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Effects of Cadmium and Zinc on Growth and Some Biochemical Parameters of Lentil Seedlings (*Lens esculenta* L.)

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Abstract: This study investigated toxic impacts of cadmium (Cd) and zinc (Zn) over a concentration gradient of 250 ppm on lentil root growth (*Lens esculenta* L. Erzurum 89). And an increased Cd concentration shoot dry weight decreased. Despite a reduction in growth of the seedlings its proline contents increased both in the roots and in the shoots under heavy metal stres corresponding to the concentration of the metal ion in the culture medium. Furthermore 500 ppm Cd concentrations decreased soluble protein and soluble phenolic content in the shoots seedlings. All Zn concentrations soluble phenolic concentartions increased in the shoots of seedlings. The proline contents in both roots and shoots of lentil seedlings that there is a decrease in proline content with 500 ppm Cd concentration when compared with that of 400 ppm Cd. Cd, in 500 ppm concentrations, inhibits of lentil growth remarkably hampering its, physiological functions and metabolism.

Keywords: Lentil, heavy metal, proline, soluble phenolics, soluble protein.

Mercimek (Lens esculenta L.) Fidelerinde Büyüme ve Bazı Biyokimyasal Parametreler Üzerine Kadmiyum ve Çinko'nun Etkileri

Özet: Bu çalışmada mercimek fidelerinin kök büyümesi üzerine 250 ppm ve üzeri Cd ve Zn ağır metal konsantrasyonlarının etkileri incelenmiştir. Artan Cd konsantrasyonlarına bağlı olarak gövde kuru ağırlığı azalmıştır. Fidelerin büyümesinde azalma olmasına rağmen kültür ortamındaki metal iyonlarının konsantrasyon artışına bağlı olarak hem kök hem de gövde de prolin miktarı artmıştır. Bununla birlikte 500 ppm Cd konsantrasyonunun gövde sürgünlerinde çözünür protein miktarı ve çözünür fenolik içeriği azalmıştır. Zn'nun tüm konsantrasyonlarında gövde sürgünlerinin çözünür fenolik miktarı artmıştır. 500 ppm Cd konsantrasyonu 400 ppm Cd konsantrasyonu ile kıyaslandığında mercimek fidelerinin kök ve gövdelerinde prolin miktarında bir azalma gözlenmiştir. 500 ppm Cd konsantrasyonunda metabolik ve fizyolojik aktivitelerin önemli ölçüde engellenmesine bağlı olarak mercimek fidelerinin büyümesinde azalma gözlenmiştir.

Anahtar Kelimeler : Mercimek, ağır metal, prolin, çözünür fenolikler, çözünür protein

Introduction

Heavy metals in high concenteration inhibits the growth and the thrive in plants. Moreover a number of biochemical and physiological parameters are adversely affected i.e. damaged cell membrane and photosynthetic organs, hampered transpiration and protein synthesis, inhibition of photosynthesis, malfunctioning of enzyme activities and rather high lipid peroxidation [1]. Accumulative capacity for free prolines is an indication of individual protection for plants exposed to environmental stress [2]. It has been suggested that proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential and the functional significance of this accumulation could be related to the water balance [3]. Kastori et al. [4], found at that free proline content increased considerably in paralel with heavy metal application in *Helianthus annuus* L. seedlins while soluble protein content decreased. On the other hand Costa and Monel [5] ascertained that Cd didn't induce proline accumulation in *Lactuca sativa* seedlings but enhanced their asparagin, metionin and lisin content. It has been suggested that proline levels were increased in soybean nodules and roots subjected to Cd treatments, these increments were not sufficient to avoid the Cd induced severe alteration in the nitrogen assimilation pathways [6].

Phenolics can be used as a potential biomarker of pollution because they participate in plant's response to accumulation of heavy metals, acting as antioxidants able to scavenge free radicals produced by metal ions [7].

In places dominated by heavy metal contamination, certain plants can tolerate rapidly heavy metal pollution while others can't. Furthermore heavy metals are accumulated in plant which will in turn jeopardise public healh through food chain. Consequently toxic metals significantly restrains agricultural production. Further studies are required to define better the molecular principles of heavy metal resistance thus making it possible to determine those varietes of agricultural plants which can tolerate heavy metal toxisity. Turkey is among the leading countries in lens production and export to world. Lentil is an annual plants with a highly nutritive products. In addition to its nutrituous feature, lentil plant has an important role in agriculture on account of its nitrogen binding characteristic. Protein quality of lentil is rather high in view of nutrition parameters, particularly due to high amino acid content [8]. Lands for agriculture are becoming imfertile due to such factors as salinity, disesase, heavy metal contamination etc. which is increasingly restraining plant production over the world [9,10], Keltjens and Beusichem [11]. Yet there is limited data available on physiological and biochemical reactions of lens plant under abiotic conditions such as salinity, drought, UV radiation, and heavy metal effects. This study, which cadmium and zinc were applied on lens seedlings as chlorine salts, aims to investigate not only these metals on root and shoot dry weight but also biochemical parameters such as free proline content and that of soluble phenolics and soluble protein in roots and shoots.

Material and Methods

Lentil seeds (*Lens esculenta* L. Erzurum 89) were used as plant material and chlorine salts of cadmium and zinc as heavy metals ($CdCl_2$ and $ZnCl_2$). Lentil seeds were let to germinate in dark in an incubator for 48 h at 24 ± 2 $^{\circ}$C located in petri dishes beetween double layers of filter paper. Seedlings grown were replanted in pots with perlite and were supplied with ½ Arnon –Hoagland nutrient solution (pH:5.8) in growth room for 7 days at 25± 2 $^{\circ}$C, 50 % humidity, in dark conditions and day light alternatively for 12 h. Nutrient solution was added to the pots as 200 ml for once in a two day period . Heavy metals were added for a 7 day period starting at the end of first 7 days in to the nutrient solution at 7 different concentrations (0, 10, 50, 100, 250, 400 and 500 ppm). Heavy metals were dissolved in nutrient solution and they were added to the pots as 200 ml for once in a two day period. Following this period plants were harvested. All essay

repeated at least three times. Fresh root and stem weight were measured, and samples having been dried at 110 °C oven for 24 h were weighed on dry basis. Free proline analysis was accomplished through extraction with 3% sulphosalisilic acid from freeze-dried material, and the calculation was made against proline standard in the spectrophotometer [12]. Soluble phenolics extraction was carried out with 80% ethanol from freeze-dried material and the quantative measurement was performed spectrophotometrically against chlorogenic acid standart in comply with Ferraris et al.[13] method. Soluble protein extraction was made from freeze-dried material according to Jordan et al. [14] method and the quantitiy was determinated in the spectrophotometer against bovin serum albumin standarts acc. to Lowry et al.[15]. All analysis and measurements repeated at least three times. Aritmetical mean numbers were handled with standart deviation norms to assess the results obtained. Duncan test was employed fort he evaluation of the data.

Result and Discussion

It was found out that the decrease in root dry weight of lentil seedlings exposed to Zn as a consequence of higher heavy metal concentration was significant than those exposed to Cd ($p<0.01$) (Fig.1). It was reported that in *Lycopersicum esculentum* and *Solanum melongena* seedlings exposed to Pb and Cd at lower concentrations, dry matter content considerably increased [16] whereas Ni, Cd, and Mo application on *Beta vulgaris* L. seedlings led to a decrease in dry matter [17]. *Mentha arvensis* seddlings exposed to Zn (0, 2.5, 5.0, 15.0 mg Zn kg^{-1}) showed enhancement in growth parameters, but the effects were nonsignificant [18]. Öncel et al.[19] pointed out a diminishing in plant lenth and an increase dry matter content of wheat seedlings . Findings in this research related to decrease in root dry weight are in confirmity with those of Kevresan et al.[17] and Öncel et al. [19].

It was found out in this research on lentil seedlings exposed to Cd that decrease in shoot dry weight in paralel with higher concentration of heavy metals was bigger than that in Zn applied seedlings ($p<0.05$) (Fig.1). Damage included roots by heavy metals lead to diminishing in essential nutrient intake resulting in immobilization of minerals in roots, which in turn causes a considerable nutrient deficiency in the stem [20].

It was found out in this research that the increse in proline content both in roots ($p<0.05$) and shoots ($p<0.01$) in Cd applied lentil seedlings was bigger than that in Zn applied ones. It was pointed out proline, a hydrophilic amino acid, played a role in regulating osmosis [21], preventing enzyme denaturation [22] and retaining carbon and nitrogen [23], Bassi and Sharma [24], reported that when compared with proline accumulation built up against water deficiency, higher salinity and low-high temperature stres. Proline accumulation following Zn and Cd application on wheat seedlings was similar to that occuring due to water deficiency [24].

This research has established about the proline content in both roots and shoots of lentil seedlings that there is a decrease in proline content with 500 ppm Cd concentration when compared with that of 400 ppm Cd (Fig.2). According to Chen et al.[2], the indication to self protection by plants growing under stress conditions is their free proline accumulative capacity. In plants subject to low Cd concentrations, free proline contens rapidly increases as a means of protection against stress. On the other hand, Cd, in higher concentrations, inhibits the plant growth remarkably hampering its, physiological functions and metabolism. It is safe to say that Cd toxicity rapidly impedes protectional functions in plants and leads to a considerable decrease in free proline content [2]. Öncel et al.[19] found out that free proline content in *T. aestivum* seedlings following the application with high Cd concentration whereas there was no chance after Pb application. Kastori et al.[4] pointed out an increase in proline accumulation in *Helianthus annuus* seedlings due to Cd. On the other hand, Costa and Monel [5] ascertained that Cd didn't induce proline accumulation in *Lactuca sativa* seedlings but triggered an increase in asparagin, methionin and lisin content. When compered with Zn applied seedlings, a bigger increase was realized in soluble phenolic content and in soluble protein content in roots of Cd applied lentil seddlings, however, the application of both metals at 500 ppm concentration led to a decrease in soluble protein content. It was found out that soluble protein content in Zn and Cd applied seedling shoots was relatively low when compered with the control plant and that the decrease was more remarkable in Cd applied seedlings. Öncel et al.[19], established that inspite of a considerable increase in total phenolics content at higher Cd concentrations, there was not any such increase following Pb application. Findings from our research are in accord with the results by Öncel et al.[19] in relation to the increase in soluble

phenolic content in shoots of lentil seedlings exposed to heavy metal application with concentrations of Zn and Cd (Fig.3-4).

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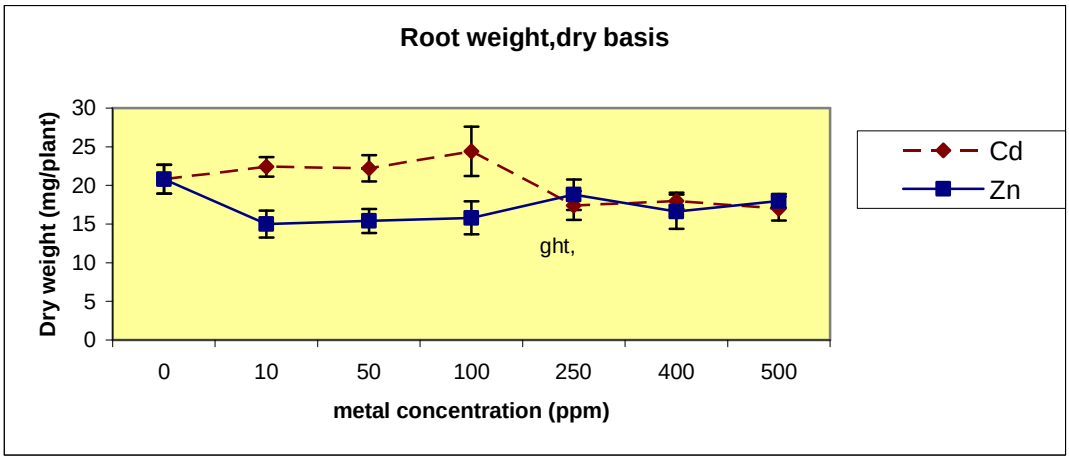


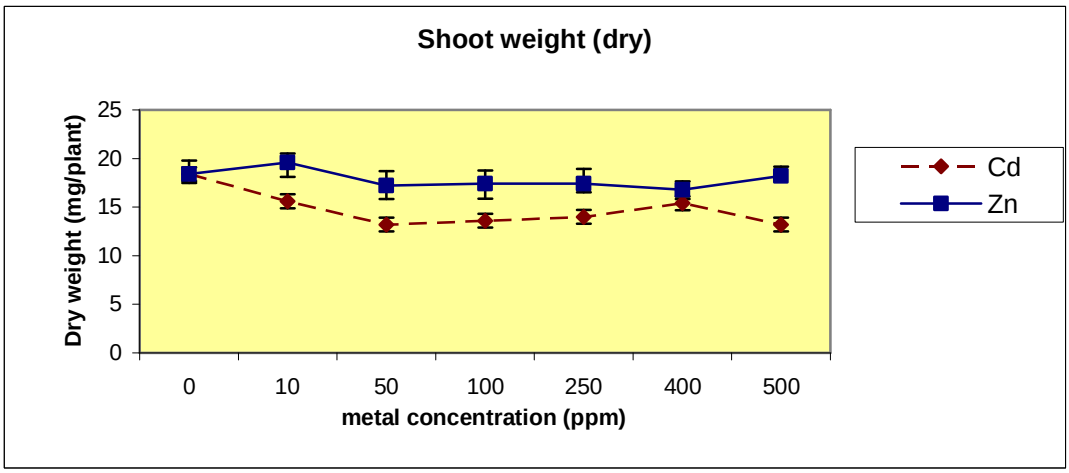
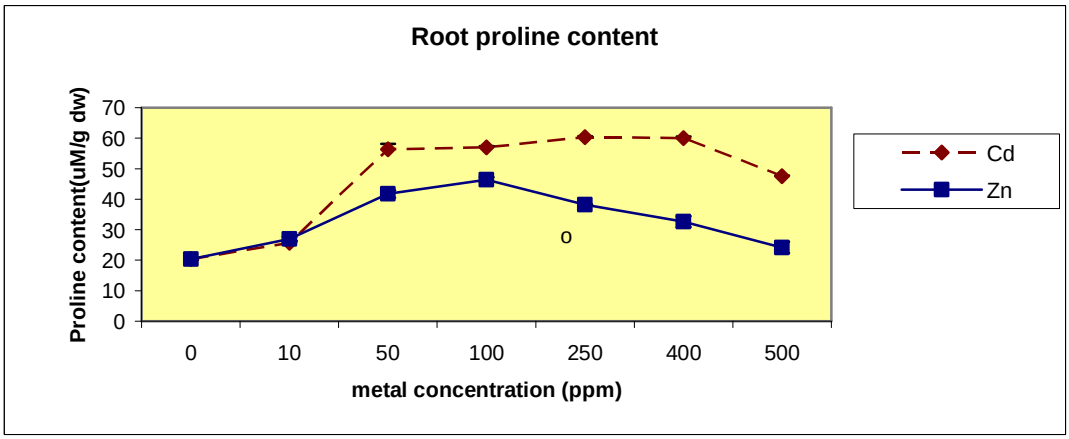


Figure 1: The effects of Cd and Zn application on lens seedlings (*Lens esculenta* L. Erzurum 89) in view of root dry weight and shoot dry weight (n=5)



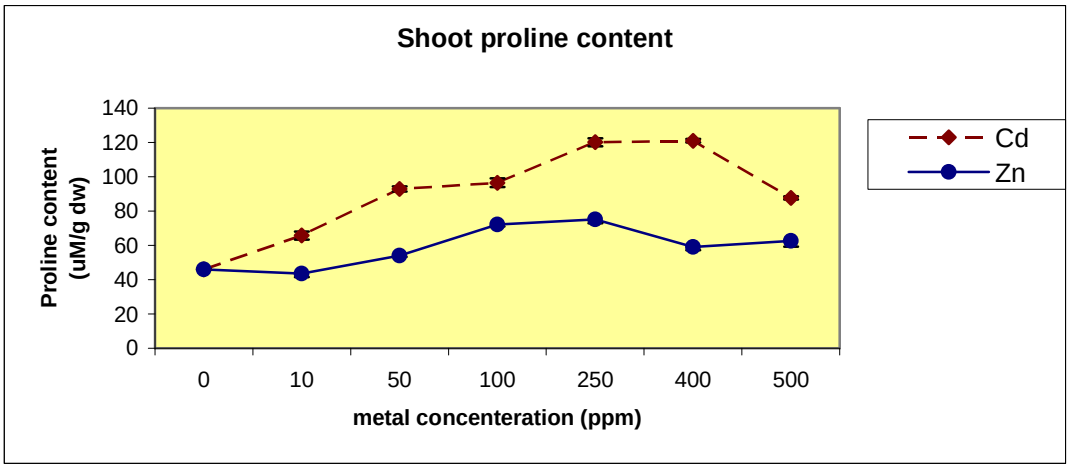
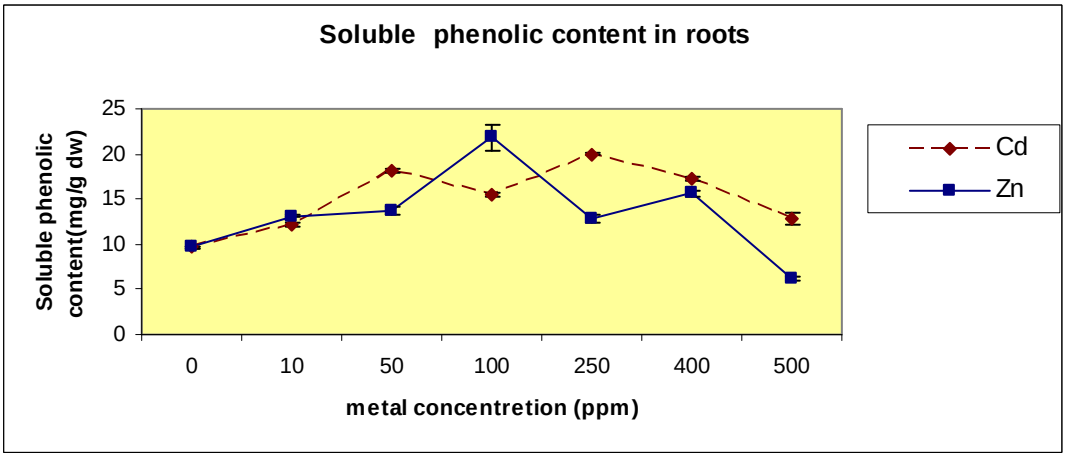


Figure 2: The effects of Cd and Zn application on lens seedlings (*Lens esculenta* L. Erzurum 89) in view of free proline content in roots and shoots (µM/g dry basis) (n=3)



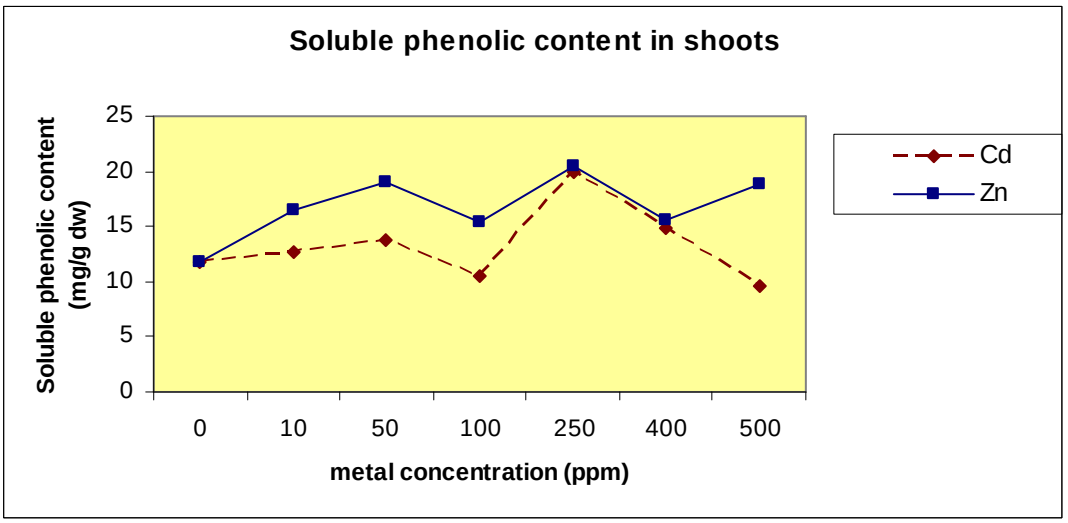


Figure 3: The effects of Cd and Zn application on lens seedlings (*Lens esculenta* L. Erzurum 89) in view of soluble phenolic content in roots and shoots (µM/g dry basis) (n=3)

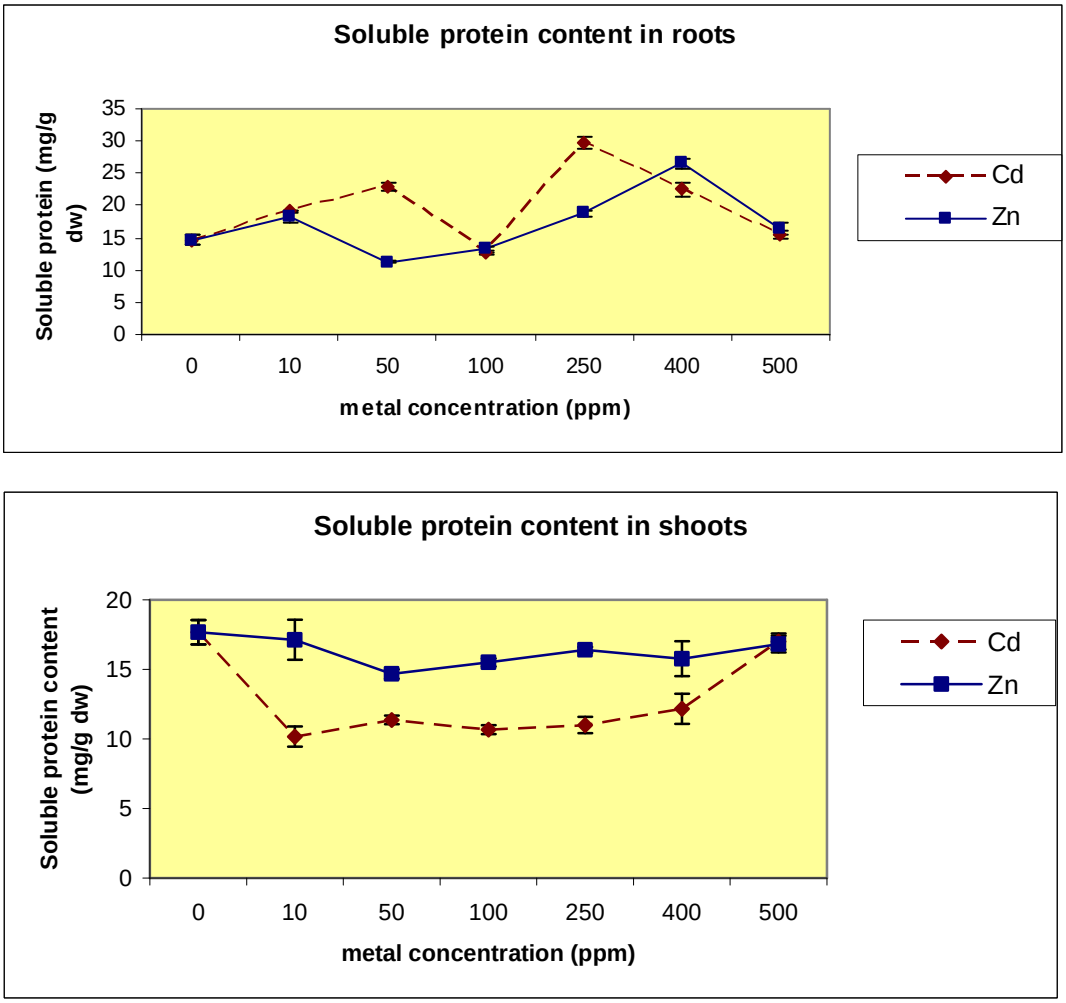


Figure 4: The effects of Cd and Zn application on lens seedlings (*Lens esculenta* L. Erzurum 89) in view of soluble protein content in roots and shoots (µM/g dry basis) (n=3)