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DETERMINATION OF CHANGES IN NUTRITIONAL ELEMENTS AND ANTIOXIDANT AMOUNTS IN THE PLANT BY APPLICATION OF HEAVY METALS (Cu, Pb, Cd) TO CORN PLANT (*Zea mays* L.)

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ABSTRACT

Excess of some heavy metals as copper lead and cadmium are significant contaminants for agricultural soils, and they affect plant life cycles by changing their defense enzymes and mineral element contents. This study was aimed to determine the effects of copper, lead and cadmium (Cu, Pb, and Cd) on the mineral concentration and antioxidant, enzymes activities in root and leaves of corn. They changed mineral elements content in roots and leaves of plants. Cd and Pb levels increased depending on applied doses in growth medium. Pb and Cd treatment showed harmful effect by limiting uptake of nutrients to the roots. Cu application increased ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities in the plant roots; whereas Pb increased APX activity and Cd increased both SOD and APX activities. Their applications decreased enzyme activities in leaves compared to control. This study showed that Cd has limited the level of calcium, copper, iron, magnesium, manganese, and zinc in roots. Pb induced the uptake of calcium, magnesium, and zinc in leaves.

Keywords: antioxidant enzymes, plant nutrients, corn, heavy metals, plant stress.

INTRODUCTION

Industrialization has caused an increase in the amount of heavy metals in soil, atmosphere, and water. It has been created one of the most important environmental problems. Cadmium, lead, and copper are the most important heavy metals increased in soil. Soil is one of the most important components of the biosphere and serves as a natural buffer task controlled transferring of chemical elements and substances to the atmosphere, hydrosphere and biota (Kabata-Pendias and Pandias, 2001). Heavy metals are essential abiotic stress factors because of accumulation, toxicity, long half-life for organisms. These metals remain in the soil for a long time and have negative effect on the natural environment, soil fertility and human health through the food chain (Gratao et al., 2008). Metal content in plants has been increased by anthropogenic factor such as fertilizers, pesticides, sewage and waste water systems (Sbartai et al., 2011). Cd, Ni and Pb as heavy metals are taken up by palnts mostly through the root system and partly, in minor amounts through the leaves (Amari et al., 2017). The accumulation of heavy metals in the roots affects the plant growth negatively by weakening the root development first in the root system. Metal toxicity decelerates water and nutrient uptake, and photosynthesis, as well as adversely affects mineral element distribution. High heavy metal concentrations may break the cell signalization and can cause irreversible damage in biological systems. The symptoms such as root tip darkening and growth slowdown are observed in the plants grown in the high heavy metal concentrations, and it can lead to death of them (Bertolini et al., 2012).

Plants respond in various ways to heavy metal pollution depending on the physical and chemical properties of metal, heavy metals lead to the formation of reactive oxygen species (ROS) (Ekmekci et al., 2008). ROS produced by respiration and photosynthesis are quite strong oxidizing

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agent and lead to damage in lipid, protein, pigment, and nucleic acid content of the cells (Verma and Dubey, 2003). Plants have developed a series of defensive strategies to overcome oxidative stress. Antioxidant defense system includes the formation of APX (Ascorbate peroxidase), CAT (Catalase), POD (Peroxidase) and SOD (Superoxide dismutase) enzymatic or non-enzymatic components such as ascorbate and α-tocopherol in plant cells. Antioxidant system can play an important protective role in the elimination of toxic radicals caused by oxidative stress (Haribabu and Sudha, 2011).

Metals are part of the terrestrial ecosystems included soil, water and organisms. Total metal concentration in the growth medium is essential to decide for toxicity of metals. Contamination of plant growth medium with heavy metals such as Cu, Cd and Pb adversely affect nutrient uptake. Plants have developed different adaptation mechanisms to prevent metal induced damages. These responses vary depending on metals and plant species. In the macro-level, metal toxicity depending on nutrient distribution is shown in growth reduction and foliar symptoms, but in the micro level, cellular symptoms are observed anatomically. Toxicity can reduce photosynthesis, transpiration, water status; affect enzyme activity, and have negative effects on cellular functions (Fageria and Barbosa Filho, 2006; Lequeux et al., 2010; Azooz et al., 2012).

The presence of minor number of heavy metals do not cause stress in the plant tissue, but also, they adversely affect intake of other nutrients, and lead to decrease total biomass. They change the mineral contents and antioxidant enzymes of plant. Studies on heavy metals (Cd, Cu, Pb) have been intensively investigated on antioxidant enzyme activities (Zengin and Munzuroglu, 2005; Rellan-Alvarez et al., 2006; Rastgoo and Alemzadeh, 2011). However, there are not enough studies comparing antioxidant enzyme activity with mineral elements and heavy metals. In the current study, we investigated the effect of heavy metal accumulation on the content of nutrients, the relationship between antioxidant enzyme activities and mineral elements. Some enzymes include mineral elements as cofactors such as copper and zinc in catalytic site, so we aim to show the effect of heavy metals in corn plant.

MATERIAL AND METHODS

Plant material

In this study, Sele F1 Corn (*Zea mays* convar. *saccharata* var. *rugosa*) variety seeds sown in multiple seedling vials filled with peat and then they were transplanted and grown in the pots contained by 10 kg mixture of peat and garden soil (1:1) in unheated greenhouse conditions. The study was carried out in greenhouses at Gaziosmanpasa University Research and Application Center in June 2016. The experiment was conducted as a randomized plot design with three replications. In the study, after the seeds emerged in the pots, the above ground and roots of the plants were sampled 50 days later. 150 ppm N, 80 ppm P and 100 ppm K were applied in sufficient quantities for plant growth. After 3 weeks, the seedlings were transferred into pots, 10, 20 and 50 ppm Cu, Pb and Cd were applied, and $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$ were used as the source of heavy metals. The amount of heavy metal and nutrient elements, APX, CAT, POD and SOD enzyme activity were analyzed in plant roots and leaves.

Determination of minerals in plant tissue and heavy metal contents

Plant tissues were dried at 65°C in the oven until they were reached constant weight. Plant samples were treated with nitric acid for wet decomposition method and then heavy metals (Cu, Pb and Cd) and other nutrients were determined by a 7700-x model Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Tokyo, Japan) equipment using an external calibration method. Dried plant samples were grinded and ashed in microwave using 2 ml of 35% H_2O_2 and 5 ml of 65% HNO_3. Following the digestions, Mg, Ca, Fe, Zn, Mn, Cu, Pb and Cd were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES; VarianVista Pro) (Doker et al., 2014).

Enzyme extraction

Corn leaves (0.3 g) were crushed in liquid nitrogen and then they were homogenized with 1 mM EDTA and 50 mM potassium phosphate buffer (pH 7) contained 1% (w / v) PVP in the mortar. They were centrifuged at 4°C with 15000 X g for 20 minutes. Supernatant was used for analysis activity of catalase, peroxidase, ascorbate peroxidase, superoxide dismutase.

Catalase activity

Catalase activity was begun with addition 50 µl crude extract in 50 mM phosphate buffer (pH 7) and 10 mM H_2O_2 mixture. Decomposition of H_2O_2 was observed at 240 nm and it was determined that absorbance changes were recorded ($E = 0.036\ mm^{-1}\ cm^{-1}$) (Aebi, 1984).

Peroxidase activity

For peroxidase activity, 30 µl enzyme extract was added into 50 mM phosphate buffer (pH 6.5), 30 mM Guaiacol, 10 mM H_2O_2 reaction mixture. It was determined by measuring of absorbance increasing with occurrence of quaiacol oxidation ($E = 6.26\ mm^{-1}\ cm^{-1}$) (Angelini et al., 1990).

Ascorbate peroxidase

The reaction mixture contains 50 mM phosphate buffer (pH 6), 2.5 mM ascorbate, H_2O_2 30 µl enzyme extract. H_2O_2 was added at the end and it was observed ascorbate oxidation at 290 nm reaction medium ($E = 2.8\ mm^{-1}\ cm^{-1}$) (Nakano and Asada, 1981).

Superoxide dismutase

Superoxide dismutase activity was determined by Beyer and Fridovich method with a slight modification (Beyer and Fridovich, 1987). It is based on creating nitroblue tetrazolium farmazon by superoxide anion and giving an absorbance at 560 nm. An amount of 50 mM phosphate buffer (pH 7), 13 mM methionine, 60 pM nitroblue tetrazolium, 0.1 mM EDTA and 100 µl enzyme extract and 2 µM riboflavin was added in the reaction mixture and then it was kept of light (4000-5000 lux) for 30 minutes. The reaction was stopped by closing the light. An enzyme unit was accepted enzyme amount, inhibited 50% for absorbance increasing at 560 nm. The amount of enzyme that inhibited 50% formazan formation is regarded as one enzyme unit.

Statistical analysis

Statistical analysis of the results was performed by ANOVA using SPSS 20.0 program package. Comparisons between means were performed with Duncan test. Significant differences were accepted if $p < 0.05$.

RESULTS AND DISCUSSION

According to the results, root and leaf nutrient contents and antioxidant defense system enzymes were affected significantly by heavy metal applications

Effects of heavy metals on mineral contents in roots

Mineral contents were affected significantly by heavy metal applications and the results are given in Figure 1. So, its increased in roots depending on Pb and Cd levels added into the growth medium. Cd amount increased from 3 ppm to 29, 61 ppm-94 ppm. Pb level increased from 49 ppm to 113 ppm-278 ppm. Nutrient's concentration was affected by heavy metal application. Mg, Ca, Mn, Fe, Zn and Cu contents were reduced by Cd and Pb stress when it was compared to control. Cadmium reduced Fe content about 65%, while Pb and Cu reduced Fe content significantly in 20 and 50 ppm. Higher Pb concentration (50 ppm) decreased approximately twice the Fe content, compared to the control. Mg, Ca, Mn, Zn, and Fe levels were increased when 10 ppm Cu was added to the growth medium, while they were decreased at higher Cu concentrations (20 and 50 ppm). Cd and Pb applications reduce root nutrient content, whereas Cu affect depending on doses.

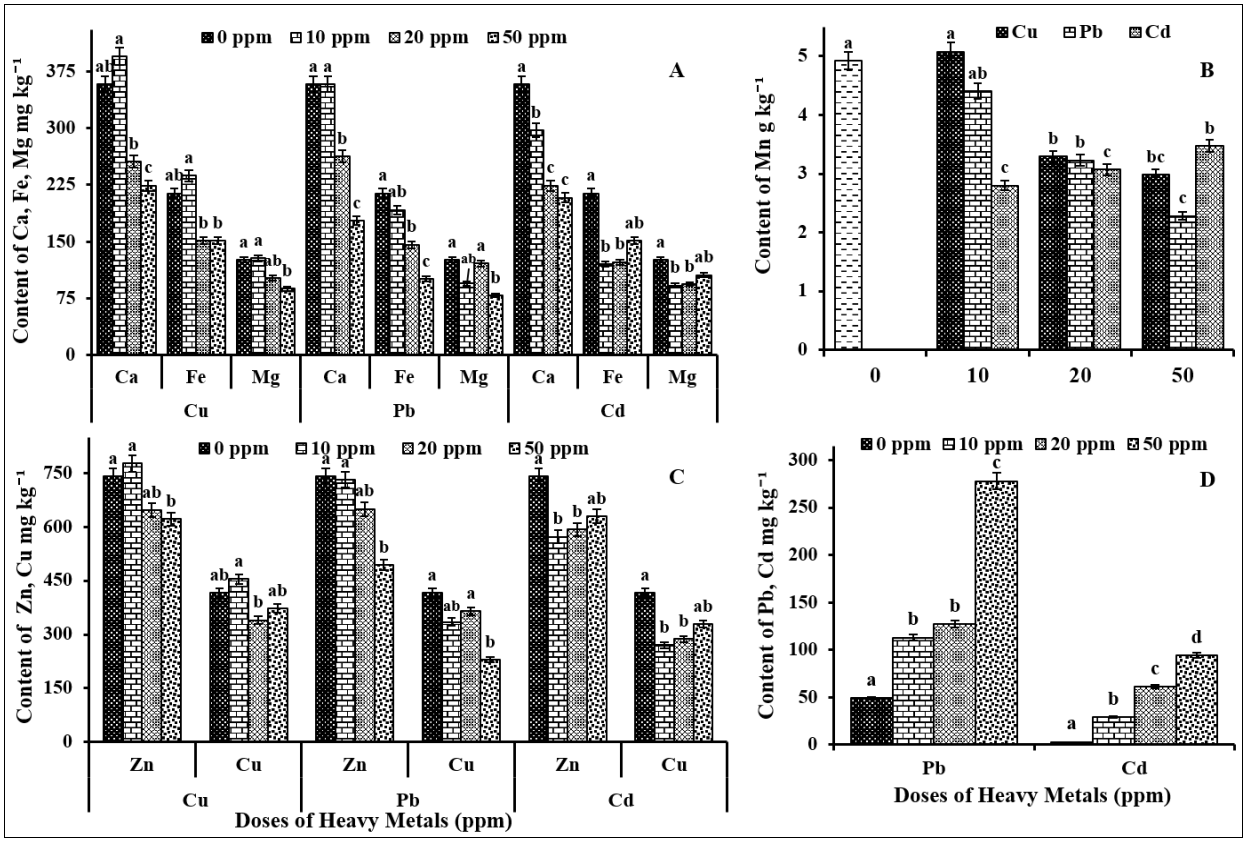

Figure 1. Effect of heavy metals on some nutrients in roots of corn. Values are the means ± sd, and data expressed as g or mg kg^{-1} DW. Bars marked with the different letters indicate significant differences at $P \leq 0.05$ (Duncan test) from each other. Letters represent separate analysis of the plant tissue exposed to different treatment of Cu, Pb and Cd (each element was analyzed within itself compared to the control group to evaluate the effect of the applied heavy metals).

Effects of heavy metals on mineral contents in leaves

Heavy metals have shown synergistic or antagonistic effect on nutrient content and the results are shown in Figure 2. Cd and Pb levels increased correspondingly depend on dose applied into the growth medium. Zinc content increased by Cd application 10%, 16% and 33% respectively according to control. Mg, Ca and Mn contents increased in various amounts. Cadmium has caused to imbalance in Ca, Fe and Cu contents. Mg, Ca and Zn contents were significantly increased by Pb doses, whereas Fe and Cu contents were reduced; Mn level was not stable. Cu is one of the essential elements for plants in low doses increased Mg level, while it reduced Fe content, and Ca, Mn, Zn contents was imbalanced.

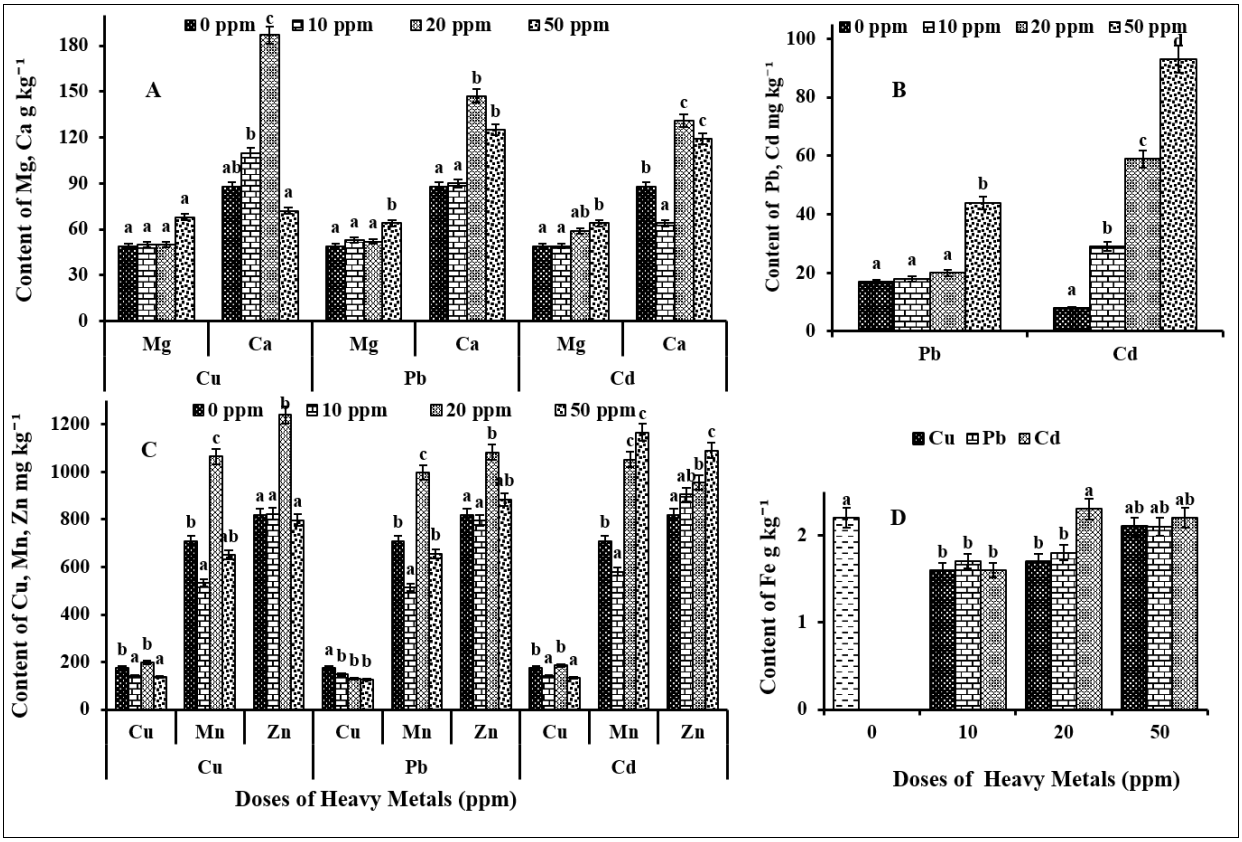

Figure 2. Effect of heavy metals on some nutrients in leaves of corn. Values are the means ± sd, and data expressed as g or mg kg^{-1} DW. Bars marked with the different letters indicate significant differences at $P \leq 0.05$ (Duncan test) from each other. Letters represent separate analysis of the plant tissue exposed to different treatment of Cu, Pb and Cd (each element was analyzed within itself compared to the control group to evaluate the effect of the applied heavy metals).

Effect of Cu, Pb and Cd on antioxidant enzymes activities

Four enzyme activities were determined related to antioxidant defense systems in roots and leaves. The results are shown in Figures 3 and 4. Enzyme activities were higher in leaves than in roots depending on metabolic rate. Cu application increased POD activity, while it was decreased by Cd and Pb in roots. APX activity was more increased by Cu, Cd and Pb than in control roots. APX activity increased by Cu application approximately 3 times more in the highest concentration. CAT activity was not affected significantly by heavy metal stress in roots. SOD enzyme activity was correlatively increased in higher Cu and Cd concentration, but it was decreased by Pb in roots. POD, APX and CAT activities were reduced by higher Cu, Pb and Cd concentration in leaves, whereas SOD activity was not affected by heavy metal stress.

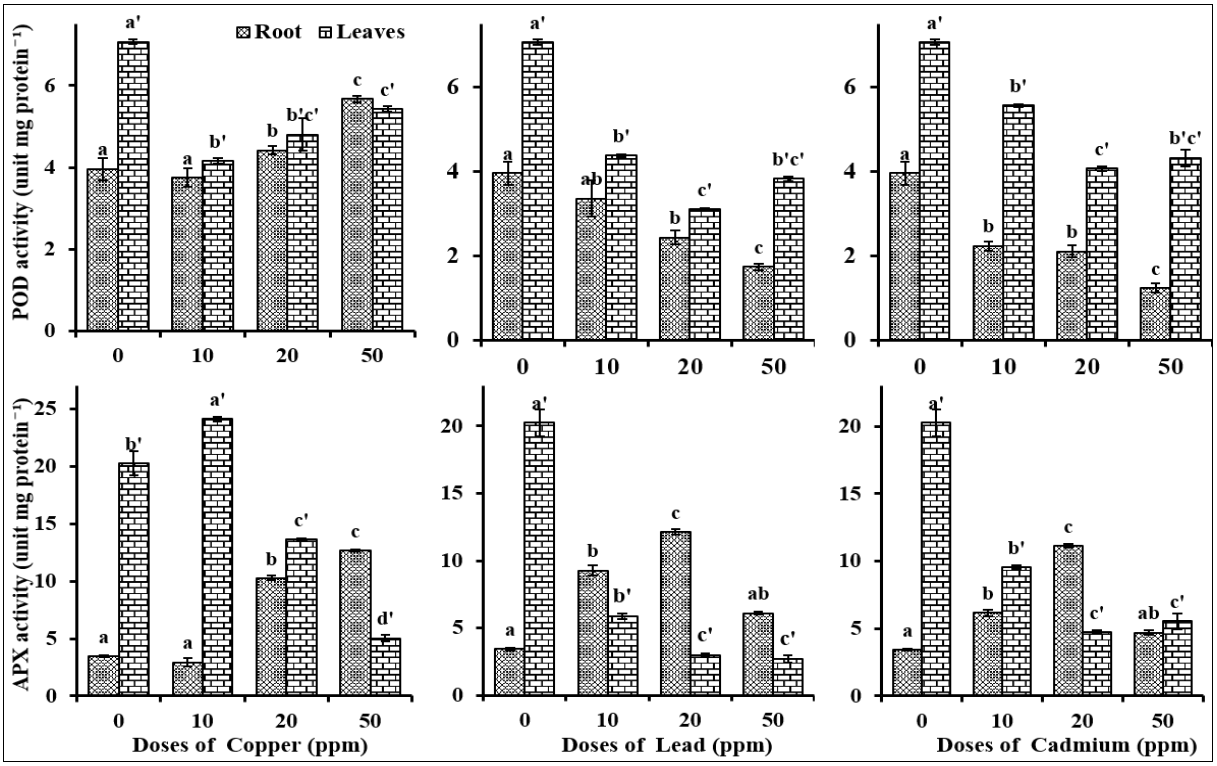

Figure 3. Effect of heavy metals on POD and APX activities**.** Values are the means ± sd, and data represents the average of three experiments with three replicates. Bars marked with the different letters indicate significant differences at $P \leq 0.05$ from each other. Letters represent separate analysis of the plant tissue exposed to different treatment of Cu, Pb and Cd (each element was analyzed within itself compared to the control group to evaluate the effect of the applied heavy metals and letters without an apostrophe for leaves, and letters with apostrophe for roots).

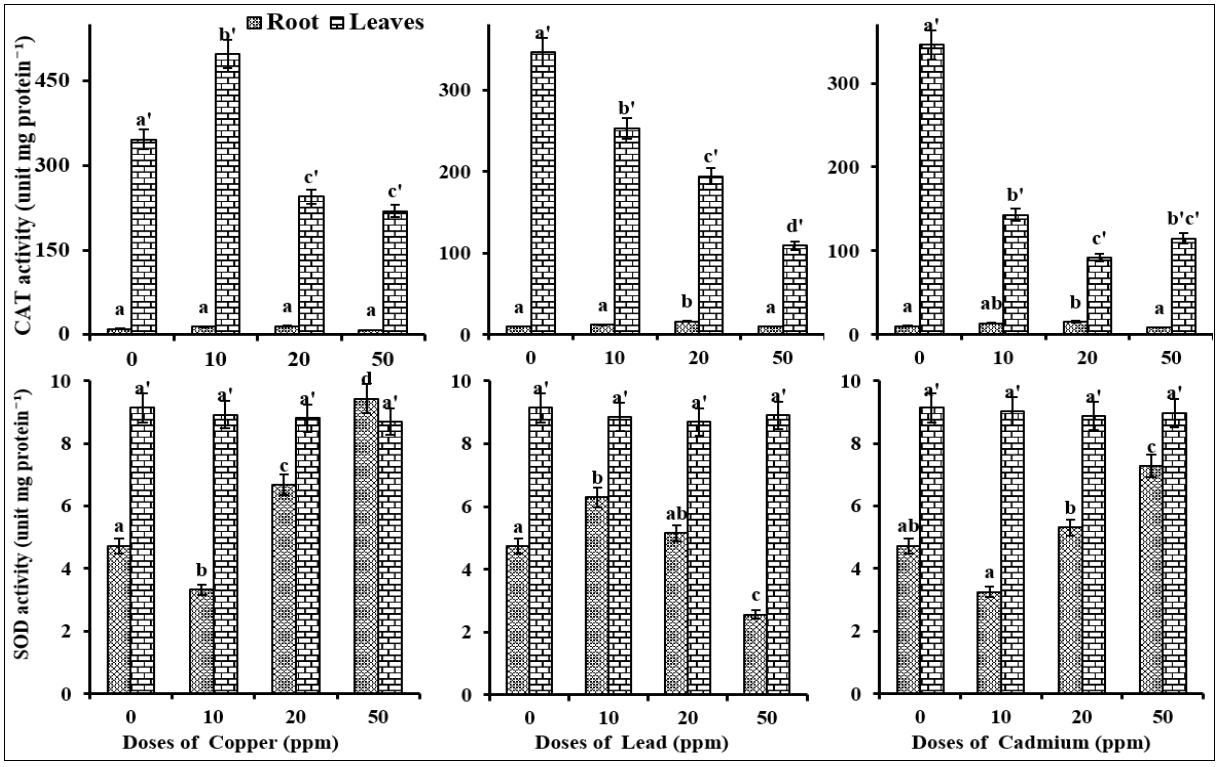

Figure 4. Effect of heavy metals on SOD and CAT activities. Values are the means ± sd, and data represents the average of three experiments with three replicates. Bars marked with the different letters indicate significant differences at $P \leq 0.05$ from each other. Letters represent separate analysis of the plant tissue exposed to different treatment of Cu, Pb and Cd (each element was analyzed within itself compared to the control group to evaluate the effect of the applied heavy metals and letters without an apostrophe for leaves, and letters with apostrophe for roots).

Changes in antioxidant enzyme activity depend on plant species, plant age, application duration and experimental conditions. Toxic metals in the cells cause activated oxygen species formation and an advanced level of oxidative damage in different cell organelles and biomolecules (Hussain et al., 2013). According to the findings, POD, APX, CAT and SOD were affected variously by Cu, Pb and Cd. SOD converts superoxide radical to hydrogen peroxide. Hydrogen peroxide is catalyzed by antioxidant enzymes such as POD, APX and CAT. Catalase converts H_2O_2 to water and molecular oxygen and it acts as a key enzyme for eliminating of toxic peroxides (Gratao et al., 2008).

Pb is one of the most polluter heavy metals existed in the soil and absorbed by plant root systems. When lead concentration increases in plant tissues, toxicity increases (Kasturi et al., 1992). If Pb is soluble in the plant nutrient solution, plant roots can receive most of it. Pb transport and accumulation is limited from roots to upper part of plants. Therefore, it is stored in roots more than in stem (Sharma and Dubey, 2005). This study indicates that Pb accumulation in roots was six times higher than in leaves. If Pb concentration is higher in the soil, it inhibits plant growth. Pb application increases APX activity, while it decreases in POD and SOD activity in roots. CAT activity was not affected significantly by Pb in roots. POD, CAT and APX activities were significantly inhibited by Pb depending concentration in the leaves. It was reported that Pb toxicity reduces CAT activity in rice plants (Verma and Dubey, 2003) and APX activity is decreased in mung bean plants (Hassan and Mansour, 2014). Pb shows specific effects, according to tissue type. This effect may be originated by differences in enzyme amount and Pb localization in tissues.

Cadmium is a non-essential element in plant nutrition that can inhibit growth and stimulate ROS production, resulting in several metabolic perturbations (Yakimova et al., 2006). It was observed that Cd is easily moved from the roots to the upper organs depending on concentration so Cd concentration in root is closely related in leaves. Cd applications decreased POD activity in roots, while it increased APX and SOD activity. However, Cd did not affect SOD activity in leaves, also; it reduced significantly POD, APX and CAT enzyme activities compared to control plants. Similarly, it was reported that Cd reduced APX activity in cucumber seedlings (Gonçalves et al., 2007). Bocova et al. (2012) reported that Cd reduces CAT activity in *Arabidopsis thaliana* leaves. Pb and Cd stress decreased CAT activity in bean leaves (Bhardwaj et al., 2009). It has been reported that superoxide radical inactivates the catalase *in vitro* and Cd can cause to inhibition of catalase when superoxide radicals increased. Cadmium directly affects the sulfhydryl groups in proteins and leads to inhibition of activity or disruption of structure, or from the displacement of an essential element, resulting in deficiency effects (Shah and Nongkynrih, 2007).

The concentrations of the applied heavy metals in the roots and leaves differed. The highest concentration of Cu, Pb and Cd applied in our study was determined as Pb>Cu>Cd, respectively. It arises from leaf Cd concentrations that the movement of cadmium in the plant is greater and that the transport in the plant is greater. This transport is a condition that can reduce the toxicity level of cadmium in the plant up to certain levels. Martinez et al. (2020) reported that as the cadmium doses in beans increased, the cadmium concentration increased in the roots and that there were no significant changes in the Cd concentrations in bean leaves. Researchers reported an increase in enzymatic activities in leaves while different changes occurred in enzymatic activities in roots with Cd applications. Li et al. (2013) reported that increases in SOD, CAT and POD activities occur in the roots of two Kenaf (*Hibiscus cannabinus* L.) plants grown under cadmium stress. POD activity of Fuhong 991 variety used in their studies gave close results in almost all Cd levels. Muradoglu et al. (2015) reported that Cd applications in strawberries

increased statistically, SOD, CAT, APX from antioxidant enzyme activities in plant roots and leaves.

Copper is not mobile in the plants; hence, its deficiency first appears in younger leaves (Fageria and Barbosa Filho, 2006). If mobility is low, it leads to Cu more accumulation in roots than leaves. Higher Cu concentrations increased POD, APX and SOD activities in roots. Cetinkaya et al. (2014) reported that Cu increases APX and SOD activities in corn seedlings. Higher Cu concentrations decreased POD, APX and CAT activities in leaves. It was reported that Cu reduces APX activity and it does not affect CAT activity in tomato (Mazhoudi et al., 1997). Catalase is sensitive to copper stress (Verma and Dubey, 2003). In this study, catalase activity was reduced in high Cu concentrations. The decline of CAT activities in leaves and in roots may be related with the Cu accumulation in tissues, and because there is Fe in the active site of catalase.

Cd can be absorbed easily by plant roots and transferred to the aerial organs (Eker et al., 2013). In this study, Cd increased its concentration in roots and leaves, but it indicated antagonistic effect on minerals because Mg, Ca, Mn, Fe, Zn and Cu levels were reduced in roots. Cd showed synergistic effect on intake Zn, Ca and Mg in leaves. Cd distribution and concentration vary according to plant species, varieties and tissues. It was reported that Cd increases intake of K, Mn and Mo in roots and Fe amount in the stem, but it decreases Fe, Mn and Mg contents in seeds of wheat (Zhang et al., 2002). Liu et al. (2006) also explained that Cd reduces intake of Fe, Mn and Cu, but mineral elements content varies depending on corn varieties. There is no correlation between Cd concentration and nutritional minerals such as P, Ca, S, Mn, Zn; but it increases Fe content (Bertolini et al., 2012). Nada et al. (2007) report that in leaf and root of almond, Cd addition reduced the concentration of macronutrients such as Ca, Mg and K in leaves and in root. Ion distributions alter depending on plant roots and leaves. One of the normal functions of roots is to take up ions selectively founded in the soils, though leaves cannot have the ability to select which ions have to take. Cd accumulation is reduced toward the inner cortex of the epidermis. Endodermis constructs barrier for ion moving; as a result, root cortex cells contain more elements than central vascular cylinder cells (Liu et al., 2006). Muradoglu et al. (2015) reported that Cd applications (0, 15, 30, 45 and 60 mg kg 1) on strawberry plant statistically affect K, Ca, Mg, Fe, Mn, Cu and Zn concentrations in the leaf and root part of the plant. While leaf and root Cu concentrations were 10.99 and 206 ppm in the control application, it was determined as 6.45 and 35.63 ppm in 60 ppm Cd application. Plant Zn concentration also decreased with Cd application. Pb uptake and accumulation was changed depending on applied Pb concentrations in roots and leaves of corn plants. Pb concentration increased accordingly content of Pb in roots and leaves. Pb is usually accumulated in roots and then a small amount is transferred to upper organs (Malkowski et al., 2005). Number of macro and micro elements was reduced in roots when Pb increased. Lead showed antagonistic effects limiting uptake of Fe and Cu in roots and leaves; Mg, Cu, Mn and Zn levels reduced only in roots. However, it showed synergistic effects encouraging Mg, Ca, and Zn intake in leaves. Lamhamdi et al. (2013) reported that Na, Ca, Mg, Fe, Cu and Zn contents were decreased by Pb stress in wheat and spinach. It was also reported that Ca, Zn and Mn were decreased in leaves, but Fe was increased in roots by Pb in *Amaranthus gangeticus*. Fe and Mn content were reduced in stem; and Ca, Zn and Mn were decreased in roots by Pb in *Amaranthus oleracea*. On the other hand, Zn contents were increased in stem, and Fe was increased in roots by Pb in *Amaranthus oleracea* (Kibria et al., 2009).

Cu is a transition metal with oxidation properties in physiological conditions. It is cofactor of several enzymes which are related to the electron transport system. When 10 ppm Cu was supplemented to the growth medium, it had a synergistic effect on uptake of Mg, Ca, Mn and Fe, but higher Cu doses had an antagonistic effect on uptake of these elements. Mg content was increased, but Fe

content was decreased by Cu in corn leaves. Similar results have been reported that copper increased Mg, Ca, Fe and Zn contents; decreased K and S contents in roots of *Arabidopsis thaliana*. Also, it decreased Ca and Mn levels, and increased Zn content in stem of Arabidopsis thaliana (Lequeux et al., 2010). Copper application showed synergistic effects, increasing Mg and Ca intake in wheat seedlings (Azooz et al., 2012).

CONCLUSIONS

As a conclusion, antioxidant enzyme activities were affected significantly by heavy metals such as Pb, Cd and Cu when they were added to the corn plant growth medium. Heavy metals which have low mobility like Cu and Pb were accumulated more in roots. Heavy metals which have high mobility such Cd were moved from roots to leaves. In particular, consumption of plants exposed to Cd toxicity creates problems at this stage. When farming in soils containing heavy metals such as Cd, it should be taken into account that these metals may be transported into the edible parts of plants.

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SS, DK and SBA conceived and designed the experiments. SS and SBA carry out nutrition of plant and analyzed of plant leaves and root. DK contributed analyzed antioxidant enzymes of plant. SS and DK wrote the manuscript.

REFERENCES

Aebi, H., 1984. *Catalase in vitro*. Methods in enzymol, 105: 121-126.

Amari, T., Ghnaya, T., Abdelly, C., 2017. *Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction.* South African Journal of Botany, 111: 99-110.

Angelini, R., Manes, F., Federico, R., 1990. *Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chickpea stems*. Planta, 182: 89-96.

Azooz, M.M., Abou-Elhamd, M.F., Al-Fredan, M.A., 2012. *Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (Triticum aestivum cv. Hasaawi) at early growing stage*. Australian Journal of Crop Science, 6(4): 688-694.

Beyer, W.F., and Fridovich, I., 1987. *Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions*. Analyt Biochem, 161: 559-566.

Bertolini, A.C., Cannata, M.G., Carvalho, R., Bastos, A.R.R., Freitas, M.P., Augusto, A.S., 2012. *Lycopersicon esculentum submitted to Cd-stressful conditions in nutrition solution: Nutrient contents and translocation.* Ecotoxicology and Enviromental Safety, 86: 176-181.

Bhardwaj, P., Chaturvedi, A., Prasad, P., 2009. *Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of Phaseolus vulgaris L*. Nature and Science, 7(8): 63-75.

Bocova, B., Huttova, J., Lıptakova, L., Mıstrık, I., Olle, M., Tamas, L., 2012. *Impact of short-term cadmium treatment on catalase and ascorbate peroxidase activities in barley root tips.* Biologia Plantarum, 4: 724-728.

Cetinkaya, H., Dınler, B.S., Tascı, E., 2014. *Investigation of comparative regulation on antioxidant enzyme system under copper treatment and drought stress in maize (Zea Mays L.)*. Not. Bot. Horti. Agrobo., 42(2): 363-371.

Döker, S., Aydemir, O., Uslu, M., 2014. *Evaluation of digestion procedures for trace element analysis of cankiri, turkey honey by inductively coupled plasma mass spectrometry*. Analytical Letters, 47(12): 2080-2094.

Eker, S., Erdem, H., Yazıcı, M.A., Barut, H., Heybet, E.H., 2013. *Effects of cadmium on growth and nutrient composition of bread and durum wheat genotypes*. Fresenius Environmental Bulletin, 22-6.

Ekmekçi, Y., Tanyolaç, D., Ayhan, B., 2008. *Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars*. Journal of Plant Physiology, 165: 600-611.

Fageria, N.K., and Barbosa Filho, M.P., 2006. *Identification and correction nutrient deficiencies in rice.* Embrapa Arroz e Feijão, 75: 7.

Gonçalves, J.F., Becker, A.G., Cargnelutti, D., Tabaldi, L., Pereira, L.B., Battisti, V., Spanevello, R.M., Morsch, V.M., Nicoloso, F.T., Schetinger, M.R.C., 2007. *Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings*. Braz. J. Plant Physiology, 19(3): 223-232.

Gratao, P.L., Monterio, C.C., Antunes, A.M., Peres, L.E.P., Azevedo, R.A., 2008. *Acquired tolerance of tomato (Lycopersicon esculantum cv. Micro-tom)*

plants to cadmium-induced stress. Annals of Applied Biology, 153: 321-333.

Haribabu, T.E., and Sudha, P.N., 2011. *Effect of heavy metals copper and cadmium exposure on the antioxidant properties of the plant Cleome gynandra*. International Journal of Plant, Animal and Environmental Science, 1-2: 80-87.

Hassan, M., and Mansoor, S., 2014. *Oxidative stress and defense mechanism in mung bean seedlings after lead and cadmium treatments.* Turkish Journal of Agriculture and Forestry, 38: 55-61.

Hussain, A., Abbas, N., Arshad, F., Akram, M., Khan, Z.I., Kafeel, A., Mansha, M., Mirzaei, F., 2013. *Effects of diverse doses of Lead (Pb) on different growth attributes of Zea mays L.* Agricultural Sciences, 4(5): 262-265.

Kabata-Pendias, A., and Pandias, H., 2001. *Trace elements in soil and plant*. Third Edition CRC Press LLC, London.

Kasturi, R., Petrovic, M., Petrovic, N., 1992. *Effect of excess lead, cadmium, copper and zinc on water relations in sunflower.* Journal Plant Nutrition, 15: 2427-2439.

Kibria, M.G., Islam, M., Osman, K.T., 2009. *Effects of lead on growth and mineral nutrition of Amaranthus gangeticus L. and Amaranthus oleracea L.* Soil & Environ., 28(1): 1-6.

Lamhamdi, M., Galiou, O., Bakrim, A., Munoz, J.C., Esteves, M., Aarab, A., Lafont, R., 2013. *Effect of lead stress on mineral content and growth of wheat (Triticum aestivum) and spinach (Spinacia oleracea) seedlings.* Saudi Journal of Biological Sciences, 20: 29-36.

Lequeux, H., Hermans, C., Lutss, S., Verbruggen, N., 2010. *Response to copper excess in Arabidopsis thaliana: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile.* Plant Physiology and Biochemistry, 48: 673-682.

Li, F., Qi, J., Zhang, G., Lin, L., Fang, P., Tao, A., Xu, J., 2013. *Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two Kenaf (Hibiscus cannabinus L.) plant seedlings*. Journal of Integrative Agriculture, 12(4): 610-620.

Liu, D.H., Wang, M., Zou, J.H., Jiang, W.S., 2006. *Uptake and accumulation of cadmium and some nutrient ions by roots and shoots of maize (Zea mays L.)*. Pak. J. Botany, 38(3): 701-709.

Malkowski, E., Kurtyka, R., Kita, A., Karcz, W., 2005. *Accumulation of Pb and Cd and its effect on Ca distribution in maize seedlings (Zea mays L.).* Polish Journal of Environmental Studies, 14(2): 203-207.

Martinez, G.P.B., Moran, T.M.I., Puartes, R.M.C., Solis, C.J., Villasenor, Z.P., Pinto, S.E., Hernandez, R.B.C., 2020. *Assesment of antioxidant enzymes in leaves and roots of Phaseolus vulgaris plants under cadmium stress.* Biotecnia, XXII(2): 110-118.

Mazhoudi, S., Chaoui, A., Ghorbal, M.H., Ferjani, E., 1997. *Responses of antioxidant enzymes to excess copper in tomato (Lycopersicon esculantum, Mill.).* Plant Science, 127: 129-137.

Muradoglu, F., Gundogdu, M., Ercisli, S., Encu, T., Balta, F., Jaafar, H.Z.E., 2015. *Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry*. Biological Research, 48: 11.

Nakano, Y., and Asada, K., 1981. *Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts.* Plant Cell Physiol., 22: 867-880.

Nada, E., Ferjani, B.A., Rhouma, A., Bechir, B.R., Imed, M., Makki, B., 2007. *Cadmium-induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture*. Acta Physiol. Plant, 29: 57-62.

Rastgoo, L., and Alemzadeh, A., 2011. *Biochemical responses of Gouan (Aeluropus littoralis) to heavy metals stress*. Australian Journal of Crop Science, 5(4): 375-383.

Rellan-Alvarez, R., Ortega-Villasante, C., Alvarez-Fernandez, A., 2006. *Stress responses of Zea mays to cadmium and mercury*. Plant and Soil, 279: 41-50.

Sbartai, H., Djebar, M.R., Rouabhi, R., Sbartai, I., Berrabbah, H., 2011. *Antioxidative response in tomato plants Lycopersicon esculantum L. roots and leaves to zinc*. American-Eurasian Journal of Toxicological Sciences, 3: 41-46.

Shah, K., and Nongkynrih, J.M., 2007. *Metal hyperaccumulation and bioremediation.* Biologia Plantarum, 51(4): 618-634.

Sharma, P., and Dubey, R.S., 2005. *Lead toxicity in plants*. Braz. J. Plant Physiol., 17(1): 35-52.

Yakimova, E.T., Kapchina-Toteva, V.M., Laarhoven L.J., Harren, F.M., Woltering, E.J., 2006. *Involvement of ethylene and lipid signaling in cadmium-induced programmed cell death in tomato suspension cells.* Plant Physiology and Biochemistry, 44: 581-589.

Verma, S., and Dubey, R.S., 2003. *Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants.* Plant Science, 164: 645-655.

Zengin, F.K., and Munzuroglu, O., 2005. *Effects of some heavy metals on content of chlorophyll, proline ans some antioxidant chemicals in bean (Phaseolus vulgaris L.) seedlings.* Acta Biologica Cracoviensia, 47/2: 157-164.

Zhang, G., Fukami, M., Sekimoto, H., 2002. *Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage*. Field Crops Research, 77: 93-98.