

## Interaction of Some Heavy Metals with Copper Content in Dock Plant

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### ABSTRACT

In this study, interaction of some heavy metals (Cr, Cd, and Pb) with copper content in the dock plant was investigated. Dock (*Rumex patientia* L.) is a medicinal aromatic plant grown in contaminated soils with some heavy metals (Cr, Cd, and Pb). For this purpose, 100 mgkg<sup>-1</sup> Cr, Cd and Pb were applied to each pot. The content of copper in test soils was found to be 1.50 mg kg<sup>-1</sup>. Increased doses of (0, 5, 10 and 15 mmolkg<sup>-1</sup>) EDTA chelate were given to soils contaminated with metals. At the end of a two-month trial, the plants were harvested. The contents of copper in the patience dock plant with a fixed dose of heavy metals were compared with that of heavy metals applied with increasing doses of chelate in the pot experiment. According to the results, increasing chelate applications, especially lead and cadmium contents increased the copper contents of the leaves of patience dock plant. However, the opposite situation was observed in the plant roots. These increases were found to be statistically significant at the level of 1%. The aromatic plant patience dock was shown to be a hyperaccumulator for heavy metals. Dock plant is useable for heavy metal remediation near highway or motorway in agricultural soils.

### Research Article

#### Article History

Received : 14.02.2020

Accepted : 07.05.2020

#### Keywords

Plant  
Copper  
Pollution  
Heavy metal  
EDTA

## Labada Bitkisinde Bakır İçeriği ile Bazı Ağır Metallerin İnteraksiyonu

### ÖZET

Bu çalışmada, Labada bitkisinde bakır içeriği ve bazı ağır metallerle (Cr, Cd ve Pb) interaksiyonu araştırılmıştır. Bazı ağır metallerle (Cr, Cd ve Pb) kirlenmiş topraklarda yetişen tıbbi aromatik bir bitki olan Labada (*Rumex patientia* L.), yetiştirilmiştir. Bu amaçla, her saksıya 100 mgkg<sup>-1</sup> Cr, Cd ve Pb uygulandı. Topraklarındaki bakır içeriği 1,50 mgkg<sup>-1</sup> olarak bulunmuştur. Metallerle kontamine olmuş topraklara artan dozlarda (0, 5, 10 ve 15 mmol kg<sup>-1</sup>) EDTA şelat verilmiştir. İki aylık denemenin sonunda, bitkiler hasat edildi. Denemede labada bitkisine artan dozlarda verilen şelat ile sabit dozda verilen ağır metaller ile bakır içerikleri karşılaştırılmıştır. Deneme sonuçlarına göre, artan dozlarda şelat uygulamaları özellikle kurşun ve kadmiyum içeriklerinin artırılması, bitki yapraklarında bakır içeriğinin azalmasına neden olmuştur. Ancak, bitki kök bölgesinde tam tersi bir durum gözlenmiştir. Bu artışlar % 1 düzeyinde istatistiksel olarak anlamlı bulunmuştur. Labada, ağır metalleri biriktirme özelliğine sahip aromatik ve hiperakümülatör bitki olarak belirlenmiştir. Toprakların ıslahında, otoyol veya karayolları kenarlarında yer alan tarım arazilerinde biriken ağır metallerin gideriminde labada bitkisi kullanılabilir.

### Araştırma Makalesi

#### Makale Tarihiçesi

Geliş Tarihi : 14.02.2020

Kabul Tarihi : 07.05.2020

#### Anahtar Kelimeler

Bitki  
Bakır  
Kirlilik  
Ağır metal  
EDTA

**To Cite** : Adiloğlu S 2020. Interaction of Some Heavy Metals with Copper Content in Dock Plant. KSU J. Agric Nat 23 (4): 1078-1084. DOI: 10.18016/ksutarimdog.vi.689443.

### INTRODUCTION

Heavy metal contamination in soils are an issue dealt with great care in recent years. The studies on heavy

metal contamination in soil have been concentrated on the sources and behaviors of the contaminants, their effects on public health, decontamination and analysis, improvement and management techniques.

In recent studies, plants like *Silene vulgaris*, *Althaea rosea*, *Thlaspi caerulescens*, *Taraxacum officinale*, *Vaccinium myrtillus*, *Aesculus hippocastanum* L., *Hypericum amblysepalum*, *Calendula officinalis*, *Solanum nigrum* L., *Urtica urens*, *Mentha* sp., *Plantago lanceolata*, *Onosma bracteatum*, have been specified to accumulate toxic heavy metals in their structure and have the ability of phytoremediation. Moreover, the heavy metals, accumulated by aromatic and medical plants, do not have a negative effect on the amount and content of the secondary metabolites (Yaldız and Şekeroğlu, 2012). The heavy metals were specified to have negative effects on seed germination, and intracellular mechanisms through retarding plant growth and development, reducing the blossoming ability and productivity of the plant, deteriorating product quality, damaging nitrogen cycle and fixation, reducing the amount of chlorophyll, impairing enzyme systems, preventing the absorption of useful elements. Therefore, it has been observed that the amount of dry matter of the plant decreases on parcels where heavy metals were applied (Pandey and Sharma, 2002; Taboada et al., 2002; Belimov et al., 2003; Peralta-Videa et al., 2004). Increasing doses of Cu application has an important effect on soil pH and the nutritious elements of the plants. Different doses of Cu application reduce the pH value of the soil and also the Mg variability and available Fe amount. It is specified that it increased the total amount of N, the amount of obtainable P, variable K, useful Zn and Cu in a laboratory experiment where maize plant in different pots was applied with different doses of Cu, and meanwhile, development of *Acaulospora mellea* was observed (Sönmez et al., 2006; Wang et al., 2007). At the end of the experiment the absorption level of useful elements was lower in plants, which was grown in heavy doses Cu applied vases. It was concluded that the reason for this was the pH of the soil. It was also observed that the structure and intensification of the organic acids like citric acid, malic acid, and oxyacid were changed by the *Acaulospora mellea*. The studies showed that *Acaulospora mellea* was inappropriate for the phytoextraction of Cu by the maize, but mycorrhizal fungi would be more suitable for phytostabilization due to their roots' ability of Cu absorption. Moreover, they hinder scattering and transportation of heavy metals through the wind, water erosion, and washing away. The plant can change the structure of the pollutants with the microbiological and chemical properties of its roots so that the pollutants cannot be dissolved and carried away in water (EPA, 2000; Yıldız, 2008). When the effects of micronutrients on living things are considered, the analysis of micro nutrient elements in the soil and water is very important. Metals are essential for all organisms, especially for anaerobic

organisms. However, high amounts of chromium, lead, cobalt and nickel endanger human health seriously. The human body cannot process or get rid of these heavy metals through excretory organs. These heavy metals cause damage by accumulate in the organs of the human body. The most common heavy metals in the environment are cadmium, nickel, mercury, chromium, silver, cobalt, tin, copper, zinc, and lead (Wase and Forster, 1997; Adiloğlu et al., 2014; Adiloğlu et al., 2015a).

In this study, the synergistic and antagonistic relationships of copper and some heavy metal with plant nutrients which are required for patience dock plant growth were analyzed.

### MATERIALS and METHODS

The experiment was conducted as a "randomized complete blok" design with triplicates (Figure 1). Three pollutants were used in the trial, namely  $\text{Cr}(\text{NO}_3)_3$ ,  $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ , and  $(\text{Pb}(\text{NO}_3)_2 \cdot 4 \text{chelate doses (EDTA)})$  (0, 5, 10, 15 mmol/kg x 3 replicate + 3 control, The experiment consisted of total of 39 pots. Each pot contained 400 cc of soil, replicated three times, with the control pots were left untreated with chelate. The plants were reduced to three plants for each pot after emerged. Before planting the patience dock plants, and having taken the favorable amount of these in the soils, for the N, P and K needs of the plants, 100 mg kg<sup>-1</sup> N as nitrogen source ammonium nitrate, 50 mg kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as phosphorus source triple superphosphate fertilizer and 50 mg kg<sup>-1</sup> K<sub>2</sub>O as potassium source, when needed, potassium sulfate fertilizers were applied into the soil in the pots. After the contaminants were applied, they were incubated for 30 days by letting the soil absorb the contaminants and then samples were taken from the pots and the extractible amounts of Cr, Cd, and Pb were analyzed. After the incubation period, the patience dock plants were planted into the pots. After that, to enable plants to absorb the heavy metals from the soil EDTA doses were applied.

### Plant and soil analysis

After a two-month growing period, the plants were harvested and roots and shoots weights were determined. Harvested patience dock plants (*Rumex patientia* L.) were brought to laboratory and their dry weights were determined. The samples were burned in the microwave oven and heavy metals in the solutions were determined with ICP-OES Spectro/SpectroBlue (Kacar and İnal, 2010). To specify the nitrogen content of the plant samples, the modified micro Kjeldahl method was used (Sağlam, 2012).

The amount of pH in the soil, (Jackson, 1967), CaCO<sub>3</sub> content (Sağlam, 2012), electrical conductivity (EC) (Sağlam, 2012), organic matter amount (Kacar, 1995)

were determined by using the above-mentioned methods, respectively. Extractable phosphorus (Olsen and Sommers, 1982), available potassium (Kacar

1995) and Zn, Fe, Mn and Cu analyzes (Lindsay and Norvell, 1978) were performed. Texture analysis was carried out according to Bouyoucos (1955).



Figure 1. Image of the experiment.

Şekil 1. Deney görüntüsü

### Some properties of plants

According to Güncan (1976), *Rumex patientia* grown in gardens, on slopes, fields, on the banks of ditches, lake sides, road sides and on prairies sustains a shoot of 60-120 cm. The lower leaves are wide and large, the mid leaves are wide and the tips are blunt and a little curly. The root of the plant is in the pile form and penetrates 30 cm or deeper into the soil. The plant spread seeds twice in one vegetation period. The first seed period is in May-June and the second is in October. The plant prefers the soil poor in lime. It is a perennial plant that is used in traditional medicine. It has an anti-inflammatory influence and is being used as a diuretic, antipyretic, and in treating wounds (Süleyman et al., 1999). According to some studies, a patience dock plant was specified of having a phytoremediation capacity. In researches, it has been found that bioaccumulation in medical and aromatic plants don't affect the content and amount of the secondary metabolites negatively so it would be beneficial to use the plants harvested after phytoremediation (Bahtiyarca and Bağdat, 2007).

### Statistical analysis

For the statistical assessment of the data, variance analysis (MSTAT 3.00/EM) was performed and the differences were compared based on 1% and 5% significance levels by LSD test (Düzgüneş et al., 1987).

## RESULTS and DISCUSSION

### Some physical and chemical properties of soil samples

Some of the physical and chemical qualities obtained from the analysis of the experimental soil were given

in Table 1.

Table 1. Some properties of the soil sample  
Tablo 1. Toprak örneklerinin bazı özellikleri

Soil characteristic	Value	Unit
pH	7.95	(1:2.5 soil:water)
EC	0.11	dSm <sup>-1</sup>
Texture	clay	%
Org. matter	0.14	%
CaCO <sub>3</sub>	6.03	%
P <sub>2</sub> O <sub>5</sub>	12.50	kg da <sup>-1</sup>
K <sub>2</sub> O	497.03	kg da <sup>-1</sup>
Chromium	0.05	mg kg <sup>-1</sup>
Cadmium	0.02	mg kg <sup>-1</sup>
Lead	0.98	mg kg <sup>-1</sup>
Copper	1.50	mg kg <sup>-1</sup>

Experimental soil was clay, very low in organic matters and pH was alkaline, phosphorus and potassium contents were sufficient (Table 1). Salt values in soil were below salinity limit and the lime content were of average.

### Comparison of heavy metals in plants

The heavy metals of chromium, cadmium, lead was of trace amount. Under the controlled conditions, 100 mg kg<sup>-1</sup> Cr, 100 mg kg<sup>-1</sup> Cd, and 100 mg kg<sup>-1</sup> Pb were applied and to enable the plant to absorb those contaminants different doses of EDTA (0, 5, 10, 15 mmolkg<sup>-1</sup>) were applied. In the face of these applications, the copper exchange in the plant was examined. The content of copper in trial soils was found to be 1.50 mg kg<sup>-1</sup>. The effects of EDTA on the copper in the roots and shoot of the plant are seen in Figures 2 and 3.

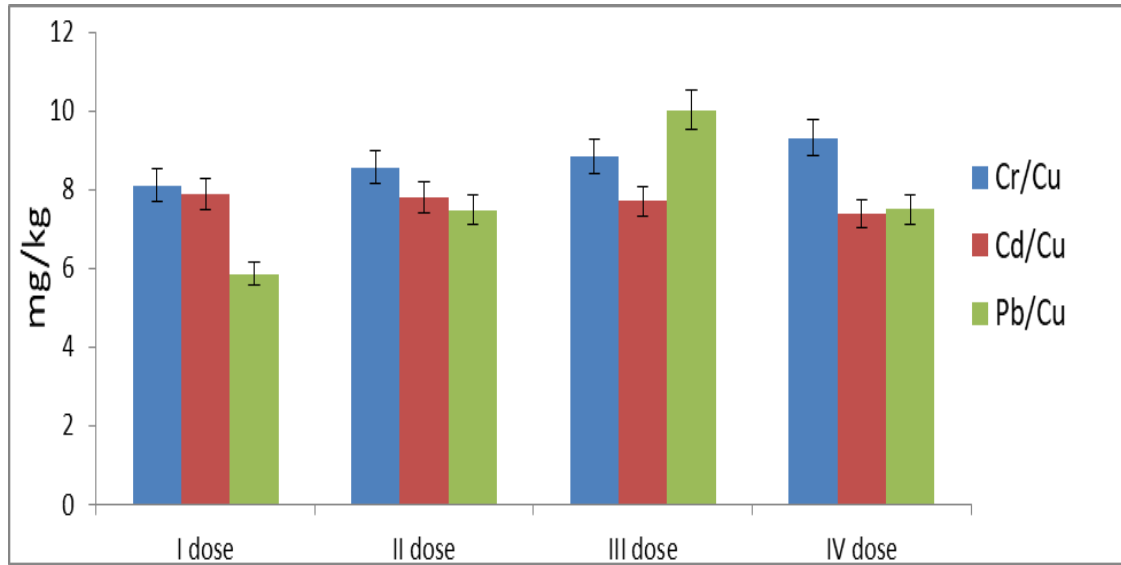


Figure 2. Mean values of EDTA doses and heavy metals (Cr, Cd, Pb) applications and relation with the Cu content of dock plant shoot ( $\text{mgkg}^{-1}$ ) \*: The doses: 0, 5, 10, 15  $\text{mmolkg}^{-1}$  EDTA.

Şekil 2. EDTA dozları ve ağır metal (Cr, Cd, Pb) uygulamalarının ortalama değerleri ve Labada bitki gövdesindeki Cu ile ilişkisi ( $\text{mg kg}^{-1}$ ) \*: Dozlar: 0, 5, 10, 15  $\text{mmolkg}^{-1}$  EDTA.

When we examine Figure 2, increasing EDTA applications, in pots that are contaminated with heavy metals (Cr, Cd, and Pb), have influenced the Cu content in the shoot of the plant. It is seen that copper and chromium contents increase together in the plant shoot. Increased solubility of pots polluted with chromium and showed a synergetic effect on the removal of chrome and copper from soils. However, the content of copper has reduced applied pots in

cadmium. An antagonistic effect was observed between cadmium and copper. On the other hand, in pots polluted with lead, copper content increased up to 10  $\text{mmolkg}^{-1}$  in EDTA applied pot. Nevertheless, copper content decreased in pots treated with 15  $\text{mmolkg}^{-1}$  EDTA. A further increase in lead solubility negatively affected the copper. The Cu content in the roots of the plants where different EDTA doses applied increased (Figure 3).

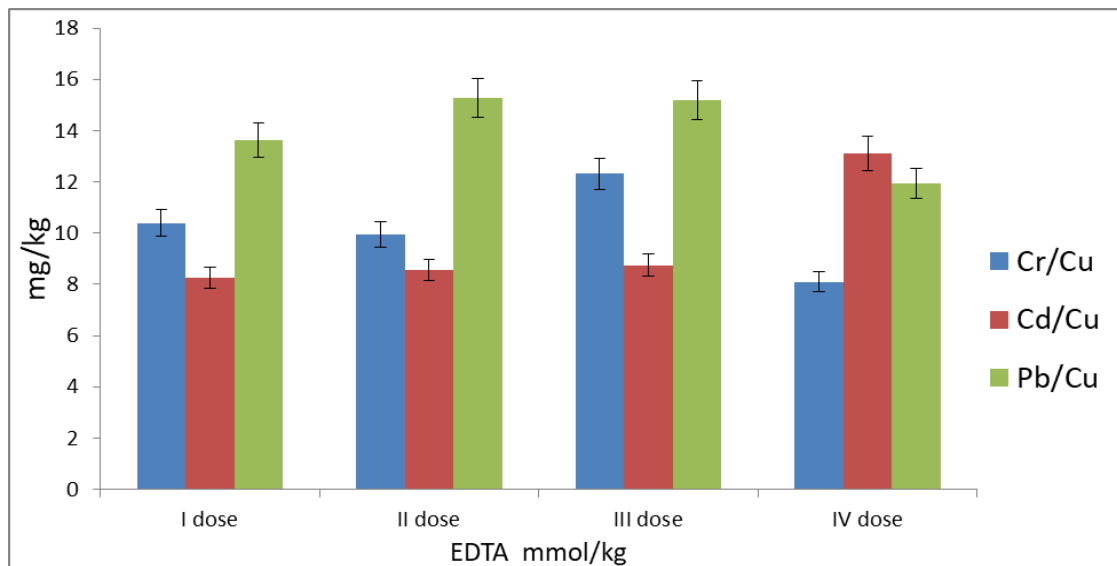


Figure.3. Relation of EDTA doses and heavy metal applications with Cu content of dock plant root mean values \*: The doses: 0, 5, 10, 15  $\text{mmolkg}^{-1}$  EDTA.

Şekil 3. EDTA dozları ve ağır metal (Cr, Cd, Pb) uygulamalarının ortalama değerleri ve Labada bitki kökteki Cu ile ilişkisi ( $\text{mgkg}^{-1}$ ) \*: Dozlar: 0, 5, 10, 15  $\text{mmolkg}^{-1}$  EDTA.

This shows that EDTA application increases the plant's intake ability of the heavy metals. These increases were found to be significant at 1%. Similar

results with patience dock (*Rumex patientia* L.) research were obtained in earlier researches (Adiloğlu et al., 2015b; Adiloğlu et al., 2016; Adiloğlu, 2017).

Contamination of the soil with copper results from fertilization, disinfestation, agricultural and household wastes and industrial emissions of elements containing Cu. Industrial pollution is an environmental problem and in the long term it also pollutes the atmosphere (Bakırcıoğlu, 2009).

In a previous research (Esringü, 2012; Wang et al., 2007) canola plant was used to remove heavy metals Cr, Cd, Pb through phytoremediation method. With increasing EDTA application, an increase was determined in the amount of heavy metals that are removed from the soil by canola (Manios et al., 2002), in their research, they studied the effects of heavy metals on total protein concentration in *Typha latifolia* plant grown in materials contain waste water compost. In a ten-week study, they applied different

concentration solutions of Cd, Cu, Ni, Pb, and Zn to the plants. As a result, they observed an increase of Ni, and Zn concentration in the leaves and shoot of *Typha latifolia* and they also recorded the same increase for the other four heavy metals. They also determined that the negative effects of the heavy metals occur with the increasing heavy metal concentration.

In Table 2 below, the heavy metal and copper interactions in the shoot, root and soil, after *Rumex patientia* was harvested from the pots where it was grown with EDTA and heavy metal applications. Table 2 reveals that the content of copper in the Dock plant which grows in chrome cadmium and lead-pollinated pots increased when the amount of pollutants increased.

Table 2. Shoot, root and soil copper contents mean values and significance groups\*\*\* in some heavy metal applications (Pb, Cr, Cd) to the patience dock plant

Tablo 2. Bazı ağır metal uygulamalarının labada bitkisi (Pb, Cr, Cd) gövde, kök ve toprak bakır içerikleri ortalama değerleri ve önem grupları

Heavy metal	EDTA (mmolkg <sup>-1</sup> )			
	0	5	10	15
Cu shoot	7.28b	7.95b	8.86a	8.07a
Cu root	10.76d	11.25b	12.08a	11.05c
Cu soil***	0.73d	0.96c	1.67b	1.92a

\*: The values average \*\*: Each element has been evaluated separately \*\*\*: after harvesting.

On the other hand, copper exchange in the soil after harvest was investigated (Figure 4). As it can be seen in Figure 4, in all cases in which heavy metal and increasing EDTA doses applied, the copper amount in the soil increased and this was found significant at a level of 1%.

It was observed that with increasing doses of EDTA application, the dissoluble amount of copper increases in the pots in which Cr, Cd, and Pb applied.

## CONCLUSION

The heavy metals, 100 mg/kg Cr, Cd, and Pb, applied to the soil, and the EDTA chelate doses of 0, 5, 10, and 15 mmol/kg increased the amount of copper significantly in the shoot and roots of the plants. These increases were found significant at 1%. This was an expected result, because the application of chelates like EDTA increases the solubility of heavy metals in the soil and increases the absorption of these metals by the plants. Removing heavy metals like Cr, Pb, and Cd, which are the results of the industrial activities through classical physico-chemical methods, is a significantly expensive technique. Therefore, we should apply some methods, in which we increase the dissolubility of the heavy metals, like Cr, Cd, and Pb, by using EDTA chelates, and remove them via natural ways like phytoremediation. Using these methods is both easy

and cheap. Using hyperaccumulator plants like *Rumex patientia* for phytoremediation to remove heavy metals from the soil has been gaining importance lately. By using this method, the amount of heavy metals that plants remove from the soil increases. In this research, it has been produced that the heavy metals in the soil like Cr, Pb, and Cd can be cleared from by using the phytoremediation method and *Rumex patientia*. The plant can absorb the heavy metals from the soil by the use of EDTA applications; it has also been observed that there is a meaningful relationship between the heavy metals and the copper intake. This shows that *Rumex patientia* can remove the heavy metals like Cr, Cd, Pb and copper from the soil with the use of EDTA applications. Consequently, of increasing doses of EDTA applications and some heavy metals (Pb and Cd) polluted soils dock cultivated in an analysis of the shoot results copper content, especially in the shoot were determined to have an antagonist effect. This situation has been determined as a synergistic effect on roots. Heavy metal content, especially Cu, of soils increased with increasing doses of EDTA application. Consequently, EDTA application in agricultural areas polluted with heavy metals, can be removed by the patience dock as there is a significant interaction between Cr, Pb, Cd and Cu contents.

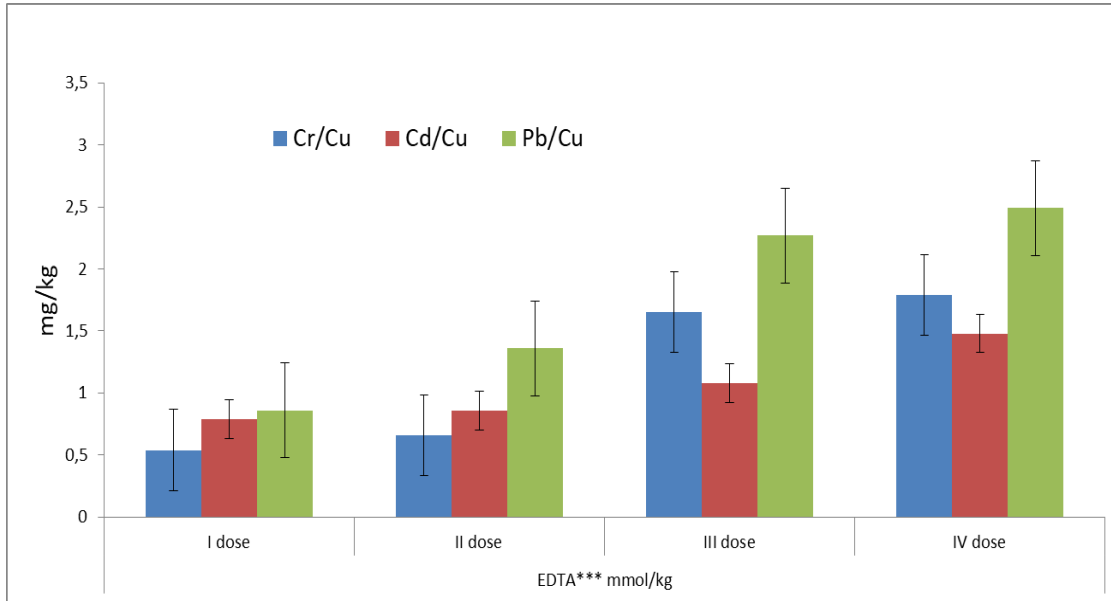


Figure 4. Relation of EDTA doses and heavy metal applications ( Cr, Cd, Pb ) with Cu content of soil after harvesting mean values (mg/kg) \*: The doses: 0, 5, 10, 15 mmolkg<sup>-1</sup> EDTA

Şekil 4. EDTA dozlarının ve ağır metal uygulamalarının (Cr, Cd, Pb) ortalama değerleri (mg / kg) hasat sonrası toprağın Cu içeriği ile etkileşimi \*: Dozlar: 0, 5, 10, 15 mmolkg<sup>-1</sup> EDTA

#### Declaration of interest

The authors declare that they have no conflict of interest.

#### ACKNOWLEDGEMENT

The abstract of this paper has been presented in the Turkey Natural Nutrition and Healthy Life Summit.

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