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# Effects of cadmium and zinc interactions on growth parameters and activities of ascorbate peroxidase on maize (*Zea mays* L. MAT 97)

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## ABSTRACT

*The aim of this research was to investigate the effects of zinc and cadmium heavy metals and the interaction of these heavy metals on mazie's (Zea mays L. cv. MAT 97) root and shoot elongation, dry weight, root/shoot dry weight ratio and ascorbate peroxidase (AP) enzyme activity. Cd's high concentration and treatment of this heavy metal with zinc had significant statistical effect on mazie root and shoot elongation. While zinc treatment had a positive effect on root elongation, Cd treatmen had a negative effect on shoot elongation. Therefore the treatment of Zn with Cd had positively effected shoot elongation. 10 and 15 µM Cd treated maize's root and shoot dry weights had significantly decreased according to control and there has no significant stataistical diffrence between 5 µM Zn or Cd+Zn treated maize regarding shoot dry weight. The differences in root and shoot water% ratio and root/shoot ratio are found to be insignificant. The increase of ascorbate peroxidase and catalase activity in 10 µM, 15 µM Cd and 5 µM Zn and Zn+Cd treated maize is found to be in significant levels according to control.*

**Keywords:** Heavy metal, ascorbate peroxidase, zinc, cadmium, maize

## INTRODUCTION

Cd pollution is a global environmental problem and it penetrates to the nature via industrial activities such as mining, release of industrial wastes [1]and man-made causes [2]. Generally, Cd remains stable and pollutes the soil. Cd is a non-base metal for plants and human; it penetrates to the plants through roots and accumulates in the stem [3,4]. Cadmium ion poisoning of plants generally manifest itself as reduced growth. Even very little amount of these toxic contaminants cause several alterations in plant cells [5,6,7,8]. However it is difficult to picture an overall mechanism chart on stress physiology. Oxidative stress caused by reactive oxygen species (ROS) is one of the underlying reasons of damages suffered by plant tissues following being exposed to Cd and Cu [9,10,11,12]. ROS ($^1O_2$, $O_2^-$, $OH^·$ and $H_2O_2$) are highly reactive, toxic molecules which are caused by various environmental stress conditions, increased oxidative stress and which cause damage on membrane, DNA, protein, photosynthetic pigments and lipids [13,14]

Cd and Zn are elements of IIB transition class and they are the best element matches to study metal-metal interaction since they have similar chemical, geochemical and environmental characteristics [15]. Zn is one of the most important essential micronutrient elements for plant systems. Zn has significant role in several critical cellular functions such as protein metabolism, gene expression, chromatin structure, photosynthetic carbon metabolism and indole acetic acid metabolism [16, 17, 18, 19]. However, higher concentrations of Zn might have toxic effect on plants. Elements having similar physical and chemical characteristics, such as Cd and Zn, might have biologically antagonistic impact on each other [20]. Accordingly, the studies on Zn and Cd concluded that Zn has an antagonistic effect on Cd [21], Zn inhibits Cd intake and accumulation in plants and prevents Cd toxicity [22, 23, 24, 21].

Cd and Zn are considered to have competition interaction in case of a common transition system on plasma membrane during plant's intake [25]. Based on this assumption, Gomes et al. [26] showed that Cd intake through Zn carrier protein on plasma membrane of yeast cell. Besides, it is observed that carrying Cd via phloem on hard wheat can be prevented with Zn. It is seen that Zn competes Cd to bind to a carrier protein in plasma membranes of sieve cells and prevents Cd intake [27]. Wu and Zhang [28] confirmed that increased use of Zn on barley reduces Cd toxicity stress, supports growth and reduces membrane damage.

There are several proofs evidencing that ROS resulting from Cd toxicity in plants cause oxidative cell damages. Thus, the plant's capacity of eliminating ROS under Cd toxicity is an important tolerance mechanism against Cd toxicity [29, 30].

Zn has important impact on preventing damages caused on cell by ROS resulted under environmental stress conditions. Thus Zn has an important role in mechanisms of ensuring plant tolerance against stress conditions such as drought, low temperature stress, high light, salinity and Fe toxicity [31]. Probably Zn enhances antioxidant enzyme activities such as Zn-containing enzyme superoxide dismutase and competes Cd to bind to –SH groups and membrane proteins of enzymes to protect plant against Cd toxicity [28]. Antioxidant enzyme is one of the defense mechanisms against formation of hydrogen peroxide ($H_2O_2$), superoxide ($O_2^{.-}$) and hydroxyl ($OH^{.}$) radicals which are ROS related [32, 33, 30, 34, 35]. Among all antioxidant enzymes, AP has an important role in ROT detoxification in cells. AP uses ascorbic acid as a specific electron transmitter and regresses to $H_2O_2$ [36].

Besides ruining the nature, heavy metals cause a severe damage on agricultural production. Contaminating agricultural lands with toxic, heavy metals such as Cd cause significant product losses in terms of agricultural plants such as wheat, barley and corn which have important functions in food chains and endanger human health. This study focuses on corn because corn is an important nutritional source, corn cultivation is common is Turkey and the Mediterranean region and there are very few studies researching Cd + Zn interaction's impact on corn and AP enzyme activities.

Bearing in mind that there are very few studies in the literature on applying Zn to corn seedlings exposed to Cd stress conditions; this thesis focuses on studying Zn's effect on corn seedlings exposed to cadmium in terms of physiological parameters such as root and root sprout lengths, dry weight and root/root sprout ratio and on enzyme activities such as ascorbate peroxide. In order to have a better understanding of metabolic ways of these two elements interaction, one metal and one nutriment, related metals interaction with each other in terms of specific parameters.

## MATERIALS AND METHODS

### Material

Corn seeds (*Zea mays* L. cv. Mat 97) seedlings which have alternative cultivation qualities as well as known to have good resistance against cold, winter and drought, are used as plant material in our research. The corn seeds are provided by West Mediterranean Agricultural Research Institution (Antalya).

### Plant Growth Conditions

During the course of cultivating seedlings used as material in our study, corn seeds are sterilized with 2 % sodium hypochloride solution for 20 minutes [37] and then they are washed with distillate water and the seeds are germinated for a period of 48 hours in an incubator of 25±2 °C between filter papers dampened with distillate water in petri plate. The germinated seeds are cultivated under greenhouse conditions in containers containing sand-perlite mixture (1:1 v:v) (average sand diameter 0.7 mm, average perlite diameter 2.8 mm) and watered with distillate water for 7 days. The seedlings are taken out of sand-perlite mixture at the end of seventh day and the roots are washed thoroughly with distillate water. Two-liter plastic containers, which are wrapped from outside to avoid being exposed to light, are used for cultivating the seedlings with water culture technique. Two liters of nutrient solution is put in each container. Full-power Arnon and Hoagland [38]solution set to pH 6 is used as nutrient solution. After being prepared in the containers as described herein, they are taken to climate chamber.

The seedlings are cultivated for 7 days by ventilation throughout the day. The solutions in the containers are thrown away on the eighth day and filled with new nutrient solutions. Heavy metal (zinc and cadmium) applications (when the seedlings are 8-day old) are made by mixing with nutrient solutions. Chemical purity grade zinc chloride ($ZnCl_2$) and cadmium chloride ($CdCl_2$) salts of heavy metals are used. These salts are added as follows; control 0 Cd 10, 15 µM and Zn 5 µM. The test is made as 3 parallels. The plants are cultivated in the climate chamber where the temperature is approximately 24±2 °C, relative humidity is 66 % and light conditions is equal to approximately 1198 gas lamps. The seedlings are harvested on the 5th day following the heavy metal application (when the seedlings are 12-day old).

Fresh materials are used for measuring plant growth after harvest. Roots of seedlings set aside for biochemical analyzes are cut from the point of root-root sprout and the root spouts are kept in -80 degrees in a freezer and in small pieces for enzyme analysis.

**Plant Growth Measurements**
After harvest, root and shoot lengths of 3 seedlings are measures in cm after being selected according to random block test pattern among the seedlings exposed to Cd, Zn and Cd + Zn applications. Then the seedlings are cut from the point of root-shoot sprout. Then plant samples are dried for 24 hours in drying ovens of 110 °C and dry weight of root and shoot are determined separately.

**Ascorbate Peroxidase Activity**
The AP activity is determined by measuring the ascorbate oxidation ratio in 290 nm according to the methods of Cakmak and Marschner [39] and Cakmak [40].

**Statistical Analyzes**
Factorial variance analysis technique is used for comparing values obtained as a result of the test. If there are statistical differences between groups compared with factorial variance analysis technique, Tukey test, which is one of the multiple comparison methods, is used for determining different groups.

## RESULTS AND DISCUSSION

The study determined that 5 µM Zn and Zn concentrations applied with 10 µM and 15 µM Cd foster root growth by $p<0{,}05$ (Figure 1.).

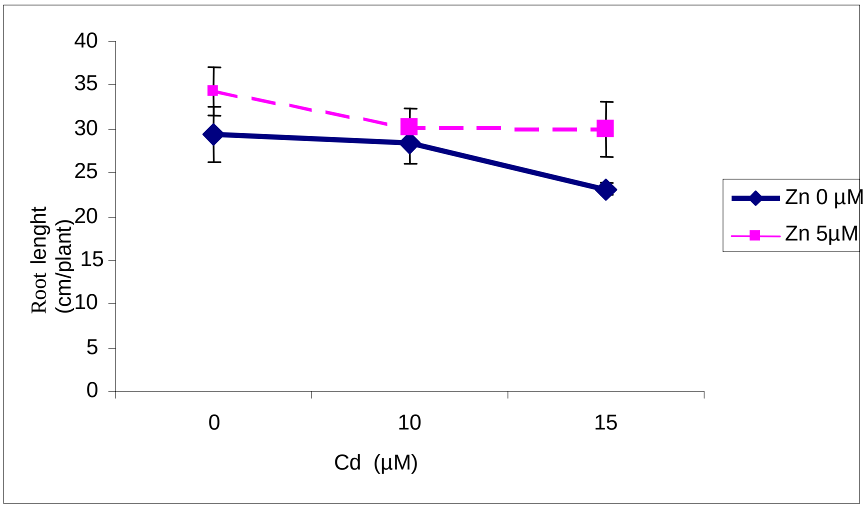


**Fig. 1. Effects of Cd, Zn and Cd + Zn interaction on the root lenght of (*Zea mays* L. MAT 97) the maize seedlings (n=3)**

Ayhan [41] argued that if we compare root lengths of corn types (Vero, Luce, Doge, DK626, DK743, 31G98, 3223 and 32D99) in early seedling and growth stages, we can see that Cd is much more toxic than Pb. It is determined that root lengths of all corn types in early seedling stage are exposed to 50 % and more inhibition in comparison to the control; 0,2-0,4 mM concentrations for Cd and 1-4 mM concentrations for Pb. Different concentrations of Hg, Cd, Co, Cu, Pb and Zn are applied to early seedling stage of *Triticum aestivum* and *Cucumis sativus* plants and it is observed that Cd inhibits root growth more than Pb in case of both plants [42].

This situation is associated with the fact that Zn, which is a plant nutrient element, is an important component of several enzymes required for lifecycle and it contributes to structures of proteins, membrane and DNA binding proteins [43]. On the other hand, Cd is a highly toxic metal with has no metabolic significance as well as being very harmful for human and biosphere health [15].

In our study, it is observed that root weight is significantly reduced when compared with control in case of 15 µM Cd ($p<0,01$) and a decrease of $p<0,05$ is observed between 10 µM Cd and 15 µM Cd concentrations (Figure 2.).

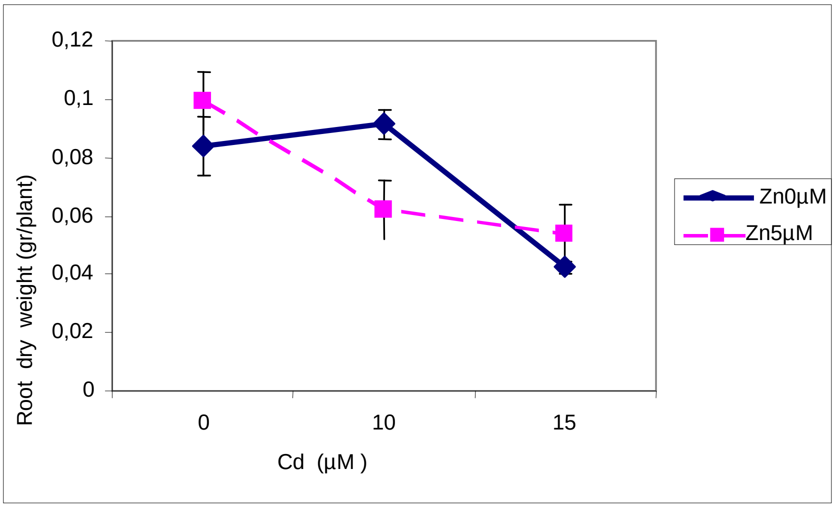


**Fig. 2. Effects of Cd, Zn and Cd + Zn interaction on the root dry weight of (*Zea mays* L. MAT 97) the maize seedlings (n=3)**

Cd is a dangerous health metal with have toxic effect on all crops and human health [3]. Although Cd is not a mineral nutrient, it is easily taken in by plant roots and stored in a plant at a level causing a food chain risk. Cd might be also toxic in cellular level and accumulation in plant tissue can restrict growth and development. Thus, it is argued that preventing Cd intake from plant roots can be crucial for minimizing biological effects of Cd. [44].

In case of plants subject to extended exposure to metals such as Pb, Cr, Al, Cu and Cd, root growth is much more effected than stem. However, it is observed that this varies according to the developmental stage of plant, cultivation conditions and type of plant [45]. Kırbağ-Zengin [46]determined that applying Hg, Cd, Cu and Pb on *Phaseolus vulgaris* L. seedlings prevents root, stem and leaf growth. Cd causes root damage [2]. Ergün and Öncel [47]confirmed that if you apply Pb, Cd and Zn to wheat seedlings, root growth of seedlings exposed to Zn enhances whereas it decreases in case of seedlings exposed to Cd, depending on the increased concentration.

The root sprout of seedlings exposed to 15 µM Cd is reduced in comparison to the control ($p<0,05$). The impact of Cd applications with Zn on length of root sprout is considered to be important at a level of $p<0,05$ (Figure 3).

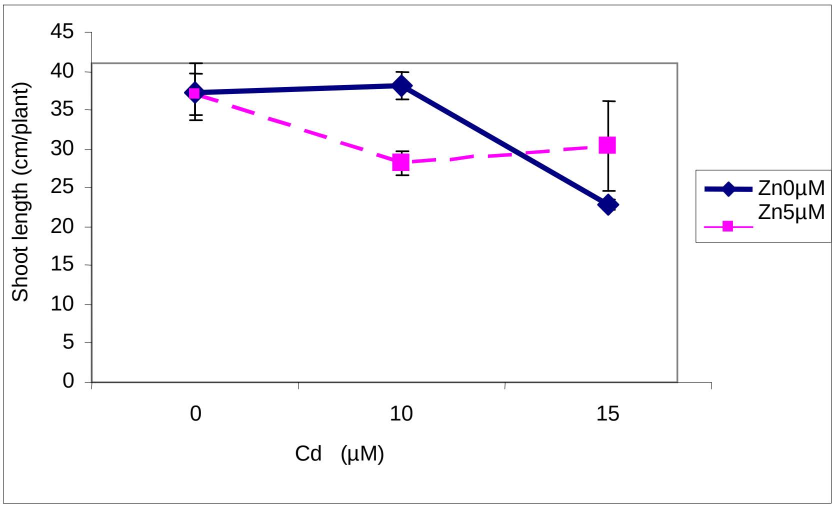


**Fig. 3. Effects of Cd, Zn and Cd + Zn interaction on the shoot lenght of (*Zea mays* L. MAT 97) the maize seedlings (n=3)**

Our study confirmed that the dry weight of root sprouts exposed to Cd application are significantly reduced in comparison to the control seedlings (p<0,05) (Figure 4) but there are no statistical differences between the Cd dosages applied.

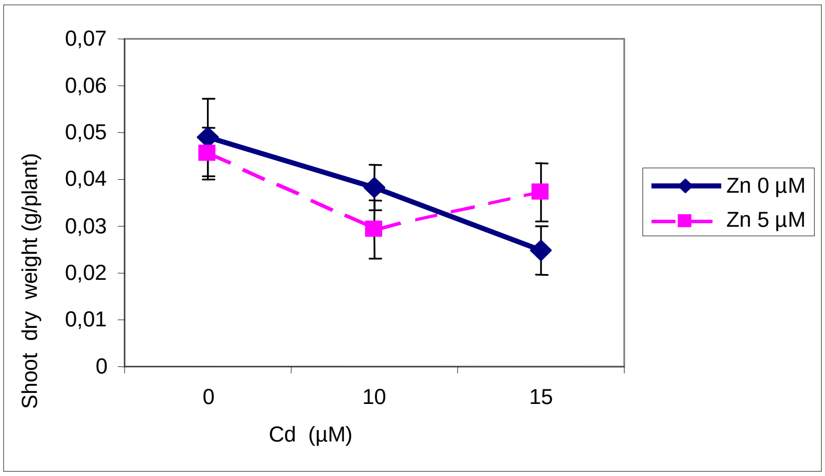


**Fig. 4. Effects of Cd, Zn and Cd + Zn interaction on the shoot dry weight of (*Zea mays* L. MAT 97) the maize seedlings (n=3)**

As for the corn types exposed to Cd and Pb (Vero, Luce, Doge, DK626,DK743, 31G98, 3223 and 32D99), the resistant types 32D99 and Vero displayed an inhibition rate of 31 % and 27 % respectively in Cd (0.9 mM) and Pb (8 mM) concentrations whereas 3223 type having less resistance displayed an inhibition rate of 25 % and 18 % respectively. It is observed that stem growth of corn types is inhibited at least as much as the root lengths in case of seedlings exposed to Cd stress[41]. Ergün and Öncel [47]determined that if we apply Pd, Cd and Zn to wheat seedlings, root sprout growth of seedlings exposed to Zn increases whereas the growth decreases on seedlings exposed to Cd, depending on the increased concentrations.

It is confirmed that *Oryza sativa* seedlings exposed to 0,1 and 0,5 mM Cd and Ni accumulated these heavy materials within the plant at significant levels and the stem length is reduced[37]. 3 µM Cd applied to the leaves of *Phaseolus vulgaris* L. cv. contender seedlings reduced growth [48]. Kırbağ-Zengin (2002)[46]determined that applying Hg, Cd, Cu and Pb causes inhibition in stem growth of *Phaseolus vulgaris* seedlings. Cd and Ni exposure inhibits stem growth of *Oryza sativa* seedlings [49].

Our findings concluded on this study about reduced root sprout caused by increased Cd concentration comply with the findings of Poschenrieder et al. (1989)[48], Rubio et al. [37], Moya et al. [49], Kırbağ-Zengin [46], Ayhan [41], and Ergün and Öncel [47].

In our study, 10 and 15 µM Cd applications and also 5 µM Zn with Cd applications are confirmed to have important impact on AP enzyme activity (p<0,01) (Figure 5). AP activity almost tripled in case of non-zinc application of and increased by almost 6 times in case of 15 µM Cd. 5 µM Zn applications reduced ascorbate peroxide activity but it almost quadrupled in case of 10 µM Cd + Zn applications. However, 15 µM Cd application is confirmed to significantly reduce the enzyme activity (p<0,01) .

Generally, Zn applications reduce Cd intake and accumulation in plants [24]. Zn's impact on plant's Cd intake and accumulation is not permanent. There are reports on Cd's and Zn's synergistic impacts. Nan et al., [50]determined that Zn concentrations increase if Cd applications increase in wheat seedlings or Zn concentration reduces in the contrary situation. Same observations are documented by Dudka et al.,[51]. Wu and Zhang [28] showed that increased Zn application in barley reduces Cd toxicity and supports growth as well as reducing membrane damage.

It is argued that Cd toxicity is the cause of oxidative cell damages resulted from plant related ROS species. Thus, the ROS elimination skill of plants under Cd toxicity is an important tolerance mechanism against Cd toxicity[29,30]. Zn has several protective roles in the process of minimizing the damages caused on cells by ROS formed under environmental stress conditions. Accordingly, Zn's role in tolerance mechanisms of plants against stress conditions such as drought, low temperature stress, high light, salinity and Fe toxicity is studied [31]. Probably Zn enhances antioxidant enzyme activities such as Zn-containing enzyme superoxide dismutase and competes Cd to bind to –SH groups and membrane proteins of enzymes to protect plant against Cd toxicity. Wu and Zhang [28]showed that Zn application can decrease reduction of root dry matter and increase of membrane damages caused by Cd toxicity.

Hard wheat's higher level sensitivity to Cd toxicity, in comparison to bread wheat, can be might explained by hard wheat's weaker detoxification mechanism at cellular level in the process of Cd elimination by Cd binding proteins or in vacuole departments [52,53].

In case of Ascorbate-Glutathione cycle, AP has an important role in eliminating $H_2O_2$. Song et al[54] confirmed role of silicone (Si) in Cd tolerance with a study on two types of *Brassica chinensis* L. with Cd tolerance and sensitivity and observed that low levels of Cd application in plants enhances AP activity whereas increased dosage of Cd applications reduce AP activity.

Aravind and Prasad [15] studied antagonistic effects of Zn and Cd on *Ceratophyllum demersum* L. (Coontail) macrophyte and confirmed that Zn application in plants suppress Cd intake. The researchers determined that SOD, CAT, GP and AP activities of plants exposed to Cd + Zn application are higher than plants exposed to only Zn or only Cd applications.

Aravind and Prasad [15] observed that 10 µM Cd in *C. demersum* L. has slight impact on AP activity but Cd + Zn applications increase ascorbate-glutathione cycle enzyme (including AP) activities.

Ayhan [41]confirmed that leaves of corn types display significantly higher AP, glutathione reductase (GR) enzyme activities in comparison to the control under Pb and Cd stress and in parallel to increased heavy metal concentration and this process has an important role in neutralizing $H_2O_2$ caused by superoxide dismutase (SOD) activity. However, the researcher argued that even if the enzyme activities show a significant increase in comparison to the control under high Cd concentrations, there is still a downwards trend when compared with 5 mM Cd application.

The data we obtained in this study regarding increased AP enzyme activity based on increased Cd concentrations are similar to the data reported by Song et al. [54], Ayhan[41], and Aravind and Parasad [15].

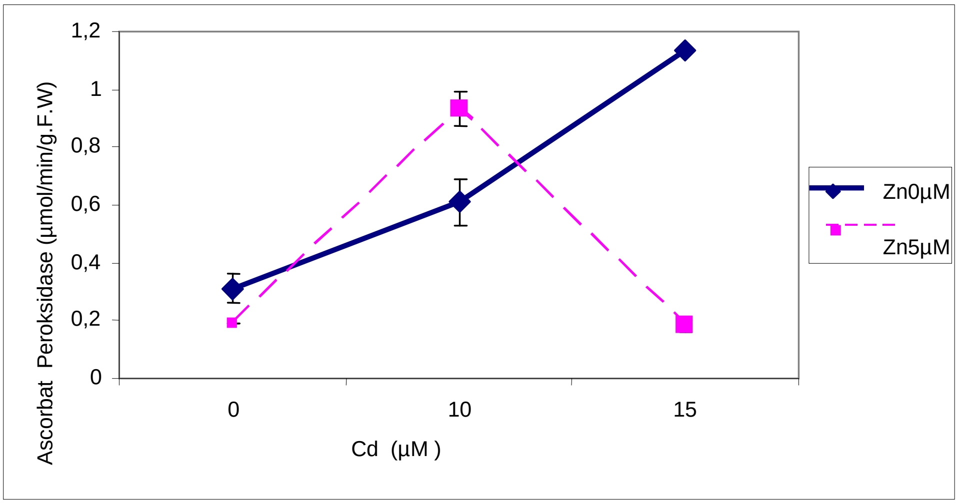


**Fig. 5. Effects of Cd, Zn and Cd + Zn interaction on the ascorbate peroxidase activity on (*Zea mays* L. MAT 97) the maize seedlings (n=3)**

Cd ions cause oxidative stress, peroxidation and disorganization and thus have direct impact on the lamellar membrane structure [55]and change the fat composition in the lamellar membrane [56]. Also Zn is known to protect bio-membrane against oxidative and peroxidative damage, reduced membrane integrity [15,31]and even change of membrane permeability [57]as well as stabilizing the bio-membrane.

In this case, we believe that the data regarding a significant increase of approximately four times in 10 µM Cd+Zn application can be associated with the fact that Zn application in case of Cd toxicity protects the plant against oxidative and peroxidative damages. Generally, Zn applications reduce Cd intake and accumulation on plants [22,23,24]. However, our study confirmed that 15 µM Cd+Zn application significantly reduced enzyme activity ($p<0,01$). In this case, we believe that Cd and Zn will show synergistic effect in Zn concentration applied with 15 µM Cd and thus reduce AP enzyme activity.

Besides, it is argued that Zn applications' impact of Cd concentration of plants might depend on type of plant, cultivation conditions and Cd concentration used [22,24,25].

## CONCLUSION

Root length growth of corn seedlings exposed to Cd is not effected but the root growth of seedlings exposed to only Zn and Zn with Cd is significantly promoted. The highest Cd concentration showed a significant reduction in root and root sprout weight and root sprout length. However using Cd with Zn has positive impact on the root sprout length but has no effect on root sprout and root dry weight. Using Cd and Zn on seedlings has no effect on root / root sprout ratio. In conclusion Cd has negative impact of growth of root sprout length whereas Zn can eliminate Cd's toxic impact on growth of length but Zn fails to eliminate Cd's influence on increase of dry weight.

In case of all Cd applications and also Zn applied with Cd, it has important effect on AP enzyme. AP activity increases in case of non-zinc applications whereas solely using Zn reduces AP activity and using 10 µM Cd+Zn almost quadrupled the results. However, applying 15 µM Cd significantly reduced enzyme activity. It is determined that AP activity is increased for eliminating Cd's toxic effects. Heavy metals cause severe damages on agricultural production as well as ruining the environment. The destruction caused by heavy metals on the earth's surface reduces productivity of several agricultural fields or entirely impairs the productivity.

The impacts of heavy metals manifest themselves with the free radicals formed within the cell; they lead to several animal and plant diseases such as tissue damage, neurodegenerative diseases, cancer and aging. Organisms have enzymatic and non-enzymatic defense systems against all radicals. Thus, these antioxidant components having the capacity repressing free radicals have become highly important. Antioxidants function by protecting the organism against multitude of reactive oxygen radicals when enzymatic defense system is insufficient [58].

According to our results, it is observed that using Cd with Zn has stimulator effect on root and stem length as well as having important impact on AP activity.

Thus, there should be further studies for studying the antioxidant defense system in details in order to have a better understanding of plant defense against heavy metal stress and for comprehending the role of Zn, which is an essential nutrient element, mineral, in preventing Cd toxicity. Further studies in this area will ensure better understanding of molecular basis of plant and animal resistance against highly toxic heavy metals such as Cd as well as enabling to choose species agricultural plants which are resistant to heavy metals, preventing plant's Cd intake and minimizing the damage caused after uptake.

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