СРАВНИТЕЛНО ПРОУЧВАНЕ НА ТОКСИЧНИТЕ ЕФЕКТИ НА КАДМИЯ И ЦИНКА ВЪРХУ ОКИСЛИТЕЛНО-РЕДУКЦИОННОТО СЪСТОЯНИЕ В КЛЕТКИТЕ НА РАСТЕНИЯ ОТ ТВЪРДА ПШЕНИЦА
COMPARATIVE STUDY OF CADMIUM AND ZINC TOXIC EFFECTS ON THE CELL REDOX STATUS OF DURUM WHEAT PLANTS

Любка Колева-Вълкова[1], Андон Василев[1], Ан Кюпърс[2], Джако Вангронсвелд[2]
Lyubka Koleva-Valkova[1], Andon Vasilev[1*], Ann Cuypers[2], Jaco Vangronsveld[2]

[1]Катедра по физиология на растенията и биохимия, Аграрен университет – Пловдив
[2]Хаселтски Университет, Център за екологични науки, Агоралаан Блок Д, Б-3590, Диипенбиик, Белгия
[1]Department of Plant Physiology and Biochemistry, Agricultural University - Plovdiv
[2]Hasselt University, Centre for Environmental Sciences, Agoralaan, Building D, B-3590 Diepenbeek Belgium

E-mail: a_vasilev2001@yahoo.com

Резюме

Млади растения от твърда пшеница, отглеждани като хидропонна култура, бяха третирани с кадмий (Cd) и цинк (Zn) в концентрации (50 μM и 600 μM, съответно), причиняващи приблизително 50% инхибиране на относителната скорост на растежа в края на експерименталния период (10-и ден). Двата метала предизвикваха развитието на визуални токсични симптоми при младите пшенични растения като кореново покафеняване и листна хлороза под въздействие на Cd и изсветляване на корените и поява на некротични петна в листата под въздействие на Zn. На биохимично ниво фитотоксичността беше придружена с прояви на окислителен стрес, като повишена липидна пероксидация и електролитно изтичане, както и с повишаване на ензимната активност на супероксидната дисмутаза и каталазата.

Abstract

Cadmium (Cd) and zinc (Zn) were applied to durum wheat seedlings on hydroponics in concentrations of 50 μM and 600 μM respectively, causing about 50% relative growth inhibition (RGI) at the end of a 10-day-exposure period. Both metals provoked visual toxicity symptoms such as root browning and leaf chlorosis in Cd-exposed seedlings and root colour lightening and leaf necrotic spots in Zn-exposed seedlings. On a biochemical level, the apparent phytotoxicity was accompanied by oxidative stress-related responses such as increased lipid peroxidation and electrolyte leakage as well as increased activities of superoxide dismutase and catalase.

Ключови думи: кадмий, твърда пшеница, ензимна активност, окислителен стрес, цинк.
Key words: cadmium, durum wheat, enzyme activity, oxidative stress, zinc.

Съкращения:
CAT - каталаза
SOD – супероксидна дисмутаза
TBA – тиобарбитурова киселина (4,6-дихидрокси-2-меркаптопиримидин)
RGR – относителна скорост на растежа

Abbreviations:
CAT - catalase
SOD – superoxide dismutase
TBA – thiobarbituric acid (4,6-Dihydroxy-2-mercaptopyrimidine)
RGR – relative growth rate

INTRODUCTION

Cadmium (Cd) and zinc (Zn) are well known environmental pollutants, which often appear together in industrially contaminated soils creating potential risks for both human and environmental health (Sanita di Toppi and Gabbrielli, 1999; Rout and Das, 2003). Phytotoxicity is one of the critical problems on metal-contaminated soils, which attracts both public and scientific attention. Presently, there exists a general assumption that Cd- and Zn phytotoxicity are partly due to induction of oxidative stress at the cellular level (Rout and Das, 2003; Khan et al., 2007; Smeets et al., 2008; Cuypers et al., 2010), which in turn is transcribed

in integral physiological responses resulting in growth inhibition and loss of productivity (Vangronsveld and Clijsters, 1994).

Due to similar symptoms of phytotoxicity provoked by both metals, an opinion exists that non-specific traits in Cd and Zn mode of action predominate over the specific ones (Clemens, 2006; Wojcik et al., 2006). In fact, the visible toxicity symptoms in Cd- and Zn-exposed plants are rather similar. They include chlorosis and necrosis (Zn mainly of young leaves and Cd mainly of older ones), stunted root branching and tip browning, and general reduction of plant growth.

However, from biological point of view, Cd and Zn are very different: Cd is a trace element without any known biological function, while Zn is an essential nutrient having significant importance for cell metabolism. Both excess Zn and Cd can cause phytotoxicity (Cuypers et al., 1999; Rout and Das, 2003; Vassilev et al., 2007). Nevertheless, it might be expected that their impact on plant functioning, including on the cellular redox state, differ to some extend. Literature survey shows that comparative studies on this aspect are quite scarce. On the other hand, part of the existing information should be handled with caution due to significant variation of the used experimental designs especially concerning concentrations and duration of the metal exposure.

Generally, oxidative stress is defined as a physiological state, where the levels of reactive oxygen species (ROS) (such as superoxide, hydrogen peroxide, hydroxyl radical, etc.) get increased causing cellular damage to all important macromolecules (lipids, nucleic acids, proteins, amino acids, carbohydrates, etc.) (Lagriffoul et al., 1998). For their protection against oxidative stress, cells are equipped with an antioxidative defence system existing of both enzymatic (e.g. superoxide dismutases (SOD), catalases (CAT), peroxidases (POD), enzymes involved in the glutathione-ascorbate cycle) and non-enzymatic (e.g. ascorbate, glutathione, phenolic compounds) compounds (Dietz et al., 1999).

Cadmium and Zn can modulate the activities of antioxidative enzymes and the levels of non-enzymatic compounds and their redox state (Foyer et al., 1997; Schutzendubel and Pole, 2002; Lin et al., 2007; Smeets et al., 2008; Cuypers et al., 2010). The effects of these metals on plant functioning strongly depend on plant species or even cultivar tolerance. In wheat plants, exposure to toxic Cd concentrations was shown to induce several enzymes such as superoxide dismutases, catalases, guaiacol peroxidases and ascorbate peroxidases and increases MDA levels (Milone et al., 2003; Zhao et al., 2005; Lin et al., 2007; Singh et al., 2008), while the information about effects of Zn is scarce (Zhao et al., 2005).

In order to elucidate weather there exists any specificity in the phytototoxic mode of action of Cd and Zn, we performed comparative studies using wheat plants. The applied metal concentrations were chosen based on the induction of equal (about 50%) inhibition of relative growth rate (RGR). Using wheat plants exposed to the same metal concentrations, we already reported some Cd induced xeromorphic changes in leaf anatomy such as increasing the number of the epidermal cells and stomata density, which were not observed in Zn exposed plants (Koleva et al., 2010). The present paper concentrates on the cellular redox state of Cd- and Zn-exposed wheat plants and on some peculiarities in metal responses.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *Triticum durum* Desf. *cv*. Beloslava were germinated on wet filter paper and subsequently transferred to plastic pots with modified half strength Hoagland nutrient solution. The solution was refreshed every other day and continuously aerated. Young plants were cultivated under controlled conditions: 250 μmol m^{-2} s^{-1} light intensity, 26/22ºC day/night temperature, 16/8 h photoperiod and a constant relative humidity (65%) for 18 days. When plants were 8-day-old, Cd and Zn were added to the nutrient solution in a sulfate form at final concentrations of 50 μM and 600 μM respectively. The experimental design consisted of three treatments in triplet: (1) untreated plants (control), (2) Cd- and (3) Zn-exposed wheat plants. In earlier range finding experiments, the applied concentrations were shown to cause approximately 50% inhibition of the relative growth rate (RGR). Plants were harvested at different time intervals after the start of exposure: 30 min, 1 h, 3 h, 7 h, 3 d, 7 d and 10 d. Plants from each treatment (control, Cd exposed and Zn exposed) were divided into roots and leaves. Each sample was immediately snap frozen in liquid nitrogen and stored at -80ºC until analysis.

Enzyme assays

Frozen material (1 g FW) was homogenized using a Polytron PT 3000 homogenizer in 5 mL of ice-cold 0.1 M Tris-HCl buffer (pH 7,8) containing 1 mM DTT and 1 mM EDTA, and centrifuged at 13 500 g (at 4ºC for 10 min). The supernatant was collected and the activities of six enzymes involved in antioxidative defence were determined spectrophotometrically.

Superoxide dismutases (SOD, EC 1.15.1.1) catalyze the conversion of superoxide radicals into dioxygen and hydrogen peroxide, the $O_2^{\cdot-}$ generating system consists of xanthine oxidase and xanthine. In the blank cuvette, cytochrome c is reduced by the formed superoxide radicals and this reaction is followed at 550 nm. Addition of the extract results in a disproportion of the superoxide radicals and hence an inhibition of the reduction of cytochrome c, which is a measure to define SOD capacity. The amount of SOD required to inhibit the rate of reduction of cytochrome

c by 50% is defined as 1 unit of activity (McCord and Fridovich, 1969).

Catalase (CAT, EC, 1.11.1.6) activity was assayed based on disappearance of H_2O_2 following the method of Aebi (1984).

Lipid peroxidation and electrolyte leakage

The TBA reactive compounds of the plant tissues were determined spectrophotometrically to estimate the level of lipid peroxidation. The amount of TBA reactive compounds (TBARC) was calculated from the difference in specific absorbance at 532 nm and non-specific absorbance at 600 nm (De Vos et al., 1989) using an extinction coefficient of 155 mM^{-1} cm^{-1}. Electrical conductivity was determined with a HANNA Instruments (HI 255 Combined Meter, Padova, Italy) conductometer. Leaves from each treatment were excised and washed twice to remove the contents of the cut cells. Samples were incubated in 5 ml of double distilled water and conductivity was measured at different time intervals.

STATISTICS

Statistical analysis of the data obtained was performed using one-way ANOVA (for $P < 0,05$). Based on ANOVA results, à Tukey's test for the main comparison at a 95% confidential level was applied.

RESULTS

Phytotoxic symptoms

The first visible toxicity symptoms after the start of exposure of the wheat plants to Cd and Zn were the reduction of both root and shoot elongation with increasing exposure time. In addition, Cd exposure resulted in browning and a stronger inhibition of root growth and branching, whereas Zn caused roots becoming lighter in color and thinner in diameter. From the third day of exposure different effects became evident in the older leaves of Cd- and Zn-exposed plants. While Zn exposure caused necrotic spots, Cd exposure induced chlorotic spots. These symptoms were most pronounced at the end of the experimental period. In the younger leaves of both Cd- and Zn-exposed plants chlorosis was occurring (Koleva et al., 2010). The observed symptoms might be caused due to the accumulation of the metals in leaves and roots, respectively at very high concentrations - 50 and 936 mg Cd kg^{-1} DW and 880 and 3029 mg Zn kg^{-1} DW) (Koleva, 2010).

Modulation of activities of antioxidative enzymes

Organisms respond to a disturbed cellular redox balance by altering their antioxidative defence system in order to reach a new equilibrium. We investigated the changes of activity of two enzymes involved in antioxidative defence including superoxide dismutases and catalases as a first line of defence. All measurements were performed at several time intervals from 30 min up to 240 h after the start of the exposure to Cd and Zn. In this way, we aimed to include the early (based on 'available' enzymes) and the later (based on *de novo* synthesis) responses.

In our experiments, Cd and Zn-exposure tended to increase the total SOD activity in roots, being significant only after longer exposure (72 h) (Table 1). After one hour of Cd exposure resulted in an increased SOD activity in leaves, while at 72 h an opposite trend was observed. The enzyme activity in leaves did not change after one hour Zn-exposure, being significantly decreased thereafter. In work with sunflower and similar concentrations of Cd (50 and 100 μM) it was reported that total SOD activity increased 48 h after the start of Cd exposure and then decreased after 96 h exposure (Hatata and Abdel-Aal, 2008). The responses of SOD could show distinctive patterns among different plants species exposed to metal stress due to presence of different isoenzymes or other AOS scavenging systems.

Cadmium exposure have a significant inhibiting effect on CAT activities in leaves measured 3 h after exposure (Table 2) which did not corresponds with results reported by Hegedus et al. (2001). The reduction of enzyme activity was almost 50% than the control. Different responses were observed in the roots – decreased CAT activity as an early effect only at 1 h after Cd exposure and enhanced activity as a late one (3-, 72- and 240 h). Leaves of Zn-exposed plants showed a noticeable increase in the activity of CAT almost in all experimental period except the very early inhibition at time 30 min after the exposure. While in roots this element had significant increasing effect on enzyme activity at time 30 min and 3 h after the exposure (Table 2).

Lipid peroxidation and electrolyte leakage

In order to verify whether exposure to Cd and Zn caused oxidative damage in durum wheat tissues, lipid peroxidation and membrane leakage were investigated. From day 3, Cd exposure increased the levels of TBA reactive compounds in both leaves and roots (not shown). Zinc exposure lead to a more severe effect at the leaf level. This might be due the preferential localization of the metals and the higher mobility of zinc (Rout and Das, 2003, Lin et al., 2007). The data presented in Figure 1 shows the results at the end of experimental period (day 10), indicating clear membrane damage.

Another important indicator of cell membrane damage is electrolyte leakage. We determined electrical conductivity (in μS) in the aqueous media at different time intervals in leaf tissue (Figure 2). Our results indicate that Cd caused more membrane damage compared to Zn; the electrolyte leakage for Cd could be described with exponential equation ($y = 71,229e^{0,3838x}$, $R^2 = 0,9766$), while for Zn the subordination was linear ($y = 94,931x - 54,998$, $R^2 = 0,9597$) (Figure 2).

Таблица 1. Изменение в ензимната активност на СОД при млади растения от твърда пшеница сорт "Белослава", изложени на въздействието на Cd и Zn в концентрации, причиняващи 50% инхибиране на относителната скорост на растежа (50 μM и 600 μM, съответно). Активността беше измервана както следва: t1 = 0,5 час, t2 = 1 час, t3 = 3 часа, t4 = 7 часа, t5 = 72 часа; t6 = 168 часа и t7 = 240 часа след началото на третирането. Данните са представени като U g^{-1} FW. Стойностите, следвани от еднаква буква (a, b или c) в колоната за всички групи, не са статистически различни при ниво на достоверност P<0,05

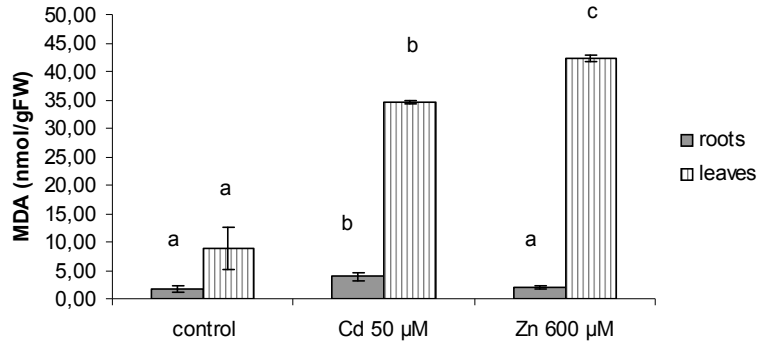
Table 1. Enzyme activity modulation of SOD in young durum wheat plants cultivar Beloslava exposed to Cd and Zn in concentration caused 50% growth inhibition (50 μM and 600 μM, respectively). The activity was measured as follow: t1 = 0,5 hour, t2 = 1 hour, t3 = 3 hour, t4 = 7 hour, t5 = 72 hour; t6 = 168 hour and t7 = 240 hour after treatment. The data are presented as U g^{-1} FW. The values followed by the same letter (a, b or c) within a column in an all groups are not significantly different at P<0,05

Групи Groups	Растителен орган Plant organ	t1	t2	t3	t4	t5	t6	t7
Контрола Control	Корен Root	7,9a	8,7a	8,2a	8,4a	8,3a	8,8a,b	8,7a
	Лист Leaf	5,1a	6,8a	6,8a	7,7a	7,1b	7,3a	6,3a
50 μM Cd	Корен Root	8,8a	9,2a	8,2a	7,8a	9,1b	7,9a	7,8a
	Лист Leaf	5,5a	9,9b	6,7a	6,7a	6,3a	5,3a	6,4a
600 μM Zn	Корен Root	8,2a	9,8a	8,7a	8,6a	9,3b	8,9b	7,7a
	Лист Leaf	5,1a	6,5a	6,4a	6,7a	5,8a	7,6a	6,7a

Таблица 2. Изменение в ензимната активност на КАТ при млади растения от твърда пшеница сорт "Белослава", изложени на въздействието на Cd и Zn в концентрации, причиняващи 50% инхибиране на относителната скорост на растежа (50 μM и 600 μM, съответно). Активността беше измервана както следва: t1 = 0,5 час, t2 = 1 час, t3 = 3 часа, t4 = 7 часа, t5 = 72 часа; t6 = 168 часа и t7 = 240 часа след началото на третирането. Данните са представени като U g^{-1} FW. Стойностите, следвани от еднаква буква (a, b или c) в колоната за всички групи, не са статистически различни при ниво на достоверност P<0,05

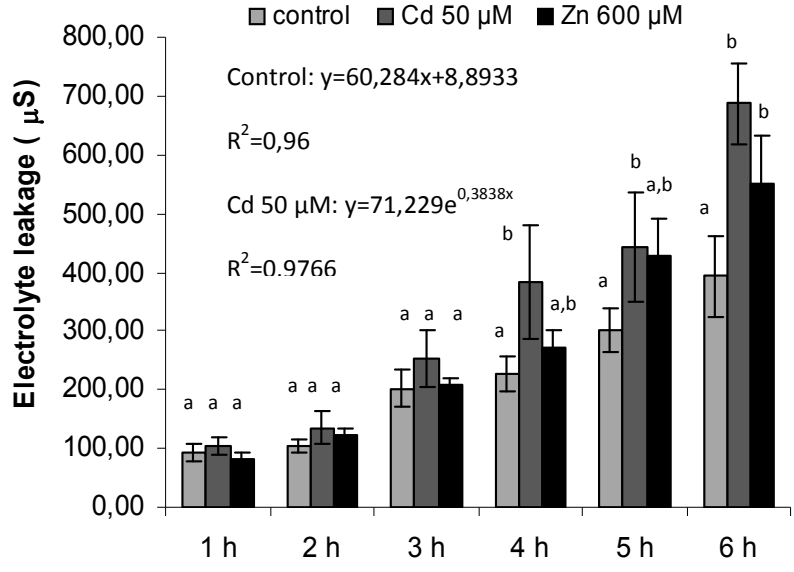
Table 2. Enzyme activity modulation of CAT in young durum wheat plants cultivar Beloslava exposed to Cd and Zn in concentration caused 50% growth inhibition (50 μM and 600 μM, respectively). The activity was measured as follow: t1 = 0,5 hour, t2 = 1 hour, t3 = 3 hour, t4 = 7 hour, t5 = 72 hour; t6 = 168 hour and t7 = 240 hour after treatment. The data are presented as U g^{-1} FW. The values followed by the same letter (a, b or c) within a column in an all groups are not significantly different at P<0,05

Групи Groups	Растителен орган Plant organ	t1	t2	t3	t4	t5	t6	t7
Контрола Control	Корен Root	2,35a	1,76b	1,14a	4,40a	4,36a	4,11a	4,3a,b
	Лист Leaf	19,3b	13,45a	16,7b	8,5a	11,35a	19,9a,b	18,4a
50 μM Cd	Корен Root	2,9a,b	1,25a	2,23c	4,14a	6,17b	3,7a	5,8b
	Лист Leaf	17,5b	13,8a	9,41a	9,54a	15,6a,b	17,7a	18,7a
600 μM Zn	Корен Root	4,1b	1,70b	1,61b	4,08a	3,98a	5,06a	3,9a
	Лист Leaf	11,8a	15,1b	21,9c	15,48b	16,9b	24,3b	20,8a



Фиг. 1. *Липидна пероксидация при млади растения от твърда пшеница, сорт "Белослава", изложени на въздействието на Cd и Zn за 10 дни в концентрации, причиняващи 50% инхибиране на относителната скорост на растежа (50 µM и 600 µM съответно). Стойностите, означени с еднаква буква (a, b или c) над колоната за корените или за листата, не са статистически различни при ниво на достоверност P<0,05*

Fig. 1. *Lipid peroxidation in young durum wheat cultivar Beloslava exposed to Cd and Zn for 10 days in concentration caused 50% growth inhibition (50 µM and 600 µM, respectively). The values marked by the same letter (a, b or c) above the bar for roots or for leaves are not significantly different at P<0,05*



Фиг. 2. *Електролитно изтичане в листата на растения от твърда пшеница, сорт "Белослава", изложени на въздействието на Cd и Zn за 10 дни. Стойностите, означени с еднаква буква (a, b или c) над колоната за различните групи, не са статистически различни при ниво на достоверност P<0,05.*

Fig. 2*. Electrolyte leakage in leaves of durum wheat cultivar Beloslava exposed to Cd and Zn for 10 days. The values marked by the same letter (a, b or c) above the bar for all groups are not significantly different at P<0,05*

DISCUSSION

In plants, toxic amounts of Cd and Zn cause severe physiological and morphological effects such as stunted growth, chlorosis, and necrosis and decreased yield (Chaney, 1993, Sanita di Toppi and Gabbrielli, 1999). At the cellular level, these metals interact with biomolecules such as proteins and nucleic acids, and are known to affect enzyme activities; extra free radicals are accumulated in the cells because the balance between pro-oxidants and antioxidants is disturbed (Cuypers et al., 2010). This causes alteration in membrane permeability, which we also observed (Figure 2). Membrane lipid peroxidation (Figure 1) is a very sensitive response induced by metal stress and is initiated by a number

of ROS and/or by the enzyme lipoxygenase (LOX) that is also the initial step in oxylipin biosynthesis (De Vos et al., 1989; Smeets et al., 2009). Besides ROS, oxylipins also seem to play a role in Cd- and/or Cu induced responses (Maksymiec and Krupa, 2006). Lipid peroxidation due to free radical action was taking place in our durum wheat plants (Figure 1). Increased levels of TBA-reactive compounds were reported in many plants under metal exposure (De Vos et al., 1989; Chen, 1991; Asada, 1994; Cuyper et al., 2010). Because of these toxic effects of ROS, it is key to keep their production and detoxification under tight control. Sequential and contemporaneous action of antioxidants, such as ascorbate and glutathione with catalases, superoxide dismutases and peroxidases makes up the cells antioxidative power that maintains the cellular redox homeostasis within certain limits (Mittler et al., 2004).

Early effects such as changes in membrane permeability and/or activities of antioxidative enzymes such as peroxidases can be detected before the appearance of any visible symptoms of toxicity (Van Assche and Clijsters, 1990; De Vos et al., 1991). The increased metal concentrations in the tissues might induce increased activities of some enzymes either as a result of *de novo* protein synthesis or by the activation of enzymes already present in plant cells. However, the high affinity of metals for sulphydryl groups (-SH) is suggested to be one of the main mechanisms of enzyme inhibition (Vangronsveld and Clijsters, 1994). In principle, two main mechanisms of enzyme inhibition can occur: (1) binding of the metal to sulfhydryl groups, involved in the catalytic action or structural integrity of enzymes and (2) deficiency of an essential metal in metalloprotein complexes, combined with the substitution of the toxic metal for the deficient element (Vangronsveld and Clijsters, 1994).

Catalase which possess low affinity to H_2O_2 and present mainly in peroxisomes, is associated with the processing of H_2O_2 generated in photorespiration (Vandenabeele et al., 2004), being also important in cases of excessive H_2O_2 formation, such as oxidative stress (Mittler, 2002). Variation in CAT activity due to Cd stress may be partly attributed to possible variation among species, growth stage, stress intensity, and duration of exposure.

The fundamental biochemical mechanisms of Zn phytotoxicity have not been identified for any plant. However, different responses have been observed. Probably Zn interferes with Fe uptake, translocation, or utilization in the leaves e.g. in chlorophyll biosynthesis (Chaney, 1993). Boawn and Rasmussen (1971) reported that plants suffering from Zn phytotoxicity had lower shoot P levels. Some studies have been conducted on the inhibition of enzymes from plants with different levels of Zn tolerance (Mathys, 1980; Woolhouse, 1983). However, it is not clear if Zn tolerance or susceptibility to Zn phytotoxicity is related to any particular enzymatic activity in any species.

In conclusion, when Cd and Zn were applied at concentrations causing 50% RGR inhibition in young durum wheat plants, these plants obviously evolved oxidative stress and showed induction of antioxidative enzymes such as SOD and CAT. These enzymes provide antioxidant protection and protect membrane integrity. Both metals caused different effects about CAT actitity in roots and leaves. Zinc mostly activated enzyme activity into the leaves, while Cd in roots. Also these metals caused membrane damages manifested with lipid peroxidation and electrolyte leakage.

REFERENCES

Aebi, H., 1984. Catalase. – In: *Methods in enzymology, eds.* L Packer, 121–126. Academic Press, Orlando.

Asada, K., 1994. Production and action of active oxygen species in photosynthetic tissues. – In: *Causes of photooxidative stress and amelioration defense systems in plants*, *eds.* C.H. Foyer and P.M. Mullineaux, 77–104. CRC Press, Boca Raton, FL.

Boawn, L.C. and P.E. Rasmussen, 1971. Crop response to excessive zinc fertilization of alkaline soil. - *Agronomy Journal,* 63, (6) 874-876.

Chaney, R.L., 1993. Zinc phytotoxicity. – In: *Zinc in Soils and Plants*, *eds.* A.D. Robson, 135-150. Kluwer Academic Publ. Dordrecht.

Chen, S.Y., 1991. Risk of membrane fat peroxidation to plant cells. *Chinese Bulletin. - Plant Physiol*, 27 (2): 82-90.

Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. - *Biochimie*, 88: 1707-1719.

Cuypers, A., J. Vangronsveld, H. Clijsters, 1999. The chemical behavior of heavy metals plays a prominent role in the induction of oxidative stress. - *Free Radical. Research,* 31: 39-43.

Cuypers, A., M. Plusquin, T. Remans, M. Jozefczak, E. Keunen, H. Gielen, K. Opdenakker, A.R. Nair, E. Munters, T.J. Artois, T. Nawrot, J. Vangronsveld, K. Smeets, 2010. Cadmium stress: an oxidative challenge. - *Biometals,* 23: 927–940.

De Vos, R.H.C., H. Schat, R. Vooijs, W.H.O. Ernst, 1989. Copper-induced damage to the permeability barrier in roots of *Silene cucubalus. - Journal of Plant Physiology,* 135: 164-169.

De Vos, R.H.C. and H. Schat, 1991. Free radicals and heavy metal tolerance. – In: *Ecological responses to environmental stress*, eds. J. Rozema and J. Verkleij, 22–30. AC Kluwer Publishers, The Netherlands.

Dietz, K.J., U. Kramer, M. Baier, 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity. – In: *Heavy Metal Stress in Plants: From Molecules to Ecosystems, eds.* M.N.V. Prasad and J. Hagemeyer, 73-97. Springer-Verlag Berlin.

Foyer, C.H., H. Lopez-Delgado, J.F. Dat, I.M. Scott, 1997. Hydrogen peroxide- and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. - *Physiologia Plantarum,* 100: 241-254.

Hatata, M. and E. Adel Abdel-Aal, 2008. Oxidative stress and antioxidant defense mechanisms in response to cadmium treatments. - *American-Eurasian Journal of Agriculture & Environmental Science,* 4 (6): 655-669.

Hegedus, A., S. Erdei, G. Horvath, 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. - *Plant Science,* 160: 1085–1093.

Khan, N.A., S. Samuillah Singh, R. Nazar, 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. - *J. Agronomy & Crop Science,* 193: 435-444.

Koleva, L., I. Semerdjiieva, A. Nikolova, A. Vassilev, 2010. Comparative morphological and histological study on zinc – and cadmium – treated durum wheat plants with similar growth inhibition. - *General and Applied Plant Physiology,* 36 (1-2): 10-13.

Koleva, L., 2010. Mineral nutrients content in zinc – and cadmium – treated durum wheat plants with similar growth inhibition. - *General and Applied Plant Physiology,* 36 (1-2): 62-65.

Lagriffoul, A., B. Mocquot, M. Mench, J. Vangronsveld, 1998. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). - *Plant Soil,* 200: 241-250.

Lin, R., X. Wang, Y. Luo, W. Du, H. Guo, D. Yin, 2007. Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). - *Chemosphere,* 69: 89-98.

Maksymiec, W. and Z. Krupa, 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. - *Environmental and Experimental Botany,* 57: 187-194.

Mathys, W., 1980. Zinc tolerance by plants. – In: *Zinc in the Environment. Part2 Health Effects, eds.* J.O. Nriagu 415-437. Wiley-Interscience, New York.

McCord, J. and I. Fridovich, 1969. The reduction of cytochrome c by milk xanthine oxidase. - *Journal Biological Chemistry,* 243: 5753-5760.

Milone, M.T., C. Sgherri, H. Clijsters, F. Navari-Izzo, 2003. Antioxidative responses of wheat treated with realistic concentration of cadmium. - *Environmental and Experimental Botany,* 50: 265-276.

Mittler, R., S. Vanderauwera, M. Gollery, F.V. Breusegem, 2004. Reactive oxygen gene network of plants. - *Trends Plant Science,* 9: 490-498.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. – *Trends Plant Science,* 7: 405-410.

Rout, G. R. and P. Das, 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc. - *Agronomie,* 23: 3-11.

Sanita di Toppi, L. and R. Gabbrielli, 1999. Response to cadmium in higher plants. *Environmental and* - *Experimental Botany,* 41: 105-130.

Schutzendubel, A. and A. Pole, 2002. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. - *Journal of Experimental Botany,* 53:1351-1365.

Singh, H.P., D.R. Batish, G. Kaur, Arora, R.K. Kohli, 2008. Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. - *Environmental and Experimental Botany,* 63: 158-167.

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

Smeets, K., K. Opdenakker, T. Remans, S. Van Sanden, F. Van Belleghem, B. Semane, N. Horemans, Y. Guisez, J. Vangronsveld, A. Cuypers, 2009. Oxidative stress-related responses at the transcriptional and enzymatic levels after exposure to Cd or Cu in a multipollution context. - *Journal of Plant Physiology,* 166: 1982-1992.

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

Vandenabeele, S., S. Vanderauwera, M. Vuylsteke, S. Rombauta, C. Langebartels, H. K. Seidlitz, M. Zabeau, M.V. Montagu, D. Inze, F.V. Breusegem, 2004. Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana*. - *Plant Journal,* 39: 45-58.

Vangronsveld, J. and H. Clijsters, 1994. Toxic effects of metals. – In: *Plants and the chemical elements,* eds. M.E. Farago, 149-177. Weinheim, Germany: VCH Verlagsgesellschaft, New York, USA, VCH Publishers.

Vassilev, A., A. Perez-Sanz, A. Cuypers, J. Vangronsveld, 2007. Tolerance of two hydroponically grown *Salix* genotypes to excess Zn. - *Journal of Plant Nutrition,* 30: 1472-1482.

Wojcik, M., E. Skorzynska-Polit, A. Tukiendorf, 2006. Organic acid accumulation and antioxidant enzyme activities in *Thlaspi caerulescens* under Zn and Cd stress. - *Plant Growth Regulation,* 48: 145-155.

Woolhouse, H. W., 1983. Toxicity and tolerance in the responses of plants to metals. – In: *Physiological Plant Ecology III: Responses to the Chemical and Biological Environment, eds.* O.L. Lange et al., 246-300. Springer-Verlag, New York.

Zhao, Z., Y. Zhu, Y. Cai, 2005. Effects of zinc on cadmium uptake by spring wheat (*Triticum aestivum* L.): long-time hydroponic study and short-time ^{109}Cd tracing study. - *Journal Zhejiang University Science,* 6A (7): 643-648.

Zhao, Z., Y. Cai, Y. Zhu, R Kneer, 2005. Cadmium-induced oxidative stress and protection by L-Galactono-1,4-lactone in winter wheat (*Triticum aestivum* L.). - *Journal Plant Nutrition and Soil Science,* 168: 759-763.

ACKNOWLEDGEMENTS

We thank the Bulgarian National Science Fund, Project № DO 02-88/2008 for the financial support.

Статията е приета на 20.12.2011 г.
Рецензент – доц. д-р Невена Стоева
E-mail: stoevanevena@abv.bg