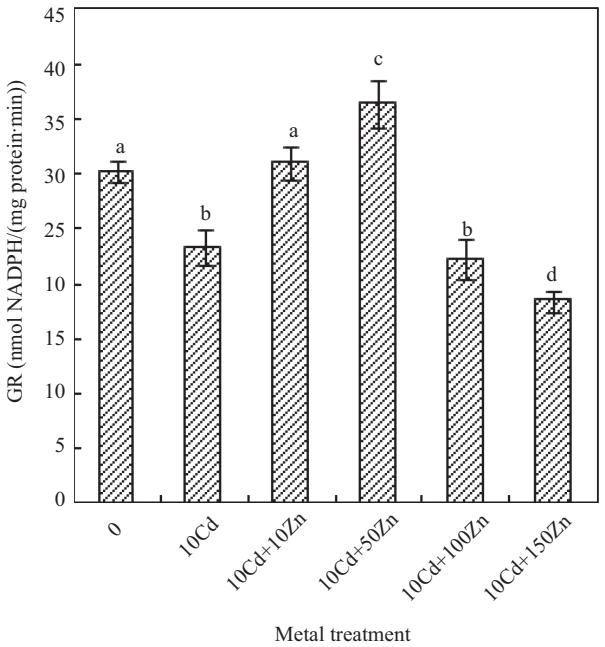

Fig. 7 Activity of GR in leaves of *Solanum lycopersicum* treated with Cd-10 μmol/L and Cd-10 μmol/L supplemented with Zn (10, 50, 100 and 150 μmol/L). Each value represents the mean ± SE of six individual replicates. Data with the same letter are not significantly different at $p < 0.05$.

was a significant reduction (Table 2).

3 Discussion

The association of Cd and Zn in the environment and their chemical similarity can lead to interaction between these two ions. The results of our study showed that exposure of tomato plants to Cd significantly inhibited growth. However, plant growth was promoted at the low concentrations of Zn (10 and 50 μmol/L) added to Cd (Fig. 1b). Thus, it can be concluded that Zn, at low concentration, had a significant effect on the alleviation of Cd toxicity on plant growth. This effect was reflected directly by analysis of Cd and Zn accumulation. Plants exposed to Cd alone showed an accumulation of Cd and a reduction in Zn content responsible for growth reduction. It is possible that such an excess of Cd affected Zn interactors, like Zn-binding domains or Zn transporters, which could then become saturated by Cd (Bovet et al., 2006). Zn addition induced a decrease in Cd uptake and simultaneously an increase in Zn accumulation, indicating a strong competition between these two metals for the same membrane-transporters. In plants, ATPase-type AtHMA4 is expressed at the plasma membrane, and studies of T-DNA insertional mutants have demonstrated that this protein is involved in Zn and Cd xylem loading and in the translocation of these metals from the roots to the shoot (Hussain et al., 2004). Further Zn and Cd share others transporters of ZIP transporters; for example, IRT1 (Plaza et al., 2007) and IRT3 (Lin et al., 2009). Several other evidences such as in bread and durum wheat (Hart et al., 2002) and *Ceratophyllum demersum* (Aravind and Prasad, 2003) also point to the depressed Cd uptake in the presence of Zn. The antagonistic effect between Cd and lower Zn concentrations on plant growth is attributable largely to reduced Cd uptake. When Zn was added alone or in combination with Cd at higher dose ⩾ 100 μmol/L, some toxicity symptoms like chlorosis and growth reduction was observed. This toxic effect due to the important accumulation of Zn in leaves. Excessive Zn in plants can profoundly affect normal ionic homeostatic systems by interfering with the uptake, transport, and regulation of essential ions (Wang et al., 2009) and results in the disruption of metabolic processes such as transpiration and photosynthesis responsible for growth reduction (Sagardoy et al., 2009).

High metals concentration can induce oxidative stress (Smeets et al., 2005). Relationship between Cd toxicity and oxidative reactions in *Solanum lycopersicum* was studied in terms of chlorophyll and TBARS variations. Our results in Chl-*a*, Chl-*b* and carotenoids content (Fig. 2) showed that, Cd induce a severe decrease in the amount of photosynthetic pigments. This decline in chlorophyll and carotenoids has been proposed to be responsible for the reduction in photosynthesis and growth produced by this metal (Maksymiec et al., 2007). Reduction in chlorophyll content was probably caused by interference of Cd to enzyme of chlorophyll biosynthesis such as δ-aminolevulinic acid dehydratase (ALA dehydratase) as well as the protochlorophyllide reductase (Myśiwa-Kurdziel and Strzałka, 2005). The reductions of chlorophyll content can be also due to the oxidation coming from an overproduction of ROS, which are generated by Cd toxicity (Laspina et al., 2005). However, when Cd-treated plants were supplemented with Zn (especially 10 μmol/L) there was full protection and restoration of the chlorophyll levels. Zn at low level probably maintains chlorophyll synthesis through sulphydryl group protection, a function primarily associated with Zn (Cakmak, 2000). Zn also plays a role in activating ALA dehydratase, and hence protochlorophyllide to chlorophyllide conversion facilitating the formation of complete chlorophyll moiety (Lebedev and Timko, 1998). In contrast, when Zn was added at 150 μmol/L with Cd, the reduction in chlorophyll contents was more severe than that of their alone. This result may be due to the synergistic interaction of excess Zn and Cd on chlorophyll content. Recent reports showed that excess Zn induces chlorosis and loss of total chlorophyll and this has been suggested to result from a Zn-induced Fe or Mg deficiency (Sagardoy et al., 2009).

Membrane destabilization is generally attributed to lipid peroxidation, due to an increased production of ROS (Smeets et al., 2005). Our results revealed that Cd may be involved in lipid peroxidation and membrane damage which was obvious from the significantly higher TBARS content. Lipid peroxidation can be also due to a Cd-mediated increase in lipoxygenases (LOXs) activity (Smeets et al., 2008). Supplementation of Zn at low concentration ⩽ 50 μmol/L to Cd treatment caused a significant decrease in TBARS content. This indicates the action of adequate Zn level against Cd at the membrane level. In this regard, Aravid and Prasad (2003) showed that Zn supplementation to Cd stress reduced lipid peroxidation

and LOX activity. Zn has a stabilizing and protective effect on the biomembrane protein and phospholipids from oxidative and peroxidative damage and loss of membrane integrity (Powell, 2000; Aravind and Prasad, 2003). It is reported that Zn-deficiency induced an increase in peroxidative damage and oxidative stress, which was alleviated under Zn-sufficient conditions (Cakmak, 2000). Whereas, TBARS content in combined Zn at high concentration (150 μmol/L) and Cd treatment were higher than that for Zn or Cd alone treatments. This result shows that the interaction effect of these two metals on membrane lipid peroxidation is mainly displayed as synergism under the combined stress of excess Zn and Cd.

Evidence that Cd causes the overproduction of ROS in *Solanum lycopersicum* came from observation that Cd exposure leads to an increase in the lipid peroxidation levels and chlorophyll breakdown. However, to neutralize the toxicity of ROS, plants activate their antioxidant enzymes (Smeets et al., 2005). Such antioxidant defences are extremely important as they represent the direct removal of free radicals. SOD catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 and play a key role in quenching ROS. In our experiments a significant increase in SOD activity after seedlings treatment with Cd was observed (Fig. 4). This enhancement in activity may be due to over synthesis of this enzyme. When Zn (especially 50 μmol/L) added in combination with Cd there was a very high induction of SOD activity, much higher than Cd-alone treatments. Possibly, Zn may also protect plants from Cd toxicity by enhancing activity of antioxidative enzymes such as superoxide dismutase, a Zn-containing enzyme, and by competing with Cd for binding to –SH groups of enzymes and membrane proteins. The H_2O_2, produced by SOD activity, is broken-down to H_2O and O_2 by CAT and ascorbate-glutathione cycle (Drazkiewicz et al., 2003). During the present study, the enzyme capacities of CAT, APX and GR in leaves were all reduced in Cd treatments, while, we noted a very high induction of SOD activity, which accelerates the spontaneous dismutation of the superoxide anion radical ($O_2^{\bullet-}$) to H_2O_2, can lead to H_2O_2 accumulation as well responsible for lipid peroxidation and chlorophyll breackdown (Laspina et al., 2005). SOD activity seems to be dependent on the intensity of oxidative stress in plant cells. From our results Cd would have inactivated same antioxidant enzymes. It has been suggested that GR is extremely sensitive to inhibition by heavy metals ions like, Cu^{2+} and Fe^{2+} (Smith et al., 1989). Furthermore, the CAT enzyme is sensitive to $O_2^{\bullet-}$ and can be inactivated by its increasing levels (Cakmak, 2000). Adding Zn at low concentration to the medium with Cd induced a very high induction of CAT, APX and GR activity especially at 50 μmol/L Zn (Figs. 5–7), indicating the efficiency of these antioxidant enzymes in the presence of Zn than without any Zn supplementation. Probably, Zn is indirectly required for high activity of the enzymes involved in ROS detoxification such as CAT, APX and GR (Cakmak, 2000). The results of this work indicate that low Zn level antagonizes Cd toxicity by maintaining the levels of antioxidant enzymes and the efficient ROS scavenging activity in cells. Zinc has been shown in numerous systems to antagonize the catalytic properties of the redox-active transition metals iron and copper with respect to their abilities to promote formation of $OH^{\cdot-}$ from H_2O_2 and $O_2^{\bullet-}$ (Powell, 2000). Cd is known to trigger the oxidation of NADPH leading to $O_2^{\bullet-}$ production. Recently, Aravind et al. (2009) showed that Zn supplementation to Cd effectively inhibited NADPH oxidation and hence $O_2^{\bullet-}$ radical thereby preventing the initiation of ROS formation. Thus, Zn protects several vital cell components such as chlorophyll, membrane lipids from oxidation. It has also been proposed that metal toxicity in plants may result from the binding of metals to protein sulphydryl groups, inhibiting enzyme activities or altering protein structure (Van Assche and Clijsters, 1990). The protection of –SH groups from thiol oxidation by Zn in Cd treatments with supplemented Zn would have strengthened the Zn-metalloprotein interaction, a function primarily associated with Zn (Cakmak, 2000; Powell, 2000).

However, the activities of CAT, APX and GR leveled off at higher supplied Zn (150 μmol/L), in parallel with remarkable breakdown of chlorophyll and significant increase in TBARS content suggesting that Zn, at high concentration, has synergistic effect to Cd toxicity on antioxidant enzymes and causes irreversible damage to plant development and function. This finding is in contradiction with that observed by Aravind and Prasad (2003) in *Ceratophyllum demersum*, they reported that Zn supplemented to Cd-treatment improve the activity of antioxidant enzymes even at 200 μmol/L. Nevertheless, the generation of ROS including hydrogen peroxide (H_2O_2) and superoxide radical $O_2^{\bullet-}$ and the reduction in the activity of antioxidant enzymes was detected in *Sedum alfredii* under excess Zn (Jin et al., 2008). Based on our results, we can conclude that Zn addition, at low level, strongly protects *Solanum lycopersicum* from Cd toxicity through reducing Cd uptake, chlorophyll breakdown and lipid peroxidation and improving the ROS scavenging antioxidant enzymes activities. Interestingly, when Zn was added at high level to the medium in combination with Cd there was an accumulation of oxidative stress, which was higher than that for Cd or excess Zn alone treatments.

References

Alia K V S K, Prasad P, Pardha S P, 1995. Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. *Phytochemistry,* 42(1): 45–47.

Aravind P, Prasad M N V, 2003. Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: A free floating freshwater macrophyte. *Plant Physiology and Biochemistry*, 41: 391–397.

Aravind P, Prasad M N V, Malec P, Waloszek A, Strzałka K, 2009. Zinc protects *Ceratophyllum demersum* L. (free-floatinghydrophyte) against reactive oxygen species induced by cadmium. *Journal of Trace Elements in Medicine and Biology*, 23: 50–60.

Arnon D J, 1949. Copper enzymes in isolated chloroplast. Polyphenolxydase in *Beta vulgaris*. *Plant Physiology*, 24: 1–14.

Beyer W F, Fridovich Y, 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analytical Biochemistry*, 161: 559–566.

Bovet L, Rossi L, Lugon-Moulin N, 2006. Cadmium partitioning and gene expression studies in *Nicotiana tabacum* and *Nicotiana rustica*. *Physiologia Plantarum*, 128: 466–475.

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

Broadley M R, 2007. Zinc in plants. *New Phytologist*, 173: 677–702.

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

Cakmak I, Welch R M, Erenoglu B, Romheld V, Norvell W A, Kochian L V, 2000. Influence of varied zinc supply on retranslocation of cadmium (109Cd) and rubidium (86Rb) applied on mature leaf of durum wheat seedlings. *Plant Soil*, 219: 279–284.

Chaparro-Giraldo A, Barata R M, Chabregas S M, Azevedo R A, Silva-Filho M C, 2000. Soybean leghemoglobilin targeted to potato chloroplasts influences growth and development of transgenic plants. *Plant Cell Reports*, 19: 961–965.

Chen G X, Asada K, 1989. Ascorbate peroxidase in pea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant and Cell Physiology*, 30: 987–998.

Drazkiewicz M, Skorzynska-polit E, Krupa Z, 2003. Response of ascorbate-glutathione cycle to excess copper in *Arabidopsis thaliana* (L.). *Plant Science*, 164: 195–202.

Foyer C H, Noctor G, 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum*, 119: 355–364.

Garnier L, Simon-Plas F, Thuleau P, Angel J, Blein J, Ranjeva R, 2006. Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity. *Plant, Cell & Environment*, 29: 1956–1969.

Hart J J, Welch R M, Norvell W A, Kochian L V, 2002. Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiologia Plantarum*, 116: 73–78.

Hassan M J, Shao G, Zhang G, 2005a. Influence of cadmium toxicity on antioxidant enzymes activity in rice cultivars with different grain Cd accumulation. *Journal of Plant Nutrition*, 28: 1259–1270.

Hassan M J, Zhang G, Wu F, Wei K, Chonghua C, 2005b. Zinc alleviates growth inhibition and oxidative stress caused by cadmium toxicity in rice. *Journal of Plant Nutrition and Soil Science*, 168: 256–261.

Hussain D, Haydon M J, Wang Y, Wong E, Sherson S M, Young J et al., 2004. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *The Plant Cell*, 16: 1327–1339.

Jin X F, Yang X E, Islam E, Liu D, Mahmood Q, Li H et al., 2008. Ultrastructural changes, zinc hyperaccumulation and its relation with antioxidants in two ecotypes of *Sedum alfredii* Hance. *Plant Physiology and Biochemistry*, 46: 1997–1006.

Laspina N V, Groppa M D, Tomaro M L, Benavides M P, 2005. Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Science*, 169: 323–330.

Lebedev N, Timko P M, 1998. Protochlorophyllide photoreduction. *Photosynthesis Research*, 58: 5–23.

Li M, Zhang L J, Tao L, Li W, 2008. Ecophysiological responses of *Jussiaea rapens* to cadmium exposure. *Aquatic Botany*, 88: 347–352.

Lin Y F, Liang H M, Yang S Y, Boch A, Clemens S, Chen C et al., 2009. Arabidopsis IRT3 is a Zn-regulated and plasma membrane localized Zn/Fe transporter. *New Phytologist*, 182: 392–404.

Maksymiec W, Wójcik M, Krupa Z, 2007. Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate and ascorbate. *Chemosphere*, 3(6): 421–427.

Myśiwa-Kurdziel B, Strzałka K, 2005. Influence of Cd(II), Cr(VI) and Fe(III) on early steps of deetiolation process in wheat: fluorescence spectral changes of protochlorophyllide and newly formed chlorophyllide. *Agriculture, Ecosystems & Environment*, 106: 199–207.

Nan Z R, Li J J, Zhang J M, Cheng G D, 2002. Cadmium and zinc interactions and their transfer in soil-crop system under actual field. *Science of the Total Environment*, 285: 187–195.

Plaza S, Tearall K L, Zhao F J, Buchner P, McGrath S P, Hawkesford M J, 2007. Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens. Journal of Experimental Botany*, 58(7): 1717–1728.

Powell S R, 2000. The antioxidant properties of zinc. *The Journal of Nutrition*, 130: 1447–1454.

Rao M V, Paliyath G, Ormrod D P, 1996. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology*, 110: 125–136.

Sagardoy R, Morales F, López-Millán A F, Abadía A, Abadía J, 2009. Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics. *Plant Biology*, 11(3): 339–350.

Smeets K, Cuypers A, Lambrechts A, Semane B, Hoet P, Van Laere P et al., 2005. Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application. *Plant Physiology and Biochemistry*, 43: 437–444.

Smeets K, Ruytinx J, Semane B, Van Belleghem F, Remans T, Van Sanden S, 2008. Cadmium-induced transcriptional and enzymatic alterations related to oxidative stress. *Environmental and Experimental Botany*, 63: 1–8.

Smith I K, Vierheller T L, Thorne C A, 1989. Proprieties and functions of glutathione reductase in plants. *Physiologia Plantarum*, 77: 449–456.

Sterckman T, Douay F, Proix N, Fourrier H, 2000. Vertical distribution of Cd, Pb and Zn in soils near smelters in the north of France. *Environmental Pollution*, 107: 377–389.

Van Assche F, Clijsters H, 1990. Effects of metals on enzyme activity in plants. *Plant, Cell & Environment*, 13: 195–206.

Waisberg M, Joseph P, Hale B, Beyersmann D, 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, 192: 95–117.

Wang C, Zhang S H, Wang P F, Hou J, Zhang W J, Li W et al., 2009. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere*, 75: 1468–1476.