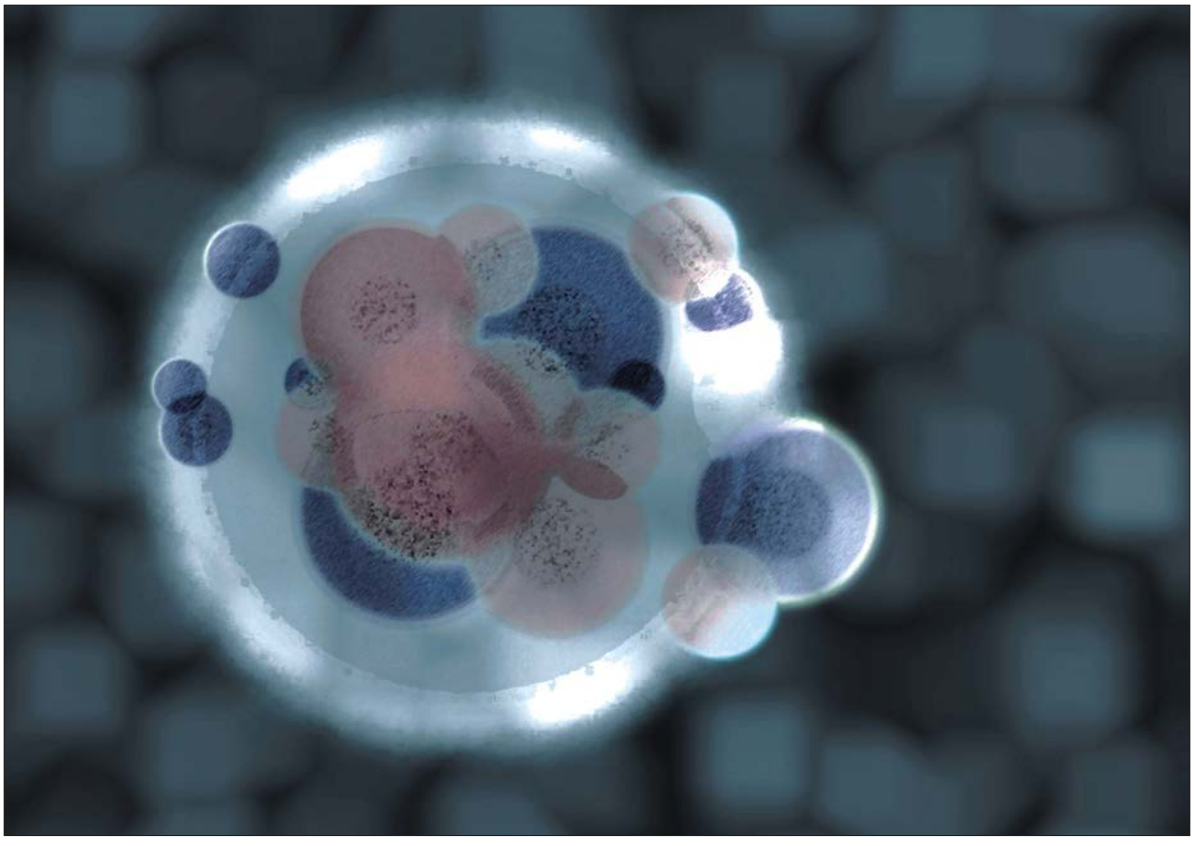

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Activity of Antioxidant Enzymes in Response to Cadmium in *Arabidopsis thaliana*

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Abstract: The effects of the heavy metal cadmium (Cd^{+2}) on growth and activities of the antioxidant enzymes, catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) have been investigated in *Arabidopsis thaliana* L. seedlings. The concentration of 50 and 100 μM $CdCl_2$ was shown to strongly inhibit the growth of roots and lipid peroxidation. Lipid peroxidation of seedlings shoots rose with increasing concentrations of Cd^{+2} as indicated by malondialdehyde (MDA) concentration. As Cd^{+2} concentration increased, catalase (CAT) activity declined progressively, while peroxidase and polyphenol oxidase activity increased when compared to the untreated plants. Close correlations between increased MDA formation and decreased root growth as well as CAT activity suggests that lipid peroxidation might caused cell damage and death proposing that applied concentrations of Cd^{+2} could be toxic to cells. It was also noted that Cd^{+2}-induced cell injury and lipid peroxidation correlated with increased peroxidase and polyphenol oxidase activities, two antioxidant enzymes involved in polyphenol peroxidation as lignification substrates. Together, the results suggest that in *Arabidopsis thaliana* reactive oxygen species (ROS) could be induced by phytotoxic concentrations of Cd^{+2} leading to increased POD and PPO activities which play a crucial role in detoxification of elevated concentrations of Cd^{+2} possibly via lignifications and physical barrier formation.

Key words: *Arabidopsis*, cadmium, polyphenol oxidase, peroxidase

INTRODUCTION

Any metallic chemical element that has relatively high density and is toxic for living organisms at low concentrations such as mercury (Hg), cadmium (Cd^{+2}), arsenic (As), chromium (Cr), thallium (Ti) and lead (Pb) are called heavy metals. Small traces of heavy metal contamination in the environment can lead to poisoning levels because they tend to accumulate in biological systems over time (Lee *et al*., 2005). Cadmium toxic properties come from its chemical similarity to zinc, an essential micronutrient for plants, animals and humans. In humans, long-term exposure to cadmium may cause renal dysfunction, lung disease and cancer, as well as bone defects. Because cadmium and zinc (or occasionally lead) occur naturally within the raw ore, it is produced as an inevitable by-product of their refining. The most usage of cadmium is in nickel/cadmium batteries, as pigments, stabilizers for PVC, in alloys and electronic compounds, as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products. A high concentration of cadmium, copper and silver in waters has been identified as a potential health and environmental hazard (Hanzlík *et al*., 2004).

Cadmium is not only a plant nutrition element (Yang *et al*., 1997; Thevenod and Friedmann, 1999) but also is a hazardous heavy metal that has high mobility in soil-plant systems and can enter the food chain very easily (Nriagu and Pacyna, 1988).

In aerobic conditions, heavy metals may induce reactive oxygen species (ROS) such as O_2^-, H_2O_2, $^{\cdot}OH$, $^1O_2$. In plants, ROS may cause damage to cell membranes, proteins, DNA replication and repair, as well as reduction of chloroplast pigments (Somashekaraiah *et al*., 1992; Drazkiewicz *et al*., 2004). Therefore, as the first level in the food chain, deduction of photosynthetic activity, leading to decline in crop yield happens by excess of cadmium (Ouzounidou, 1996; Maksymiec, 1997; Siedlecka *et al*., 2001).

Treatment of plants with Cd^{+2} causes leaf roll, chlorosis, reduced growth (Krupa, 1988); as well as inhibition of chlorophyll synthesis and various reactions in Calvin cycle (Padmaja *et al*., 1990). The experimental evidences suggest that exposure of plants to Cd^{+2} inhibits activities of some enzymes including hexokinase, glucose-6-phosphate dehydrogenase and alcohol dehydrogenase (Chugh and Sawhney, 1999), nitrate and nitrite reductase (Boussama *et al*., 1999; Kumar and Dubey, 1999)

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and enzymes involved in sulfate assimilation (Lee and Leustek, 1999). Many of these inhibitory effects are thought to be caused by ROS created by Cd^{+2} (Sandalio *et al*., 2001).

Plants possess two very efficient antioxidant defense systems: (i) the enzymatic pathway including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), polyphenol oxidase (PPO), glutathione oxidase (GSHOx) and (ii) non-enzymatic antioxidant defense systems such as ascorbate, glutathione, α-tocopherol and carotenoids. Both of these pathways allow for scavenging of ROS leading to protection of plant cells from oxidative damage (Gratão *et al*., 2005; Blokhina *et al*., 2003).

In these studies, the effect(s) of cadmium, as a destructive heavy metal in environment, on some aspects of enzymatic anti-oxidant systems of *Arabidopsis* studied and showed that *Arabidopsis* can be employed as powerful indicator of Cd^{+2} contamination in the environment.

MATERIALS AND METHODS

Plant materials and growth conditions: *Arabidopsis thaliana* L. wild type seeds were surface-sterilized by washing with 70% (v/v) ethanol for 2 min and sodium hypochlorite solution (20%) for 8 min in Gorgan University of Agricultural Sciences and Natural Resources (Golestan University) in Iran (2006-2007).

Seeds were then washed 3 times in sterile distilled water and germinated on agar plates containing MS (Murashige and Skoog, 1962) salts, supplemented with or without cadmium chloride (50, 85 and 100 mM). Plates were kept at 4°C in the dark for 4 days to synchronize germination then, transferred to a climate room at 22°C and 65% relative humidity with 16 h light (60 μmol m^{-2} sec^{-1})/ 8 h dark cycles. Twenty one day old plants were used for biochemical analysis.

Lipid peroxidation: Lipid peroxidation was measured by the thio-barbituric assay for malondialdehyde (MDA) as lipid peroxidation index (Shalata and Neumann, 2001) with some modifications as follows. 0.05 g of plant material was ground in 2 mL of 0.1% (w/v) cool trichloro-acetic acid (TCA) on ice. The homogenate was centrifuged at 10,000 x g for 5 min at 4°C and then 250 μL of supernatant was mixed with 2 mL TCA/TBA reagent (0.25% TBA containing 10% TCA). The mixture was incubated at 95°C for 30 min and kept on ice for 15 min. Then, it was centrifuged at 10,000 x g for 10 min. Absorbance of sample was read at 532, 600 and 440 nm. Lipid peroxidation were calculated (Du and Bramlage, 1992) as the malonyldialdehyde (MDA) equivalents according to this equation:

$$LP\ (nmol\ mL^{-1}) = [[(A_{532} - A_{600}) - [(A_{440} - A_{600})\ (MA\ of\ sucrose\ at\ 532\ nm\ /MA\ of\ sucrose\ at\ 440\ nm)]]/157000]10^{6}$$

Trypan blue exclusion and cell viability: Cell viability was measured by trypan blue exclusion (Pasqualini *et al*., 2003). Leaves were plunged in lactophenol trypan blue (30 mL ethanol, 10 mL glycerol, 10 mL lactic acid, 10 mg trypan blue and 10 mL distilled water) and boiled at 95°C for 2-3 min and then at room temperature for 1 h. Samples were transferred into hydrate chloral solution (2.5 g mL^{-1}) and boiled for decolorized about 20 min. After several exchanges of hydrate chloral solution, samples were equilibrated in 50% (w/v) glycerol and visually observed under stereomicroscopy; non-viable cells were blue-stained.

Enzyme activities assay: For preparation of crude enzyme extracts, a 0.05 g sample of fresh leaves was ground in 2 mL of 0.1 M cool phosphate buffer (pH 6.8) on ice bath (Kar and Mishra, 1976). Crude extract was centrifuged at 15,000 x g for 15 min at 4°C. The supernatant was used for catalase, peroxidase and polyphenol oxidase activity assays. The protein concentration of the supernatant was measured according to Lowry *et al*. (1951). Catalase and peroxidase activities were measured according to Erdeil *et al*. (2002) as follows:

Catalase: The assay mixture (3 mL) contained 15 mM H_2O_2, 50 mM phosphate buffer (pH 6.8) and 100 μL enzymes extract. The decline at absorbance in 40 nm was scanned automatically with a spectrophotometer (Shimidzo UV-160) in kinetic mode. Molar extension coefficient of catalase is 40 L $mM^{-1}cm^{-1}$.

Peroxidase: The assay mixture (3 mL) contained 20 mM guaiacol, 25 mM phosphate buffer (pH 6.8), 40 mM H_2O_2, 10 μL enzyme extract. Guaiacol peroxidase activity was scanned in 470 nm; molar extension coefficient of peroxidase is 26.6 L $mM^{-1}cm^{-1}$.

Polyphenol oxidase: The assay mixture (3 mL) of contained 10 mM pirogalol, 25 mM phosphate buffer (pH 6.8) and 200 μL enzyme extract. Enzyme activity determined in 420 nm, its molar extension coefficient is 2.47 L $mM^{-1}cm^{-1}$.

Statistical analysis: Leaves of all developmental stages of control and treated plants were sampled separately for

analyses and the experiments were repeated four times. The data presented in figures are the mean of four independent experiments with calculated standard deviations. A Duncan test was used to identify statistical differences between pairs of means at a confidence level of 95% for each set of data.

RESULTS AND DISCUSSION

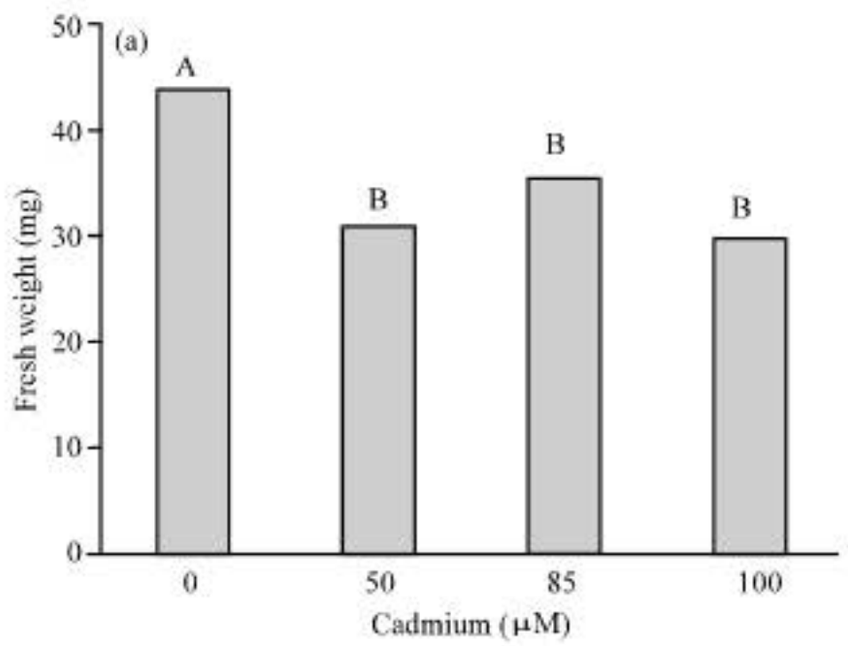
The effect(s) of cadmium on *Arabidopsis thaliana* growth: *Arabidopsis thaliana* was cultured on MS medium with different concentration of cadmium. The result shown, increased concentration of cadmium affects on *Arabidopsis thaliana* growth parameters like as fresh weight, length of roots, chlorophyll content and etc. Figure 1 showed the samples with the best difference among them. The Fig. 2 (average of measurement) revealed cadmium inhibited the growth of Arabidopsis thaliana both fresh weight and length of roots (100 µM). Also, high concentration of cadmium decreased amount of lateral roots of plants (Fig. 2).

Lipid peroxidation and cell death: Malonyldialdehyde (MDA) content as lipid peroxidation index of twenty-one day-old *Arabidopsis thaliana* under the effect of cadmium was established. Present results that showed MDA content increased in treated plants. Its maximum was belonging to treated plant grown on media completed with 100 µM of cadmium compared to control (Fig. 3). The results of cell membranes damage is structure and function perturbation and finally cell death. Trypan blue staining showed the most coloring as cell death parameter was occurred under the effect of 100 µM of cadmium and shown significance increase compared with control (Fig. 4).

Fig. 1: Twenty one day old seedlings of *Arabidopsis thaliana* exposure to Cd compared with control. The seeds of *Arabidopsis thaliana* cultured on MS media containing with several concentration (0, 50, 85 and 100 µM) of Cd and after 21 days analyzed

Antioxidant enzymes activity: The activity of catalase, guaiacol peroxidase and polyphenol oxidase assessed in *Arabidopsis thaliana* treated with cadmium and control. Cd^{+2} treated plants showed statistically significant decrease in catalase activity compared with controls (Fig. 5). Whereas, increasing Cd^{+2} concentration in medium was causing enhanced the activity of peroxidase and exposure to 100 µM Cd^{+2} were caused a marked increased enzyme activity with statistically significant between treated plant and control. However, the plant exposure to Cd^{+2} don't show the alterations but only on 100 µM Cd^{+2} supplement, PPO activity of *Arabidopsis thaliana* seedling statistically significant increased.

The results showed that oxidative stress was produced in *Arabidopsis thaliana* seedling under the



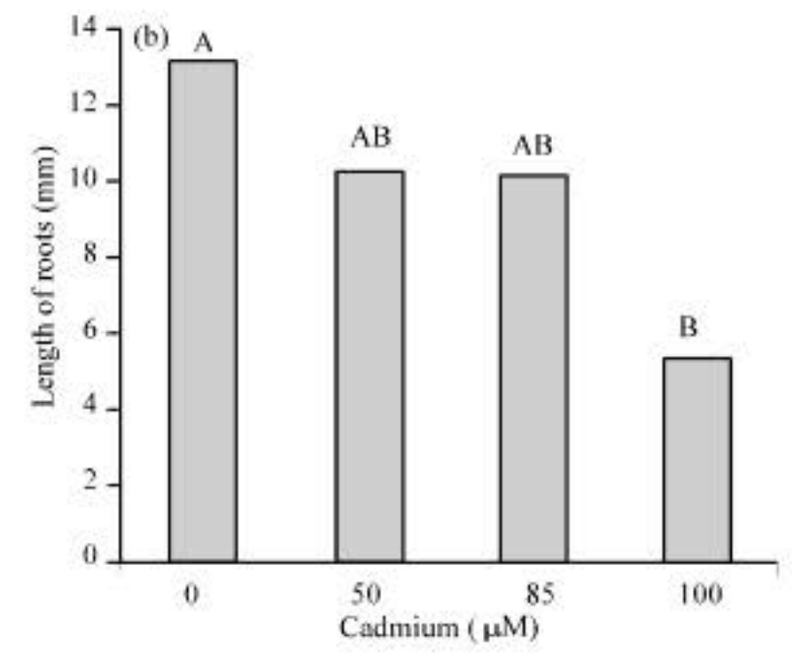


Fig. 2: The effects of Cd on fresh weight (a) and length of root (b) compared with control. Twenty one day old of *Arabidopsis thaliana* seedlings Cd treated were shown decreasing biomass (fresh weight) and also length of root in comparison to control. Data analyzed with Duncan test to identify statistical differences between samples at confidence level of 95%. Different letters indicate statistically significant differences among treatments ($p<0.05$)

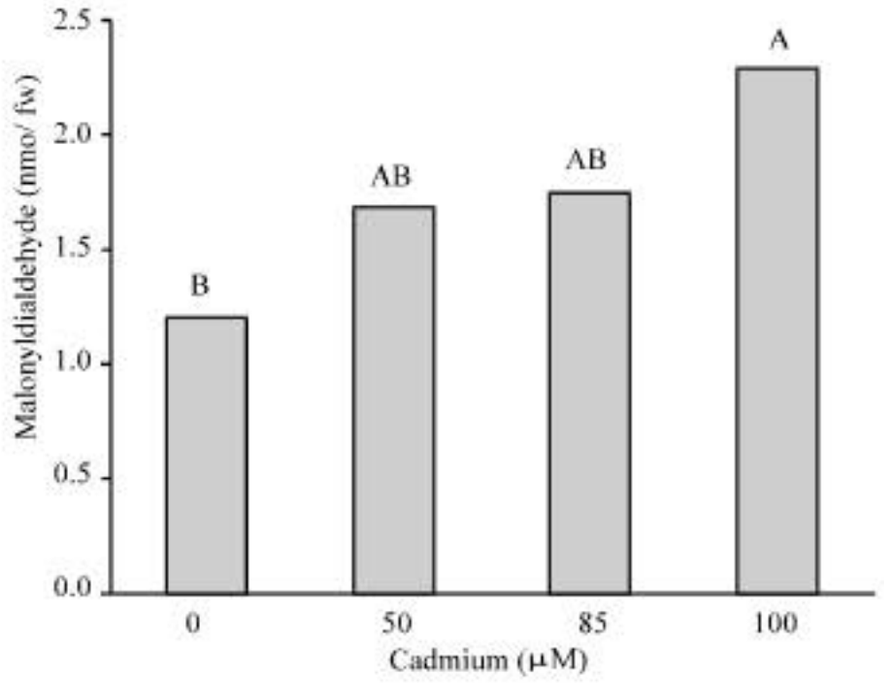


Fig. 3: Malonyldialdehyde (MDA) contents in shoots of *Arabidopsis thaliana* as affected by exogenous applications of cadmium at 0, 50, 85 and 100 μM. This test was performed with three replicate cultures per treatment. Different letters indicate that mean values are significantly different at $p<0.05$

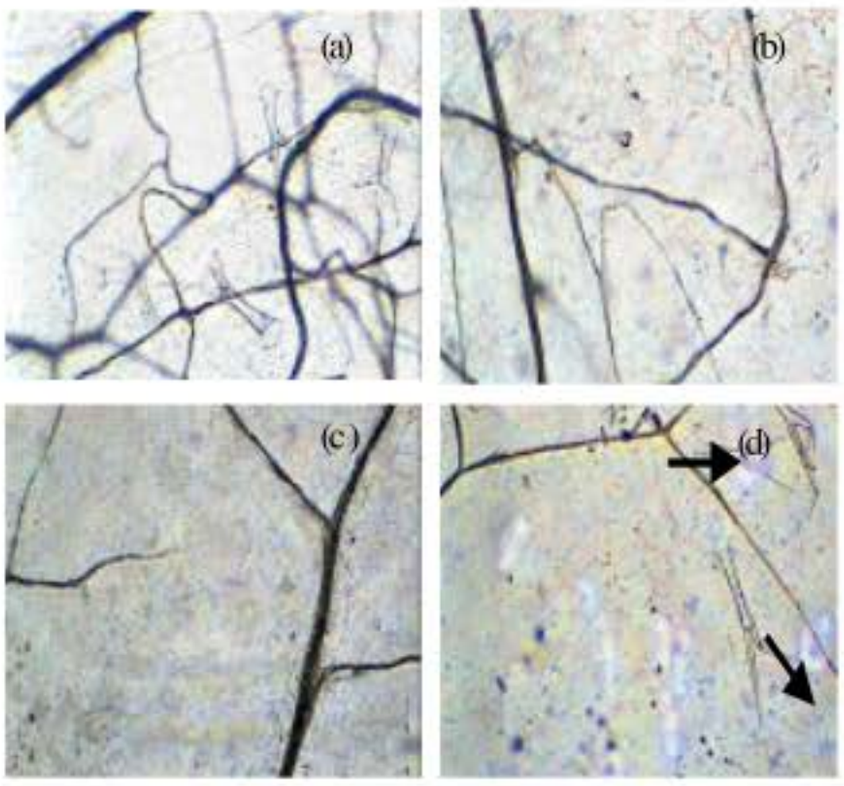


Fig. 4: Trypan blue staining of intact leaves of *Arabidopsis thaliana* control and treated with cadmium. Cell death was shown as dark spots (indicated by arrows)

effect of Cd^{+2}, hazardous heavy metal. ROS can react with amino acids, unsaturated fatty acids and other molecules to cause peroxidation of essential lipids in the membranes and proteins and etc. Lipid peroxidation damages membrane integrity (cell death) resulting in increases in the permeability of the plasma membrane, which leads to leakage of K^+ ions and other solutes so plant growth was inhibited. Figure 1 shown, Cd^{+2} excess in medium were caused inhibition of roots growth, reduction of

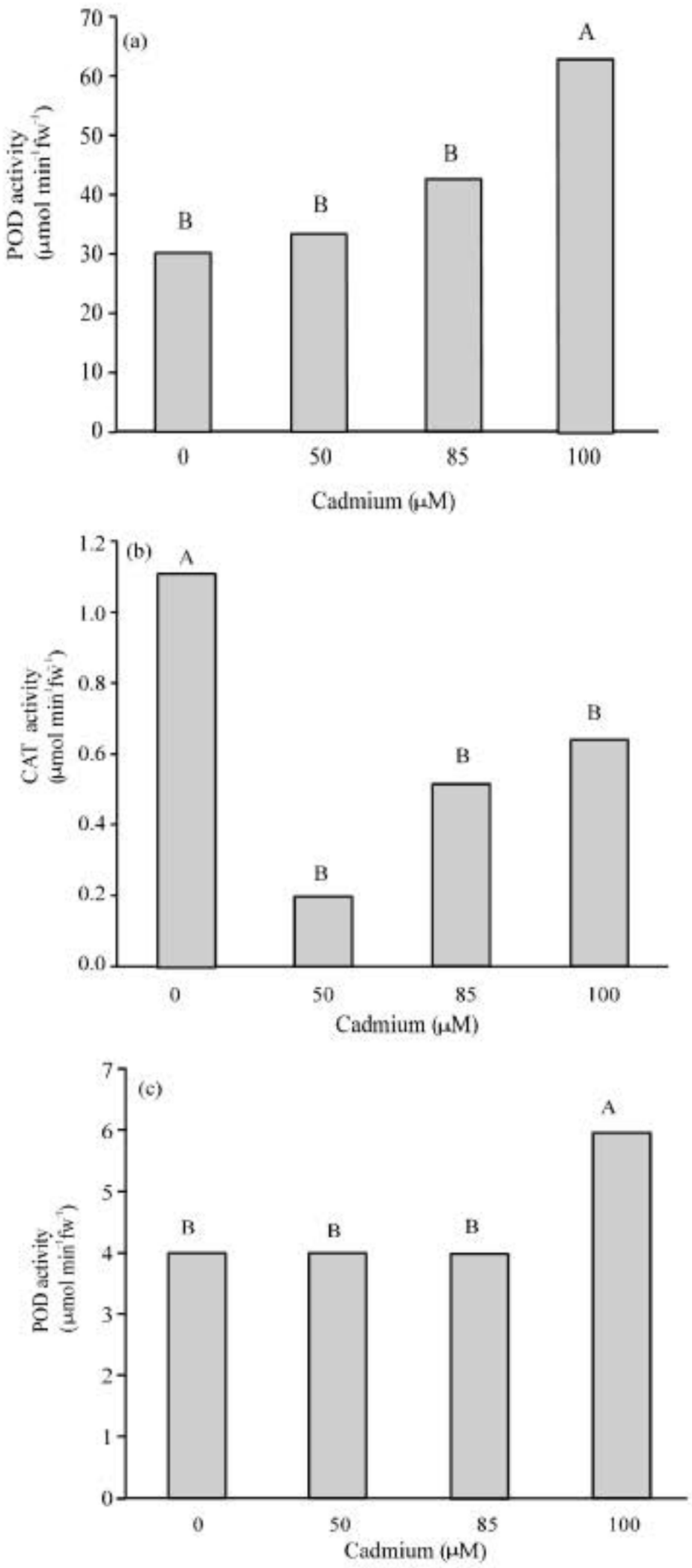


Fig. 5: Peroxidase (a), Catalase (b) and Poly phenol oxidase (c) activities of *Arabidopsis thaliana* leaves. *Arabidopsis thaliana* seeds were grown on MS medium that was supplemented with Cd and anti-oxidant enzymes activities in leaves of 21 days old seedling were assayed. For each compound analyzed, different characters indicate that mean values are significantly different at $p<0.05$

roots number and fresh weight as growth parameters. It was thinking reduction of surface area of leaves and photosynthesis affects on plant growth. It also has been indicated the high concentration of Cd^{+2} probably could inhibits essential metal absorption like Fe^{+2} and Zn^{+2} and then plant growth (Rogers *et al*., 2000). Although, chlorophyll content wasn't measured in treated plant but chlorophyll reduction become observable especially on 100 μM of Cd concentration. Therefore, reduction of chlorophyll content and photosynthesis could be other reason for inhibition of plant growth. In the other Cd^{+2} treated plants, destruction of chlorophyll or disturbance of chlorophyll biosynthesis introduced as an important agent of reducing of photosynthesis and plant growth (Somashekaraiah *et al*., 1992; Barcelo *et al*., 1988). A significant reduction of total root length of *Arabidopsis thaliana* seedling under the effect of Cd^{+2} was shown (Fig. 1), this is under the effect of inhibition of cell enlargement or cell division exposure to Cd^{+2}. Aidid and Okamoto (1993) reported that Cd^{+2} is an irreversible inhibitor of proton pump responsible for elongation growth rate, turgor pressure and cell wall extensibility. So, reduction of cell enlargement caused the root growth of *Arabidopsis thaliana* to be inhibited.

According to these results, MDA content in Cd^{+2} treated *Arabidopsis thaliana* seedling increased, this result is in agreement with data obtained on other Cd^{+2} treated plant for example bean plants (Somashekaraiah *et al*., 1992), sunflower (Gallego *et al*., 1996) and maize and pea plants (Lozano-Rodrigues *et al*., 1997). MDA is one of several low molecular weight end products formed via decomposition of certain primary and secondary lipid peroxidation products and MDA enhancement is lipid peroxidation and oxidative stress indicator. So, phytotoxicity of Cd^{+2} was stablished by enhancement of free radical and lipid peroxidation.

According to Mashoudi *et al*. (1997) responses of antioxidative enzymes to metal stress like excess Cd^{+2} vary among plant species and even different plant tissues. Moreover, H_2O_2 can accumulate in plant tissues where the antioxidant activities are suppressed and detoxify due to catalase and peroxidase activities. Cd^{+2} significantly reduced Catalase activity in treated plants compared to control (Fig. 5). Such a result is in agreement with data obtained by Sandalio *et al*. (2001) in pea leaves, Somashekaraiah *et al*. (1992) in bean plants and Gallego *et al*. (1996) in sunflower. CAT activity doesn't change after incubation with 100 μM Cd^{+2}, this evidence indicated, direct addition of Cd^{+2} don't affect on CAT activity. Immunoaffinity purification of pea plant catalase with the anti-catalase antibody revealed the decline of CAT activity is under the effect of reduction of protein (Sandalio *et al*., 2001).

Cd^{2+} induced significantly enhancement in guaiacol peroxidase activity compared to control. Activation of peroxidase-dependent reactions involved in oxidation of many organic compounds like polyphenols in suberine and lignin deposition (Lagrimini, 1991; Schopfer, 1996; Quiroga *et al*., 2000; Lin and Kao, 2001). Schützendübel *et al*. (2001) reported enhancement of peroxidase activity, oxidation of phenols, and lignification in Scots Pine Roots exposure to 50 μM Cd^{+2}. Lignification process as a defense response induced in response to pathogen invasion (Cosgrove, 1997), salinity stress (Lin and Kao, 2001), and heavy metal toxicity (Schützendübel *et al*., 2001). Therefore, toxicity of Cd^{+2} was decreased by peroxidase consumption of H_2O_2 (Wang *et al*., 2004) and lignification process as a physical barrier against heavy metals (Hegedus, 2001). The capability of adaptation to stress can relate to the increase or sustained ability to form defense barriers for reinforcing the cell structure by lignification-related enzymes. However, increase in the activities of lignification-related enzymes, particularly PPO activity, might be the result from prolonged heavy metal stress.

REFERENCES

Aidid, S.B. and B. Okamoto, 1993. Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc. BioMetals, 6: 245-249.

Barcelo, J., M.D. Vazquez and C.H. Poschenrieder, 1988. Structural and ultrastructural disorders in cadmium-treated bush bean plants. New Phytol. 108: 37-49.

Blokhina, O., E. Virolainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation Stress: A review. Ann. Bot., 91: 179-194.

Boussama, N., O. Ouariti and M.H. Ghorbal, 1999. Changes in growth and nitrogen assimilation in barley seedlings under cadmium stress. J. Plant Nutr., 22: 731-752.

Chugh, L.K. and S.K. Sawhney, 1999. Effect of cadmium on activities of some enzymes of glycolysis and pentose phosphate pathway in pea. Biol. Plant, 42: 401-407.

Cosgrove, D.J., 1997. Assembly and enlargement of the primary cell wall in plants. Ann. Rev. Cell Biol., 13: 171-201.

Drazkiewicz, M., E. Skorzynska-Polit and Z. Krupa, 2004. Copper-induced oxidative stress and antioxidant defense in *Arabidopsis thaliana*. Biometals, 17: 379-387.

Du, Z. and W.J. Bramlage, 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem., 40: 1566-1570.

Erdeil, S., A. Hegedûs , G. Hauptmann, J. Szalai and G. Horváth, 2002. Heavy metal induced physiological changes in the antioxidative response system. Plant Physiol., 46: 89-90.

Gallego, S.M., M.P. Benavides and M.L. Tomaro, 1996. Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. Plant Sci., 121: 151-159.

Gratão, P.L., A. Polle, P.J. Lea and R.A. Azevedo, 2005. Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol., 32: 481-494.

Hanzlík, P., J. Jehlicka, Z. Weishauptová and O. Šebek, 2004. Adsorption of copper, cadmium and silver from aqueous solutions onto natural carbonaceous materials. Plant Soil Environ., 6: 257-264.

Hegedus, A., S. Erdei and G. Horvath, 2001. Comparative studies of H2O2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci., 160: 1085-1093.

Kar, -M. and D. Mishra, 1976. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57: 315-319.

Krupa, Z., 1988. Cadmium-induced changes in the composition and structure of light harvesting complex II in radish cotyledons. Plant Physiol., 73: 518-524.

Kumar, R.G. and R.S. Dubey, 1999. Glutamine synthetase isoforms from rice seedlings: Effects of stress on enzyme activity and the protective roles of osmolytes. J. Plant Physiol., 155: 118-121.

Lagrimini, L.M., 1991. Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase. Plant Physiol., 96: 577-583.

Lee, J.Y., J.C. Choi and K.K. Lee, 2005. Variations in heavy metal contamination of stream water and groundwater affected by an abandoned lead-zinc mine in Korea. Environ. Geochem. Health, 27: 237-257.

Lee, S.M. and T. Leustek, 1999. The effect of cadmium on sulphate assimilation enzymes in *Brassica juncea*. Plant Sci., 141: 201-207.

Lin, C.C. and C.H. Kao, 2001. Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings. Plant Soil, 230: 135-143.

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

Lozano-Rodrigez, E., L.E. Hernandez, P. Bonay and R.O. Carpena-Ruiz, 1997. Distribution of cadmium in shoot and root tissues of maize and pea plants: Physiological disturbances. J. Exp. Bot., 48: 123-128.

Maksymiec, W., 1997. Effect of copper on cellular processes in higher plants. Photosynthetica, 34: 321-342.

Mashoudi, S., A. Chaoui, M.H. Ghorbal and E.E. Ferjani, 1997. Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill). Plant Sci., 127: 129-137.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Nriagu, J.O. and J.M. Pacyna, 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature, 333: 134-139.

Ouzounidou, G., 1996. The use of photoacoustic spectroscopy in assessing leaf photosynthesis under copper stress: Correlation of energy storage to photosystem II fluorescence parameters and redox change of P700. Plant Sci., 113: 229-237.

Padmaja, K., D.D.K. Prasad and A.R.K. Prasad, 1990. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. Seedlings by cadmium acetate. Photosynthetica, 24: 399-405.

Pasqualini, S., C. Piccioni, L. Reale, L. Ederli, G.D. Torre and F. Ferranti, 2003. Ozone-induced cell death in tobacco cultivar Bel W3 Plants. The role of programmed cell death in lesion formation. Plant Physiol., 133: 1122-1134.

Quiroga, M., C. Guerrero, M.A. Botella, A. Barceló and I. Amaya *et al*., 2000. A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiol., 122: 1119-1128.

Rogers, E.E., D.J. Eide and M.L. Guerinot, 2000. Altered selectivity in an arabidopsis metal transporter. Proc. Natl. Acad. Sci. USA., 97: 12356-12360.

Sandalio, L.M., H.C. Dalurzo, M. Go´mez, M.C. Romero-Puertas and L.A. Del-Ri´o, 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot., 52: 2115-2126.

Schopfer, P., 1996. Hydrogen peroxide-mediated cell-wall stiffening *in vitro* in maize coleoptiles. Planta, 199: 43-49.

Schützendübel, A., P. Schwanz, T. Teichmann and K. Gross, 2001. Cadmium-induced changes in antioxidative systems, hydrogen peroxide content and differentiation in scots pine roots. Plant Physiol., 127: 887-898.

Shalata, A. and P.M. Neumann, 2001. Exogenous ascorbic acid (vitamin C). increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot., 52: 2207-2211.

Siedlecka, A., A. Tukendorf, E. Skorzynska-Polit, W. Maksymiec and M. Wojcik *et al*., 2001. Angiosperms (Asteraceae, Convolvulaceae, Fabaceae and Poaceae: Other Than Brassicaceae). In: Metals in the Environment. Analysis by Biodiversity. Prasad, M.N.V. (Ed.). Marcel Dekker, Inc., New York, pp: 171-215.

Somashekaraiah, B.V., K. Padmaja and A.R.K. Prasad, 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiol. Plant, 85: 85-89.

Thevenod, F. and J.M. Friedmann, 1999. Cadmium-mediated oxidative stress in kidney proximal tubule cells induces degradation of Na/K-ATPase through proteasomal and endolysosomal proteolytic pathways. FASEB J., 13: 1751-1761.

Wang, S.H., Z.M. Yang, H. Yang, B. Lu, S.Q. Li and Y.P. Lu, 2004. Copper-induced stress and antioxidative responses in roots of *Brassica juncea* L. Bot. Bull. Acad. Sin., 45: 203-212.

Yang, C.F., H.M. Shen, Y. Shen, Z.X. Zhuang and C.N. Ong, 1997. Cadmium induced oxidative cellular damage in human fetal lung fibroblasts (MRC-5 cells). Environ. Health Perspect., 105: 712-716.