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# Uptake, accumulation and some biochemical responses in *Raphanus sativus* L. to zinc stress

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The responses of radish (*Raphanus sativus* L.) to increasing concentrations of ZnCl<sub>2</sub> (1, 5 and 10 mM) in Hoagland nutrient medium were studied. Under the conditions of these increasing zinc concentrations, the highest zinc accumulation was obtained in the roots of the plants treated with 10 mM applications. The zinc concentration in the vegetative parts, was highest in the root and was lowest in the cotyledons. The highest bioconcentration factor (BCF) value was detected in the roots in 5 mM zinc applications. When compared with the control, total zinc uptake was observed to increase in 1, 5 and 10 mM ZnCl<sub>2</sub> treatments. The total accumulation rate (TAR) for zinc was highest in 10 mM ZnCl<sub>2</sub> treatment, while the lowest TAR was observed in radish plants exposed to 1 mM ZnCl<sub>2</sub>. Plants treated with 5, 10 mM ZnCl<sub>2</sub> showed significant decreases in chlorophyll (Chl a, Chl b, Chl a/b) and carotenoid content compared with the control. Peroxidase (POD) activity especially in radish roots increased significantly with increasing concentrations of ZnCl<sub>2</sub> (5 and 10 mM) while the total protein amount decreased when compared with the control. The results of this study showed that, radish plants could tolerate the negative effects of zinc stress up to 1 mM ZnCl<sub>2</sub> concentration and that in zinc concentrations of 5 mM and above toxic effects were existent.

**Key words:** Radish, *Raphanus sativus*, zinc, metal toxicity, uptake, accumulation, peroxidase, pigment.

## INTRODUCTION

Soil pollution by toxic metals is a serious problem for the environment and is also one of the environmental stresses for higher plants. Agricultural lands are contaminated with heavy metals due to industrialization, urbanization and agricultural activities (Kabata-Pendias and Pendias, 1986; Singh et al., 2004). Metal toxicity is related to bioavailability rather than the total concentration of metal in the soil and may reduce both quality and productivity of plants (Gayoor et al., 1999).

Zinc is usually in low concentrations in soil. Zinc is also an essential micronutrient in higher plants; however, in higher concentrations it is highly toxic for plants (Rengel, 1999). However, anthropogenic sources can increase soil zinc to a level toxic to plants. Zinc has been a major

industrial pollutant of the terrestrial and aquatic environment over the last few decades. On the other hand, being an essential micronutrient, zinc is involved in numerous physiological processes such as redox reaction, structural configuration of several enzymes and nucleic acid metabolism (Rengel, 1999). The phytotoxic zinc concentration range is generally 100 to 400 mg kg<sup>-1</sup> and depends on the plant species and on the development stage, and on the other hand, zinc concentration is around 27 to 150 mg kg<sup>-1</sup> in normal healthy plants (Stevenson and Cole, 1999). However, at high concentrations, zinc becomes strongly poisonous thus, causing inhibition of growth and metabolism and even death of organisms (Zenk, 1996; Vaillant et al., 2005). Excess of Zn is indicated by a decrease in growth, development and metabolic activity and an induction of oxidative damage in various plant species (Panda et al., 2003). In addition, zinc toxicity leads to chlorosis in young leaves and inhibits photosynthesis at various steps

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and through different mechanisms (Chaney, 1993). The oxidative stress results in generation of toxic free radicals in plants (Asada and Takahashi, 1987). Heavy metals accelerate generation of reactive oxygen species (ROS) that have the capacity to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids (Halliwell and Gutteridge, 1999; Sharma et al., 2007). To protect themselves from heavy metal toxicity, the metal ion, entering the cytosol of the cell, is immediately complexed and inactivated. In addition, plant cells are also equipped with oxygen radical detoxifying enzymes such as superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) (Prasad et al., 1999; Bonnet et al., 2000; Sharma et al., 2007).

This research was designed to investigate the uptake of zinc, total accumulation rate (TAR), secondary transport index (STI), primary transport index (PTI) and bioconcentration factor (BCF) by *Raphanus* at early growth stage. In addition, the second objective of the study was to establish the effects of zinc stress on pigment (chlorophyll, carotenoid) content, total protein amount and detoxifying enzyme activity (guaiacol peroxidase (POD), EC 1.11.1.7).

## MATERIALS AND METHODS

### Plant material and growth conditions

Radish seeds (*Raphanus sativus* L.) which belong to the Brassicaceae family were used as the experimental material. After surface sterilization with 70% ethanol, radish seeds were imbibed in the experimental solutions at room temperature for 24 h and then were transferred into Petri dishes containing filter paper soaked with experimental solutions. The ZnCl<sub>2</sub> concentrations chosen for the study were based on other researchers (Alia et al., 1995; Prasad et al., 1999; Kösesakal, 2005). Radish plants (*R. sativus* L.) were grown in Hoagland and Arnon (1938) medium containing 0, 1, 5 and 10 mM of ZnCl<sub>2</sub>. The seedlings were raised in a growth chamber (12 h light period) under white fluorescent light providing a light intensity of 300 μmol m<sup>-2</sup> s<sup>-1</sup>, under day/night temperature of 25±2°C and 65±5% relative humidity. The roots, hypocotyls and cotyledons excised from 7-day-old seedlings were used for all the investigations.

### Zinc analysis

For zinc analysis, seeds and various plant parts (roots, hypocotyls and cotyledons) were thoroughly washed with deionized water in order to remove adsorbed metal ions. Samples were dried at 70°C (Heraeus) to a constant weight and pulverized. Each dry plant sample was digested with concentrated HNO<sub>3</sub> on a hot-plate for 96 h. Zinc concentrations in the digests were determined by flame atomic absorption spectrophotometer (Shimadzu AA 680, air-C<sub>2</sub>H<sub>2</sub>, background correction mode). The concentrations for each sample solution were measured three times. The results obtained were defined as mg kg<sup>-1</sup> DW (Izzo et al., 1991).

### Zn-uptake, total accumulation rate (TAR), transport indices and bioconcentration factor (BCF)

To study the transport behavior of zinc, indices such as primary

transport index (PTI) and secondary transport index (STI) were calculated following Moral et al. (1994). Primary transport index is the ratio of the heavy metal concentration in the hypocotyl to the heavy metal concentration in the root. Secondary transport index was calculated by dividing the heavy metal concentration in the cotyledons by the heavy metal concentration in the hypocotyls. The bioconcentration factor (BCF) was calculated by dividing the metal concentration in the plant tissue (μg g<sup>-1</sup> DW) at harvest by the metal concentration in the Hoagland solutions. Heavy metal uptake was calculated using the following formula:

$$\text{Uptake } (\mu\text{g plant}^{-1} \text{ d}^{-1}) = (M_2W_2 - M_1W_1) / (T_2 - T_1)$$

Where, M<sub>1</sub> and M<sub>2</sub> are metal concentrations in the plant tissue, W<sub>1</sub> and W<sub>2</sub> are the plant biomass at time T<sub>1</sub> (initial sampling) and T<sub>2</sub> is the final sampling.

Total accumulation rate (TAR) (μg plant<sup>-1</sup> d<sup>-1</sup>) was calculated using the following formula (Zhu et al., 1999):

$$\text{TAR} = [(H_{\text{agp}} \times B_{\text{agp}}) + (H_{\text{bgp}} \times B_{\text{bgp}})] / [(B_{\text{agp}} \times B_{\text{bgp}}) \times 7]$$

Where H<sub>agp</sub> and H<sub>bgp</sub> are the heavy metal concentrations in the above (hypocotyls and cotyledons) and below (roots) ground plant parts and B<sub>agp</sub> and B<sub>bgp</sub> are the above- and below-ground plant biomass, respectively.

### Chlorophyll and carotenoid analysis

The plant materials (cotyledons) were extracted in 90% acetone and after that, the extracts were kept in the dark at 4°C for 24 h. The samples were then centrifuged (Heraeus Labofuge 400 R) at 3000 g (4°C) for 15 min. The pigment contents (chlorophyll a and b, total chlorophyll and carotenoid) were measured (Shimadzu 1601 UV-Visible spectrophotometer) and determined in μg g<sup>-1</sup> FW (Parsons and Strickland, 1963).

### Total protein assay

Plant samples (roots, hypocotyls and cotyledons) were homogenized with ice-cold 0.1 mM sodium phosphate buffer (pH 6.8). The homogenates were then centrifuged at 13,000 rpm for 30 min at 4°C and supernatants were used for the determination of total soluble protein content and total peroxidase enzyme assays. Protein content of the extracts were determined according to Bradford (1976) using bovine serum albumin (BSA) as standard and were defined quantitatively as μg ml<sup>-1</sup>.

### Guaiacol peroxidase (POD) activity assay

POD (guaiacol: H<sub>2</sub>O<sub>2</sub> oxidoreductase, EC 1.11.1.7) activity was measured according to the method of Birecka et al. (1973). The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.0), 0.25% guaiacol, 0.1% H<sub>2</sub>O<sub>2</sub> and enzyme extract. Increase in the absorbance due to oxidation of guaiacol ( $E=26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ) was measured at 470 nm (Shimadzu 1601 UV-Visible spectrophotometer). Enzyme activity was defined in terms of μmol of guaiacol oxidized min<sup>-1</sup> g<sup>-1</sup> fresh weight.

### Statistical analysis of data

All the experiments were performed in triplicates. Data were analyzed by the analysis of variance (ANOVA). Bars and "±" represented the standard deviations.

## RESULTS

### Zinc concentrations

Zinc content was assessed in the dormant (without imbibition) and control (Hoagland solution without zinc). The treatment seeds were exposed to increasing zinc concentrations (Table 1). Zinc content was almost identical in the dormant seeds ( $52 \text{ mg kg}^{-1}$ ) and in the seeds of the control group ( $50 \text{ mg kg}^{-1}$ ) grown for 24 h in Hoagland nutrition. However, zinc concentration in the seeds of the treatment group remarkable increased with increasing  $\text{ZnCl}_2$  concentrations. The concentrations of zinc measured in the seeds treated with especially 10 mM  $\text{ZnCl}_2$  were significantly higher ( $5426 \text{ mg kg}^{-1}$ ) than those measured in the control seeds. The lowest zinc concentration ( $1255 \text{ mg kg}^{-1}$ ) in radish seeds was observed in 1 mM  $\text{ZnCl}_2$  treatment, whereas zinc content in the seeds with 5 mM  $\text{ZnCl}_2$  concentration was detected as  $3352 \text{ mg kg}^{-1}$ .

The amount of zinc in the roots, hypocotyls and cotyledons of the 7-day old seedlings showed a parallelism with the increase in  $\text{ZnCl}_2$  concentrations (Table 2). The highest zinc concentration in the roots ( $46223 \text{ mg kg}^{-1}$ ) was observed in the highest zinc treatment (10 mM  $\text{ZnCl}_2$ ) when compared with the control ( $187 \text{ mg kg}^{-1}$ ). The lowest zinc concentration ( $123 \text{ mg kg}^{-1}$ ) in radish hypocotyls was found in the control treatment. Zinc concentration in radish hypocotyls in the lowest zinc treatment was only  $1366 \text{ mg kg}^{-1}$ , which increased progressively with increasing zinc application and reached  $20103 \text{ mg kg}^{-1}$  in the highest zinc treatment. Also, the zinc concentration of the cotyledons increased with increasing zinc doses and reached  $10673 \text{ mg kg}^{-1}$  in the 10 mM zinc treatment when compared with the control cotyledon ( $153 \text{ mg kg}^{-1}$ ). There was no difference in the zinc content in the cotyledon between 5 and 10 mM  $\text{ZnCl}_2$ .

### Uptake, total accumulation rate (TAR) and transport indices

In comparison with the control, uptake of zinc and TAR increased considerably with the increasing zinc concentration in the medium. The highest zinc uptake was observed in 10 mM zinc application and the uptake level remained steady in this application. Zinc uptake and TAR in the control radish plants were obtained as  $103 \text{ mg kg}^{-1} \text{ d}^{-1}$  and  $37 \text{ mg kg}^{-1} \text{ d}^{-1}$ , respectively. Compared with the control, total zinc uptake increased by 17% in 1 mM and 65% in 5 and 10 mM  $\text{ZnCl}_2$  treatments (Table 3).

Compared with the control, TAR as well as zinc uptake increased significantly with the increasing Zn concentration. The TAR for zinc showed a trend similar to that of zinc uptake. The TAR for zinc was highest in 10 mM  $\text{ZnCl}_2$  ( $4527 \text{ mg kg}^{-1} \text{ d}^{-1}$ ) treatments, while the lowest TAR ( $558 \text{ mg kg}^{-1} \text{ d}^{-1}$ ), was observed in radish plants

exposed to 1 mM  $\text{ZnCl}_2$ . Transport indices, such as primary and secondary transport, changed according to the increasing zinc concentrations (Table 3). In this study, the PTI of Zn decreased initially in 1 mM zinc treatment compared with the control, but thereafter gradually increased in the plants treated with 5 and 10 mM  $\text{ZnCl}_2$ . It was significantly lower in all the treatments applications than that in the control plants. The secondary transport of Zn was higher in 1 mM zinc treatment compared with the control, but decreased significantly at higher concentrations.

### Bioconcentration factor (BCF)

The effects of different treatments on BCF for Zn in the several plant parts are represented in Table 4. When compared with 1 mM  $\text{ZnCl}_2$  application, BCF for Zn was highest in the roots, followed by hypocotyl and then the cotyledon at higher concentrations (5 and 10 mM  $\text{ZnCl}_2$ ).

### Chlorophyll content

Application of zinc had a marked effect on the pigment content of radish plant. The pigment content ( $623 \mu\text{g g}^{-1}$  chlorophyll a,  $208 \mu\text{g g}^{-1}$  chlorophyll b,  $831 \mu\text{g g}^{-1}$  total chlorophyll and  $359 \mu\text{g g}^{-1}$  carotenoid) increased slightly in the cotyledons of the 7-day radish seedlings raised in 1 mM  $\text{ZnCl}_2$  medium, while the chlorophylls a, b, total chlorophyll and carotenoid content in the control group cotyledons were detected as 580, 206, 786 and  $337 \mu\text{g g}^{-1}$ , respectively. At 5 and 10 mM  $\text{ZnCl}_2$ , the total chlorophyll content and chl a/b ratio markedly decreased with increasing concentration of  $\text{ZnCl}_2$  in the medium (Table 5). Total chlorophyll and carotenoid content were slightly higher in the cotyledons of 1 mM  $\text{ZnCl}_2$  exposed radish seedlings compared with the control (Table 5). However, application of 5 and 10 mM  $\text{ZnCl}_2$  did significantly reduce the total amount of chlorophyll and carotenoid pigments in the cotyledons of radish seedlings. Total chlorophyll ( $464 \mu\text{g g}^{-1}$ ) and carotenoid content ( $191 \mu\text{g g}^{-1}$ ) at 5 mM  $\text{ZnCl}_2$  gradually decreased, reaching a minimum of total chlorophyll ( $404 \mu\text{g g}^{-1}$ ) and carotenoid ( $169 \mu\text{g g}^{-1}$ ) content in cotyledons at 10 mM  $\text{ZnCl}_2$ .

### Total soluble protein amount

The stress-induced total soluble protein amount was assessed in various parts of the seedlings grown in  $\text{ZnCl}_2$ -supplemented medium (Figure 1). The total protein amount in radish roots decreased with increasing concentration of  $\text{ZnCl}_2$  in the medium when compared with the control (no stress), but it remained steady in the cotyledons. The total protein content of the radish

**Table 1.** Zn concentrations of the seeds after 24 h of growth in the Hoagland nutrient solutions (control, 1, 5 and 10 mM ZnCl<sub>2</sub>) (mg kg<sup>-1</sup> DW).

Treatment	Zn content (mg kg <sup>-1</sup> DW)
Dormant seed	51.87 ± 8.03
Control	50.04 ± 8.86
1 mM	1254.82 ± 78.61*
5 mM	3351.55 ± 166.11*
10 mM	5425.54 ± 120.04*

Data are means ± standard deviations (SD); \* indicates values that differ significantly from the control at P < 0.0001.

**Table 2.** Zn concentrations in the roots, hypocotyls and cotyledons of the 7-day-old radish seedlings following the treatments in Hoagland nutrient solutions (control group and 1, 5 and 10 mM ZnCl<sub>2</sub> applications) (mg kg<sup>-1</sup> DW).

Treatment	Zn Content (mg kg <sup>-1</sup> DW)		
	Root	Hypocotyl	Cotyledon
Control	187.89 ± 23.52	123.86 ± 14.19	153.89 ± 9.98
1 mM ZnCl <sub>2</sub>	3654.53 ± 636.39*	1366.61 ± 236.17*	2595.20 ± 83.52*
5 mM ZnCl <sub>2</sub>	34489.09 ± 3988.32*	12343.58 ± 2866.97*	9611.36 ± 2007.68*
10 mM ZnCl <sub>2</sub>	46223.41 ± 5574.21*	20103.50 ± 1857.33*	10673.30 ± 3217.09*

Data are means ± SD; \* indicate values that differ significantly from the control at P < 0.0001.

**Table 3.** Total accumulation rate (TAR), uptake, primary transport index (PTI) and secondary transport index (STI) of zinc in radish plants raised in the medium with 1, 5 and 10 mM ZnCl<sub>2</sub> supplements.

Treatment	Uptake (mg kg <sup>-1</sup> d <sup>-1</sup> )	TAR (mg kg <sup>-1</sup> d <sup>-1</sup> )	PTI	STI
Control	103.42 ± 1.58	36.94 ± 3.76	0.65 ± 0.09	1.23 ± 0.22
1 mM ZnCl <sub>2</sub>	1716.41 ± 38.75*	558.13 ± 59.36*	0.37 ± 0.03*	1.90 ± 0.33*
5 mM ZnCl <sub>2</sub>	6489.04 ± 63.86*	3160.75 ± 98.88*	0.39 ± 0.16*	0.63 ± 0.13*
10 mM ZnCl <sub>2</sub>	6512.38 ± 06.86*	4527.78 ± 767.53*	0.45 ± 0.03*	0.53 ± 0.12*

Data are means ± SD; \* indicate values that differ significantly from the control at P < 0.0001.

**Table 4.** BCF for zinc in radish plants raised in the medium with 1, 5 and 10 mM ZnCl<sub>2</sub> supplements.

Treatment (mM ZnCl <sub>2</sub> )	Bioconcentration factor (BCF)		
	Root	Hypocotyl	Cotyledon
1	55.87 ± 9.73	20.89 ± 3.61	39.68 ± 1.28
5	105.69 ± 14.92	39.67 ± 9.65	25.04 ± 0.68
10	69.38 ± 9.26	30.74 ± 2.84	16.32 ± 4.92

Data are means ± SD.

hypocotyls increased slightly with increasing ZnCl<sub>2</sub> concentration in the medium. At 1 mM ZnCl<sub>2</sub>, protein content of the radish hypocotyls was 2449 µg/ml, whereas at 5 and 10 mM ZnCl<sub>2</sub>, it was 2840 and 2812 µg/ml, respectively. In the control, it was 2367 µg/ml (Figure 1).

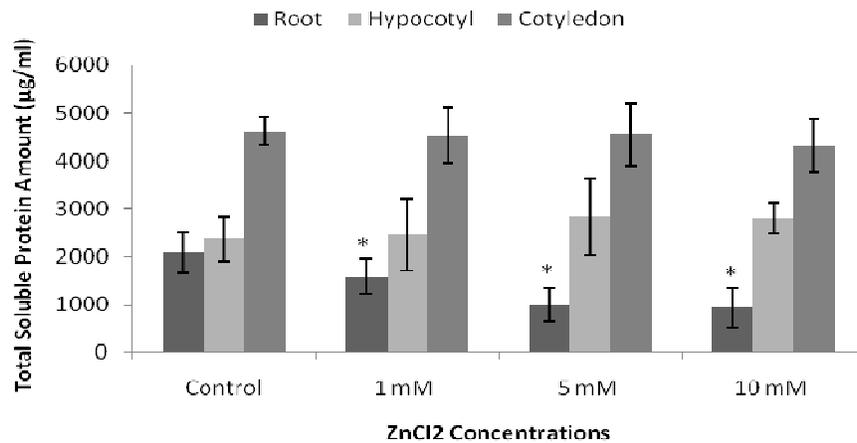
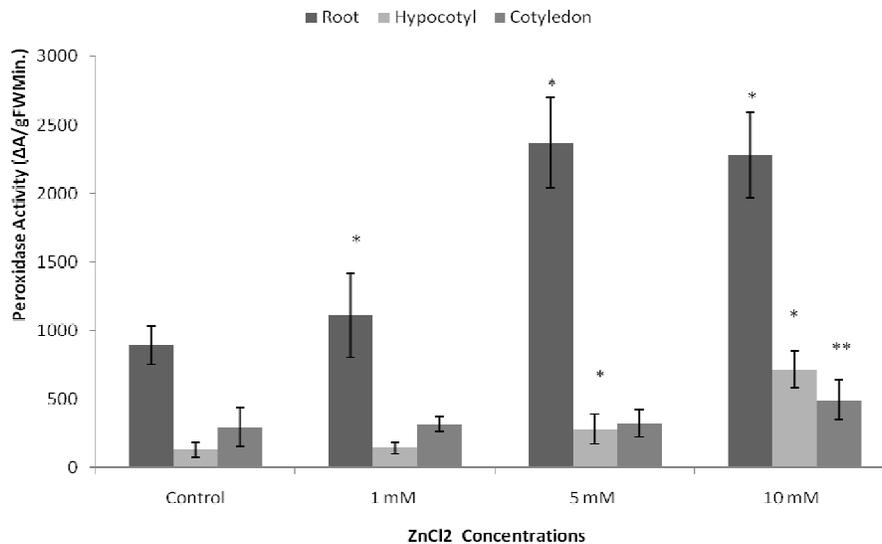
### Guaiacol peroxidase (POD) activity

As seen in Figure 2, POD activity especially in the radish roots increased significantly with increasing concentration of ZnCl<sub>2</sub> (5 and 10 mM) in the medium compared with the control. The activity of POD at 5 mM zinc reached 2369

**Table 5.** Chlorophyll a, chlorophyll b, total chlorophyll and caretenoid contents in the cotyledons of the 7-day-old radish seedlings following the treatments in Hoagland nutrient solutions (control group and 1, 5 and 10 mM ZnCl<sub>2</sub> applications) (µg/g FW).

Treatments	Chlorophyll a	Chlorophyll b	Chl a / Chl b	Total chlorophyll	Carotenoid
Control	580.00 ± 73.06	205.71 ± 23.84	2.82	785.71 ± 94.33	337.43 ± 40.18
1 mM ZnCl <sub>2</sub>	623.23 ± 130.95	208.15 ± 34.60	2.99	831.38 ± 163.48	358.62 ± 81.93
5 mM ZnCl <sub>2</sub>	334.63 ± 51.22*	129.63 ± 12.01*	2.58	464.25 ± 61.71*	191.00 ± 33.12*
10 mM ZnCl <sub>2</sub>	289.67 ± 58.73*	113.92 ± 21.94*	2.54	403.58 ± 71.68*	168.91 ± 20.65*

Data are means ± SD \* indicate values that differ significantly from the control at P < 0.0001.

**Figure 1.** Total protein contents in the roots, hypocotyls and cotyledons of the 7-day-old radish seedlings following the treatments in Hoagland nutrient solutions (control group and 1, 5 and 10 mM ZnCl<sub>2</sub> applications). Bars represent the SD; \* indicate values that differ significantly from the control at P < 0.0001.**Figure 2.** Peroxidase activity in the roots, hypocotyls and cotyledons of the 7-day-old radish seedlings following the treatments in Hoagland nutrient solutions (control group and 1, 5 and 10 mM ZnCl<sub>2</sub> applications) (ΔA/g FW min.). Bars represent the SD; \*, \*\* indicate values that differ significantly from the control at P < 0.0001 and P < 0.001, respectively.

and remained steady at 10 mM zinc in the roots and on the other hand, the roots of seedlings raised in the medium supplemented with 1 mM zinc showed 124% higher POD activity than that of the control. During the investigation, no significant change in the activity of POD was existent in the hypocotyls and cotyledons of the seedlings grown in the presence of 1 and 5 mM zinc compared with the control. However, an increase in POD activity by about 541 and 167% was observed in the hypocotyls and cotyledons of the seedlings raised in the presence of 10 mM zinc, respectively, when compared with the POD activity observed in the control.

## DISCUSSION

The radish seeds used for this study were treated with ZnCl<sub>2</sub>-supplemented medium for 24 h. Zinc amounts in the dormant and control radish seeds were approximately the same. However, significant differences were established when the radish seeds treated with increasing zinc concentrations were compared with the control group. The increase of zinc dose up to 10 mM caused an increase in zinc content in radish seeds. The highest content of zinc (5426 mg kg<sup>-1</sup>) was found in the seeds raised with 10 mM ZnCl<sub>2</sub> supplemented medium. Radish seeds effectively accumulated zinc. Stefanov et al. (1995) reported that, wheat seeds accumulated Zn (59.4 to 73.2 mg kg<sup>-1</sup>) when the concentrations of zinc in the soil was 30 mg kg<sup>-1</sup>.

The seedlings of radish were grown in Hoagland solutions with increasing ZnCl<sub>2</sub> supplements (treatment group) for 7 days. After 7 days, the roots, hypocotyls and cotyledons of the seedlings were harvested and their zinc content was analyzed. Zinc was found in all the plant organs, especially in the roots. With the increasing zinc concentrations, the amount of zinc in the plant parts also increased. The zinc concentration in the vegetative parts (root, hypocotyl and cotyledon) was in the order roots > hypocotyls > cotyledons. At 10 mM ZnCl<sub>2</sub>, zinc content in roots, (fold 247) and hypocotyls (fold 163) tissues increased considerably compared with the control. Under conditions of mild zinc toxicity (1 to 5 mg Zn kg<sup>-1</sup>) zinc accumulation was observed in all the plant parts with the highest accumulation in the roots. Vaillant et al. (2005) established that, zinc increased in the roots and the leaves of *Datura* species with increasing ZnSO<sub>4</sub> concentrations. In addition, they found that for the four *Datura* species, with 5 mM of zinc in the nutrient solution, the concentrations of zinc measured in the roots were higher than those measured in the leaves. The sufficient Zn concentration in the shoots of plant species ranges between 10 and 30 mg Zn kg<sup>-1</sup> dry weight (Marschner, 1993). The roots of higher plants were considered as a barrier against heavy metal translocation to the top parts (Wallace and Romney, 1977), reflecting a potential tolerance mechanism operating in the root cells.

Hagemeyer (1999) stated that, the organs which were in direct contact with zinc, such as the roots, were generally sensitive. In this study, even under conditions of severe Zn toxicity (10 mM ZnCl<sub>2</sub>), radish seedlings were able to survive up to the increased ZnCl<sub>2</sub> concentrations. It can be concluded that, the roots of radish seedlings were able to endure high levels of zinc and improved to a certain extent, the defense capability.

When compared with the control, uptake of zinc as well as TAR tended to increase significantly with increasing zinc concentration in the medium. Zn uptake and TAR were higher at the higher metal application rate than at the lower ones. Transport indices, such as primary and secondary, changed according to increasing zinc concentrations. The effects of different treatments on BCF for Zn in several plant parts are represented in Table 3. When compared with 1 mM ZnCl<sub>2</sub> application, BCF for Zn was higher in the 5 mM Zn concentration; it was highest in the roots, followed by the hypocotyls and then the cotyledons.

Plants treated with high zinc concentrations (5 and 10 mM ZnCl<sub>2</sub>) showed significant decreases in chlorophyll (Chl a, Chl b, Chl a/b) and carotenoid contents compared with the control, while (Table 3) the chlorophyll and carotenoid contents in the plants grown in nutrient solution with low zinc concentration (1 mM ZnCl<sub>2</sub>) increased (Table 5). With increasing zinc concentrations, the amount of chlorophyll pigment (total chl) in the cotyledons of radish seedlings decreased (Tables 3 and 5). The results are consistent with the findings of Kösesakal (2005), where this researcher showed that the chlorophyll and carotenoid contents of cotyledons in *Lycopersicon esculentum* Mill. increased at 1 to 5 mM ZnCl<sub>2</sub> concentrations, and decreased at 7 mM ZnCl<sub>2</sub>-supplemented medium. High zinc concentration caused the chlorophyll content in the upper and lower leaves of *Mentha spicata* to decrease (Bekiaroglou and Karataglis, 2002). Khurana and Chatterjee (2001) showed that, an increase in zinc from a low concentration (0.00065 mg l<sup>-1</sup>) to a high concentration (0.65 mg l<sup>-1</sup>) increased the content of chlorophyll a and b (up to 0.065 mg l<sup>-1</sup>) and it decreased from 0.65 to 65 mg l<sup>-1</sup> in sunflower leaves. Kaya and Higgs (2002) reported that, *L. esculentum* L. plants that were treated with 0.15 µmol l<sup>-1</sup> Zn in the nutrient solution and with high levels of zinc (3.5 mmol l<sup>-1</sup>) as a foliar spray, showed a significant decrease in the chlorophyll content when compared with those grown in the nutrient solution with 7.70 µmol l<sup>-1</sup> zinc. A significant decrease was seen in the total chlorophyll amount and chl a/b ratio, suggesting that the chlorophyll synthesizing system and chlorophyllase activity were affected at the higher concentrations of zinc (Van Assche and Clijsters, 1990).

As can be seen in Figure 1, there were no differences on the total soluble protein amounts between the control and zinc concentrations in all of the plant organs except the roots. Total protein content in *Bacopa* regenerants,

considerably increased in response to enhanced levels of ZnSO<sub>4</sub> (Ali et al., 1999). Khurana and Chatterjee (2001) also found that, total protein amount decreased in sunflower leaves with increasing ZnSO<sub>4</sub> concentrations. Farshian et al. (2007) showed that, the total protein content reduced in lettuce plants, which might be caused by the toxic effects of Zn on protein synthesis. The protein concentration decreased in *Brassica juncea* seedlings due to metal treatment, particularly at the highest Zn concentration (Sharma et al., 2007).

Zinc treatments caused significant increases in the POD activity in all the parts of the radish seedlings, especially in the roots (Figure 2). Chaoui et al. (1997) reported an increase in POD activity in bean plants under zinc toxicity. Significant increase in the activity of POD was observed in the shoots of 10-day-old *B. juncea* seedlings under zinc toxicity (Prasad et al., 1999). Sharma et al. (2007) also reported that, different concentrations of Zn-metal treatment decreased the POD activity in the 7-d-old seedlings of *B. juncea* L. In plants, the detoxification of H<sub>2</sub>O<sub>2</sub> has been known to be an important function of the peroxidases (Asada, 1994). An increase in POD activity in radish seedlings exposed to ZnCl<sub>2</sub> suggested its role in the detoxification of H<sub>2</sub>O<sub>2</sub>. However, under the conditions which promote the production of active oxygen species, a concurrent enhancement in the expression of the genes for the synthesis of various antioxidant enzymes was observed (Foyer et al., 1994, 1997; Prasad et al., 1999).

In conclusion, we found that there was a parallelism between the increasing zinc concentrations and zinc uptake as well as TAR in the early growth stages of the radish seedlings. In particular, it was thought that the biomass of cotyledons may be negatively affected in the advanced growth stages due to the phytotoxic effects of zinc on the pigments in the cotyledons. It is suggested that, radish plant could be a tolerant species due to the reduction of the amount of protein in the roots, and the increase in the POD activity in the higher zinc accumulation.

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