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Effects of EDDS application on phytoextraction of cadmium, lead and zinc contaminated soil with *Brassica napus*

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Abstract: Cadmium (Cd), lead (Pb) and zinc (Zn) contaminations of soils are a serious worldwide problem that affects human health and environmental quality. Phytoremediation, use of green plants to remove, sequester or detoxify contaminants, offers an environmentally friendly alternative solution for soil remediation. The effect of canola (*Brassica napus* L.) and a biodegradable chelate, ethylenediamine dissuccinate (EDDS), were tested on remediation of multi metal (Cd, Pb, and Zn) contaminated soil. In the pot experiment, plants were grown for two months on five soil mixtures obtained by mixing an uncontaminated soil sample with 0, 25, 50, 75, and 100% of multiple metal contaminated soil. The biomass of the plants were weighed, and the uptakes of Cd, Pb and Zn in the shoot were determined using ICP-MS. The addition of multi metals to the soil led to the increase of multi metal contents in plants. Consequently, the dry weights of the plants were increased with EDDS treatments in 25 and 50 % multi-metal doses compared to the control treatment. EDDS has significantly increased the uptake of metals (Cd, Pb, and Zn) from the soil and their accumulations in shoots of the plants. The Cd concentration of plant was higher than the hyperaccumulation limit of Cd (>100 mg kg^{-1}) with EDDS treatment in 25 and 50% multi-metal doses (141 and 174 mg Cd kg^{-1}, respectively) except for Zn and Pb concentrations.

Key words: *Brassica napus*, EDDS, phytoremediation, multiple metal, contaminated soil, decontamination

Kadmiyum, Kurşun ve Çinko ile Kirlenmiş Toprağın *Brassica Napus* ile Fitoksraksiyonuna EDDS Uygulamasının Etkisi

Özet: Toprakların kadmiyum (Cd), kurşun (Pb) ve çinko (Zn) ile kirlenmesi insan sağlığını ve çevre kalitesini etkileyen dünya çapında ciddi bir sorunudur. Fitoremediasyon, bitki kullanılarak kirleticilerin uzaklaştırılması, giderilmesi veya detoksifiye edilmesi yöntemi, toprakların ıslah edilmesinde çevre dostu alternatif bir çözüm sunar. Bu çalışmada, biyo-bozunabilir bir şelat olan etilendiamin disüksinatın (EDDS) kanola (*Brassica napus* L.) ile çoklu-metal (Cd, Pb ve Zn) ile kirlenmiş bir toprağın fitoremediasyonu üzerindeki etkisi araştırılmıştır. Saksı denemesinde, kanola bitkileri çoklu metallerle kirlenmiş toprağın temiz toprakla karıştırılmasıyla elde edilen beş toprak karışımı (%0, %25, %50, %75 ve %100) toprakta iki ay süreyle yetiştirilmiştir. Bitkilerin biyokütleleri tartıldı ve yeşil aksamın Cd, Pb ve Zn alımları ICP-MS kullanılarak belirlenmiştir. Toprağa çoklu metallerin eklenmesi, tüm bitkilerde çoklu metal içeriklerinin artmasına neden olmuştur. Sonuç olarak, kontrole kıyasla bitkilerin kuru ağırlıkları % 25 ve % 50 çoklu metal dozlarında EDDS uygulaması ile artmıştır. EDDS, topraktan metallerin (Cd, Pb ve Zn) alınmasını ve bitkinin yeşil aksamında birikmesini önemli ölçüde artırmıştır. Bitkinin, Zn ve Pb konsantrasyonları hariç EDDS uygulaması ile Cd konsantrasyonu % 25 ve % 50 multimetal dozlarında sırasıyla 141 ve 174 mg Cd kg^{-1}'e ulaşmıştır. Bu değer Cd hiperakümülasyon sınırından (<100 mg kg^{-1}) daha fazladır.

Anahtar kelimeler: *Brassica napus*, EDDS, fitoremediasyon, çoklu metal, kirlenmiş toprak, arıtma

Introduction

The increasing world population from the 20th century to the present day, the intensive agriculture, industrial, and mining, etc. activities cause soil pollution that became an important worldwide environmental problem (Dağhan 2007; Wuana and Okieimen 2011; Sumiahadi and Acar 2018). Contaminants cause an undesirable change in the physical, chemical and biological properties of the soil. Heavy metals are one of the most important kind of the contaminant in the soil. They are long-term persistence in the soil since they cannot be broken down to non-toxic forms. Heavy metal contamination is especially a problem of the industrial societies. The main source of heavy metal pollution is fossil fuel consumption. Other sources are industrial wastes, mining and smelting wastes, fertilizers, pesticides, household wastes and sewage sludges (Wuana and Okieimen 2011; Sumiahadi and Acar 2018). These metals also naturally exist in the soil. Some of the heavy metals zinc (Zn), manganese (Mn), iron (Fe), copper (Cu) are essential for living organism in very low concentrations. Other heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), mercury (Hg), etc., are toxic to living organisms and they have a harmful effect on them even in very low concentrations. Heavy metals are highly toxic (mutagenic and/or carcinogenic) to human health. Plants grown in heavy metals contaminated soils exposed to heavy metals, which are consumed by the human can cause serious health problems such as damages on the lungs, kidneys, liver, and other vital organs (Evangelou et al. 2007; Dağhan 2011; Singh and Kalamdhad 2011; Wuana and Okieimen 2011; Sumiahadi and Acar 2018).

There are different soil remediation techniques such as isolation and immobilization, mechanical separation, pyrometallurgical isolation, biochemical, electro kinetic, soil washing, phytoremediation methods using to clean up heavy metal contaminated soils (Dağhan 2007; Angelova et al. 2015). Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost-effectiveness, aesthetic advantages and long-term applicability (Evangelou et al. 2007; Angelova et al. 2015; Sumiahadi and Acar 2018). Phytoremediation is the use of metal-accumulating plants to clean up toxic metals from soils. This technique is environmentally friendly, cheap, aesthetically pleasing, and long-term and wider range applicability than physical and chemical remediation technology (Tangahu et al. 2011; Angelova et al. 2015; Sumiahadi and Acar 2018). The main groups of this method are phytoextraction (using plant to uptake contaminants, particularly toxic metals from the contaminated soil by a plant), phytostabilization (the use of plants to limit the mobility and bioavailability of metals in soil) and rhizofiltration (removal of pollutants from groundwater via absorption by plant roots). Phytoextraction is widely utilized method to remediate heavy metal contaminated soils. This method is achieved by two ways: continuous (natural) phytoextraction and induced (assisted) phytoextraction.

Specially selected plants, known as hyperaccumulators, can extract and accumulate exceptionally high levels of contaminants from soil. There are approximately 450 types of natural hyperaccumulator plants exist in the world (Evangelou et al. 2007). Heavy metal accumulation limits of these plants are 100 times more than cultivated plant's concentration (accumulation). Hyperaccumulator plants can accumulate 10 000 mg kg^{-1} Zn and Mn; 1000 mg kg^{-1} As, Cr, Pb, cobalt (Co), Cu, nickel (Ni), selenium (Se) and 100 mg kg^{-1} Cd (Evangelou et al. 2007; Wuana and Okieimen 2011). On the other hand hyperaccumulator plants have low biomass and their growth period is long. *Brassica* species are commonly using in phytoextraction of heavy metal contaminated soil because of their high biomass production, deeply rooted and higher heavy metal accumulation ability (Kasiuliene et al. 2016). Different synthetic (such as ethylene diamine tetraacetic acid (EDTA), hydroxylethylene diamine tetraacetic acid (HEDTA), diethylene triaminee pentaacetic acid (DTPA), ethylenebis[oxyethylenetrinitrilo] tetraacetic acid (EGTA)) or natural chelates (such as EDDS, nitrilotriacetic acid (NTA)) are used to increase metal uptake capacities of plants (Evangelou et al. 2007). When the literature is examined, it is seen that EDTA is the most commonly used chelating agents in the removal of metals from the soil (Evangelou et al. 2007; Kasiuliene et al. 2016; Attinti et al. 2017). Similar to EDTA, EDDS increased the uptake of heavy metals, but as in the case of EDTA, only a fraction of the mobilized metals is effectively absorbed by the plant and subsequently translocated to the shoot, as much higher amounts of heavy metals were phytoavailable (Evangelou et al. 2007; Kasiuliene et al. 2016). However, EDTA is quite persistent in the environment due to its low-level biodegradability. The investigations reveal that EDTA has been destroyed of soil structure. Soil-applied EDTA can adversely impact soil enzymatic and microbial activities and at high concentrations, EDTA can negatively affect soil fungi and plants (Ullah et al. 2014; Attinti et al. 2017). For this reason, biodegradable chelating agents such as [S, S] -ethylenediamine succinic acid ([S, S]-EDDS) have begun to gain more attention as an environmentally friendly alternative to EDTA. Ethylenediamine disuccinic acid, $C_{10}H_{16}N_2O_8$, is an isomer of EDTA that produces either artificially or naturally by various microorganisms (Takahashi et al. 1999; Ullmann et al. 2013; Attinti et al. 2017).

The aim of this study is to investigate effects of EDDS treatment on phytoextraction of multi-metal (Cd, Pb, and Zn) contaminated soil using canola (*Brassica napus*) plant.

Material and Methods

The soil sample contaminated with multiple metals (Cd, Pb and Zn) was taken from Kayseri-İncesu (38 ° 42 '43 "N and 35 ° 15' 55" E) and the uncontaminated soil sample was taken from Çopurlu village on Mersin Gözne road (36 ° 87 '32 "N and 34 ° 56' 50" E) from 0-30 cm depth. Some physical and chemical properties of contaminated and uncontaminated soil samples were given in Table 1. The particle size distribution of uncontaminated soil was determined as 20.5%, 27.0%, 52.5% for sand, silt and clay, respectively. The soil texture was clay (C) according to the texture triangle. The particle size distribution of contaminated soil was determined as 84.6%, 8.4%, 7.0% for sand, silt and clay, respectively. The soil texture was loamy sand (LS). The contaminated and uncontaminated soils demonstrated the following properties, respectively; pH 8.23 and 8.04, lime 28.34% and 7.26%, organic matter 3.04% and 5.27%. Both soils were alkaline, but uncontaminated soil has a high lime content than contaminated soil.

Soil samples were sieved from 4 mm sieve. Five soil mixtures (0, 25, 50, 75 and 100% of contaminated soil) were obtained by mixing the multiple metals (Cd, Pb and Zn) contaminated soil with a uncontaminated soil. The pot experiment was a randomized complete block design containing with and without EDDS treatments and three replications.

Table 1. Some physical and chemical properties of soil samples (from Çiftçi 2016)

Soil Properties	*Uncontaminated Soil*	*Contaminated Soil*	*References*
Sand (0.02-2 mm) (%)	20.5±1.1	84.6±2.3	Bouyoucos 1962
Silt (0,002-0,02 mm) (%)	27.0±0.9	8.4±0.5	
Clay (<0.002 mm) (%)	52.5±1.0	7.0±0.6	
Texture class	C (Clay)	LS (Loamy sand)	
pH (in the saturated soil	8.23±0.02	8.04±0.01	Soil Survey Staff 1951
Lime ($CaCO_3$) (%)	28.34±0.08	7.26±0.41	
Organic matter (%)	3.04±0.48	5.27±0.24	
Total Cd (mg kg^{-1})	1.92±0.10	483±32.9	USEPA 1995
Total Zn (mg kg^{-1})	92.4±3.35	13412±0.1	
Total Pb (mg kg^{-1})	19.1±0.01	25755±0.54	
DTPA extractable Cd (mg kg^{-1})	0.05±0.02	26.8±0.16	Lindsay and Norvell 1978
DTPA extractable Pb (mg kg^{-1})	1.03±0.21	31.1±1.72	
DTPA extractable Zn (mg kg^{-1})	0.75±0.33	128±11.6	

The seeds of canola (*Brassica napus* L.) were used as plant material in the study. Total 8 kg mixed soils (0, 25, 50, 75 and 100% of contaminated soil) filled into the pots and 10 of canola seeds were sown in pots. Those seedlings reduced to one seedling in each pot. Canola plant was grown for 2 months under controlled environmental conditions with 10 klux light intensity, 25°C day and 20°C night temperature cycle, 16 h light and 8 h dark period and 60% humidity.

At the beginning, canola seeds were planted in plastic pots in the absence or presence of 10 mg EDDS kg^{-1} soil, added in the form of EDDS ($C_{10}H_{13}N_2Na_3O_8$). A basal treatment of 200 mg kg^{-1} nitrogen (N) as calcium nitrate ($Ca(NO_3)_2$), and 100 mg kg^{-1} phosphorus (P) and 125 mg kg^{-1} potassium (K) as potassium dihydrogen phosphate (KH_2PO_4) were also applied to all pots. Before harvest, the chlorophyll (in old, middle and young leaves) content as SPAD (Soil, Plant Analysis Development) value was measured by using Konica Minolta SPAD-502 meter . The SPAD meter measures the optical density difference in 2 mm × 3 mm field, 2 wavelengths (650 nm and 942 nm) (Çiftci 2016). The numerical SPAD value refers to the proportional content of chlorophyll in the measured area in µg/cm^2 (Markwell et al. 1995). Accordingly, 1 SPAD unit is approximately 0.001 µg cm^2. After the SPAD measurement, plants were harvested. The samples were rinsed in distilled water and dried at the 65 °C in oven for two days. After measurement of the dry weight (DW) of the plants, samples were ground in agat mill and then digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in microwave oven (MarsXpres CEM). Total multi metals (Cd, Pb, and Zn) concentrations of the samples were determined in ICP-MS (Inductively Coupled Plasma- Mass Spectrometry; Agillent 7500ce). All results were given based on dry plant matter at 65 °C. Certified reference materials (*SRM 1573A, SRM 1547*) were also analyzed in order to check the accuracy of the extraction technique used in this study. All data were presented as an average ±standard deviation (SD).

Results and Discussion

Chlorophyll Content

Chlorophyll content of canola was decreased with increasing multi metal (Cd, Pb, and Zn) concentration treatments (Figure 1). Plants did not grow in 75 and 100% multimetal doses. The lowest chlorophyll content (13.60 and 14.60 SPAD values) was measured in young leaves of the canola plants in 50% multimetal dose with and without EDDS treatment, respectively. On the other hand, the highest chlorophyll content (28.77 and 29.77 SPAD value) was measured in the middle leaves of the plants in 25% multimetal dose with and without EDDS treatment, respectively.

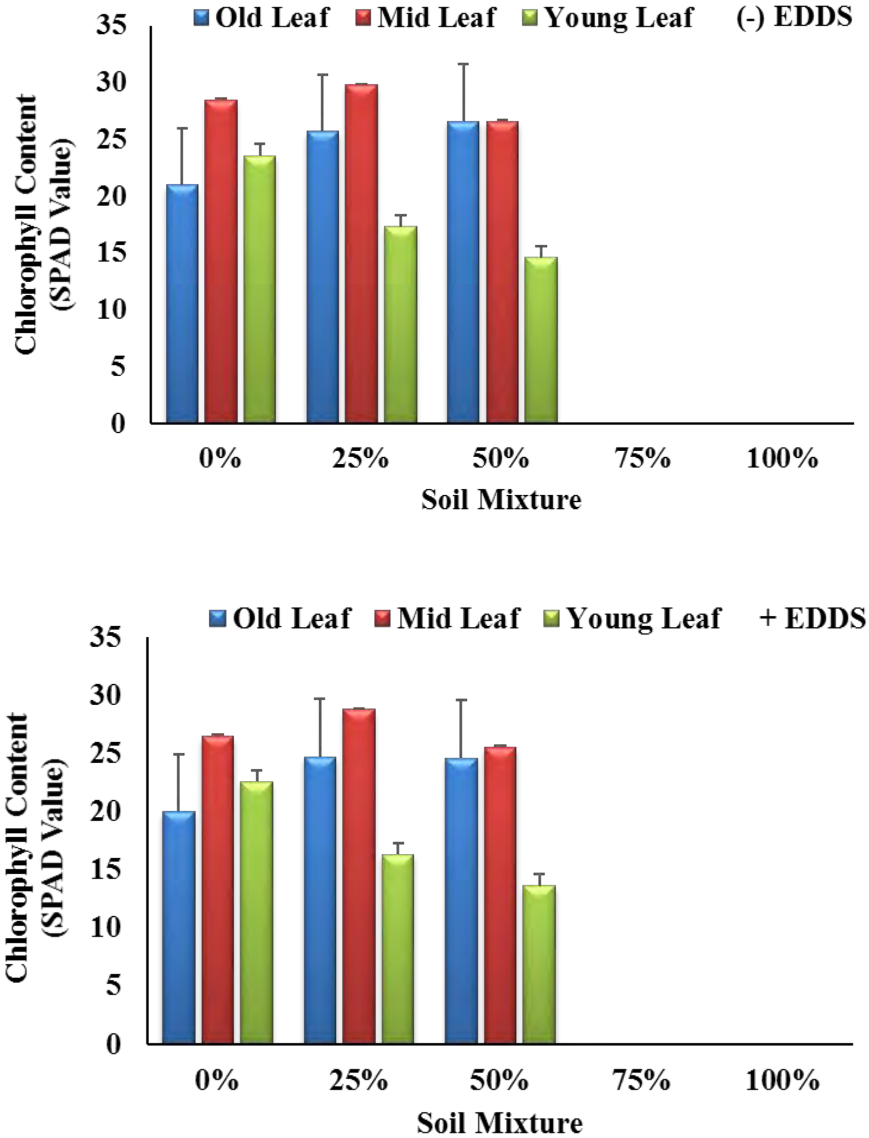


Figure 1. Effects of multi metal (Cd, Pb, and Zn) application on chlorophyll contents of canola plants without and with EDDS treatments (mean ±SD, *n*=3).

Chlorophyll content may be decreased because of the toxic effects of multi metals. Luo et al. (2005), reported that with the application of 5 mmol kg^{-1} EDDS to the soil, plants showed toxicity symptoms and biomass production decrease 14 days after treatment. Grčman et al. (2003) observed chlorosis and necrosis symptoms on *Brassica rapa* leaves grown in multi metal contaminated soil treated with 10 mmol kg^{-1} EDDS.

Dry Weights

EDDS treatments were increased dry weights of plants in the 25 and 50 % multi metal doses containing soils compared to the control treatment (Figure 2). On the other hand, dry weight of plants was lower with EDDS treatment than without EDDS treatment. This is due to the fact that the EDDS application reduces the dry weight of the plant by enhancing the heavy metal uptake by the plant. Similar results were reported by, Luo et al. (2005), who explained that the application of 5 mmol EDDS and EDTA to the 1 kg soil significantly inhibited plant growth and 14 days after applications of EDDS and EDTA, the shoot dry weights decreased to 60% and 52% of the control plants for corn, and 76% and 61% of the control plants for bean, respectively.

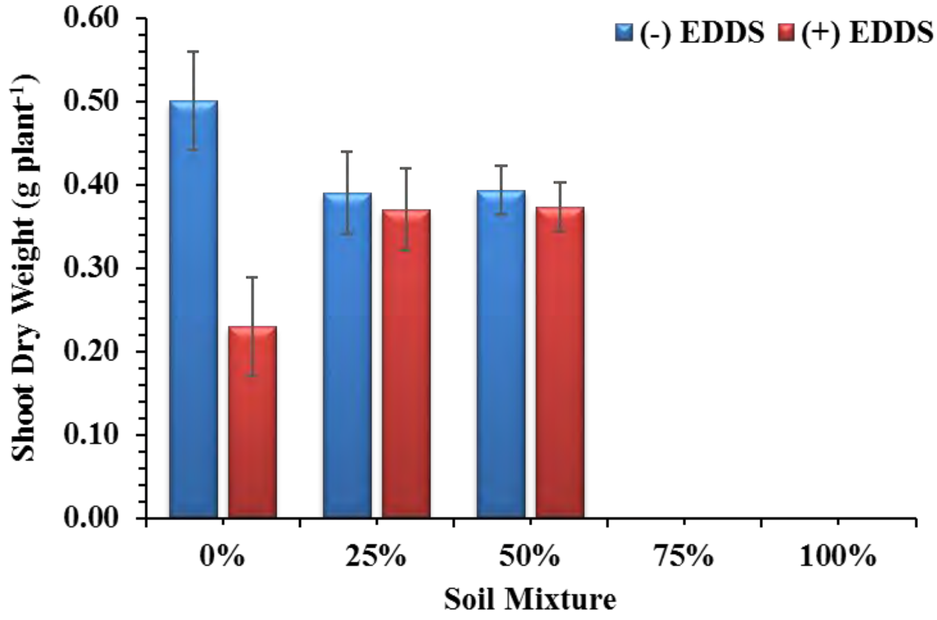
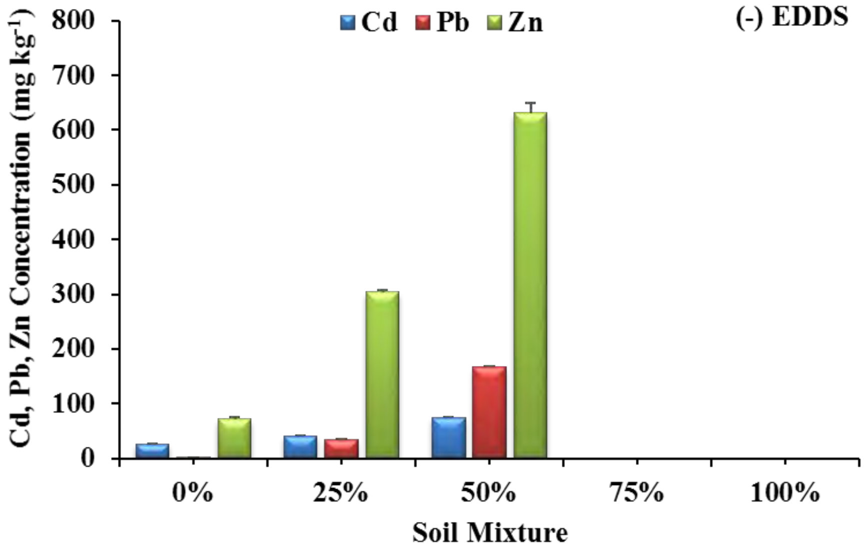


Figure 2. Effects of multi metal (Cd, Pb, and Zn) application on the dry weights of canola plants without and with EDDS (mean ±SD, *n*= 3).

However, the dry weight of canola was decreased due to the toxic heavy metal without EDDS treatments at 25 and 50% (0.39 g plant^{-1}) multi metal doses compare to the control (0.50 g plant^{-1}) treatments (Figure 2). Because of the toxic effects of heavy metals (Cd, Pb, and Zn) plants did not grow in the 75 and 100% multi metal doses.

Cd, Pb, and Zn Concentrations of Canola Plant

Plant multi metal concentrations were increased with increasing multi metal doses (Figure 3).



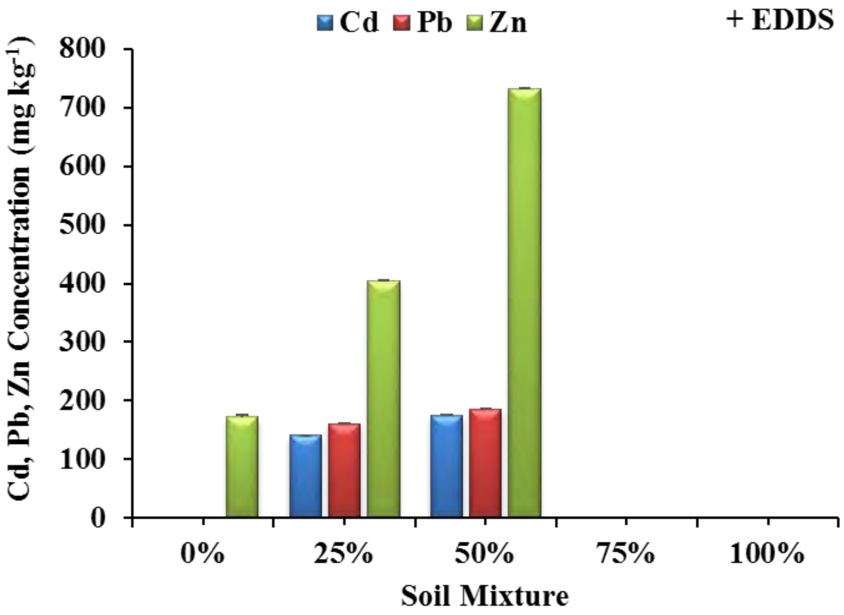


Figure 3. Effects of multi metal (Cd, Pb, and Zn) application on heavy metal concentrations in shoots of canola without and with EDDS (mean ±SD, *n*= 3).

The highest metal concentration of the plants were as follows Zn>Pb>Cd. The highest multimetal concentrations were obtained EDDS treatment at 50% multi metal doses as follows; 730 mg Zn kg^{-1}, 186 mg Pb kg^{-1} and 174 mg Cd kg^{-1}. Only Cd concentration was 1.4- and 1.7-fold higher than hyperaccumulation limit (>100 mg kg^{-1} DW, Baker 1981) with EDDS treatment in the 25 and 50% multimetal doses (141 and 174 mg Cd kg^{-1}, respectively). However, Zn and Pb concentrations in shoot of canola plants were lower than the critical toxicity level of hyperaccumulator (Pb: 1000 mg kg^{-1} and Zn: >10000 mg kg^{-1}). Grčman et al. (2003), obtained a 3-fold increase in Cd and Zn and 10.3-fold in Pb uptake by *Brassica rapa* with 4 times 10 mmol kg^{-1} EDDS treatment in multi metal contaminated soil. Luo et al. (2005) reported that 5 mmol kg^{-1} treatment of EDDS and EDTA were significantly enhanced heavy metal (Cd, Zn, Cu, and Pb) uptake of corn and white bean plants. Lee and Sung (2015) was reported similar results with us. They were investigated the effects of EDDS (5 mmol kg^{-1}) on the heavy metals (4 mg Cd kg^{-1}, 150 mg Cu kg^{-1}, 200 mg Pb kg^{-1}, 100 mg Ni kg^{-1}, and 300 mg Zn kg^{-1}) uptake of *Brassica campetris* and *Sorghum biocolor* plants. According to their results, EDDS could increase Pb, Cu, Ni, Cd, and Zn concentrations in the roots and shoots of *Brassica campetris* and *Sorghum biocolor* plants. *Cannabis sativa* was accumulated 105-fold Pb, 2.3-fold Zn and 31.7-fold Cd with 10 mmol kg^{-1} EDDS treatments than the control plant (Kos et al. 2003).

Conclusion

Although canola plant is a hyperaccumulator for a single heavy metal, but it is not suitable for phytoextraction of multi metal (Cd, Pb, and Zn) contaminations. EDDS treatment was enhanced multi metal uptake of the plants. Except for Zn and Pb, Cd concentration of *Brassica napus* was 1.4 and 1.7-fold higher than hyperaccumulation limit (>100 mg kg^{-1}) with EDDS treatment in the 25 and 50% multimetal doses. Nevertheless, it should be considered that elevated EDDS may be washed from the soil profile and contaminate the groundwater. According to Paracelsus, the father of toxicology, "The dose makes the poison" (Latin: sola dosis facit venenum) is indicating a basic principle of toxicology. It means harmless substances can be dangerous when consumed in large quantities. Due to this reason, suitable EDDS application in the soil and its movement in the soil profile should be monitored.

References

Angelova VR, Akova VI, Krustev SV, Ivanov KI (2015). Potential of safflower (*Carthamus tinctorius* L.) for phytoremedation of soils contaminated with heavy metals. World Academy of Science, Engineering and Technology, International Science Index 102, International Journal of Agricultural and Biosystems Engineering, 9(6), 607 - 614. http://waset.org/publications/10001509

Attinti R, Barrett KR, Datta R, Sarkar D (2017). Ethylenediaminedisuccinic acid (EDDS) enhances phytoextraction of lead by vetiver grass from contaminated residential soils in a panel study in the field. Environ. Pollut. 225: 524-533.

Baker AJM (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. J. Plant Nutr. 3: 643-654. DOI: 10.1080/01904168109362867.

Bouyoucos GJ (1962). Hydrometer method improved for making particle size analysis of soils. Agron J. 54: 464-465.

Çiftçi A (2016). Çoklu Metal (kadmiyum, kurşun ve çinko) ile Kirlenmiş Bir Toprağın Arıtımında Yabani Hint Yağı (*Ricinus communis*) ve Aspir (*Carthamus tinctorius*) Bitkilerinin Fitoremediasyon Kapasitesinin Araştırılması Mersin Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi (Multimetal (Cadmium, Lead and Zinc) Treatment of Soil Contaminated with a Wild İndian Oil (*Ricinus comminus*) and Safflower (*Carthamus tinctorius*) Investigation of Phytoremediation Plant Capacity), Master Thesis, Graduate School of Natural and Applied Sciences, University of Mersin (in Turkish).

Dağhan H (2007). Fitoremediasyon: Bitki Kullanılarak Kirlenmiş Toprakların Temizlenmesi (Phytoremediation: using plant to clean up contaminated sites). GAP V. Tarım Kongresi, Bildiriler Kitabı, s.362-367, 17-19 Ekim 2007, Şanlıurfa, Türkiye (in Turkish).

Dağhan H (2011). Doğal Kaynaklarda Ağır Metal Kirliliğinin İnsan Sağlığı Üzerine Etkileri (The effects of heavy metal contaminations in natural sources on human health). MKU Ziraat Fakültesi Dergisi (Journal of Agricultural Faculty, MKU) 16 (2):15-25 (in Turkish).

Evangelou MWH, Ebel M, Schaeffer A (2007). Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68: 989-1003.

Grčman, H, Vodnik D, Velinkonja-Bolta Š, Leštan D (2003). Ethylenediamine dissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. J. Environ. Qual. 32:500–506.

Kasiuliene A, Paulauskas V, Kumpiene J (2016). Influence of nitrogen fertilizer on Cd and Zn accumulation in rapeseed (*Brassica napus* L.) biomass. Agron. Research 14(2): 418–427.

Kos B, Grčman H, Leštan D (2003). Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil Environ. 49(12): 548-553.

Lee J, Sung·K (2015). EDDS effects on heavy metal uptake by bioenergy plants. J. Soil Groundw. Environ. 20(4): 8-14.

Lindsay WL, Norvell WA (1978). Development of a DTPA test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. 42: 421-428.

Luo C, Shen Z, Li X (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59:1-11.

Markwell J, Osterman JC, Mitchell JL (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosyn. Res. 46(3): 467-472.

Singh J, Kalamdhad AS (2011). Effects of heavy metals on soil, plants, human health and aquatic life. Int. J. Res. Chem. Environ. 1 (2):15-21.

Soil Survey Staff (1951) Soil Survey Manual. U. S. Dept. Agr. Handbook No:18, U.S Goverment Print Office, Washington

Sumiahadi A, Acar R (2018). A review of phytoremediation technology: heavy metals uptake by plants. IOP Conf. Ser.: Earth Environ. Sci. 142 012023: 1-9.

Takahashi R, Yamayoshi K, Fujimoto N, Suzuki M (1999). Production of (S,S)-Ethylenediamine-N,N'-disuccinic Acid from Ethylenediamine and Fumaric Acid by Bacteria. Biosci Biotechnol Biochem.;63(7):1269-73. DOI: 10.1271/bbb.63.1269

Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int. J. Chem. Eng. Volume 2011, Article ID 939161, 31 pages, DOI: 10.1155/2011/939161.

USEPA (1995) Method 3051, Microwave assisted acid digestion of sediments, sludges, soils and oils. In: Test Methods for Evaluating Solid Waste, 3rd ed, U.S. Environmental Protection Agency, Washington DC.

Ullah S, Shahid M, Zia-ur-Rehman M,Sabir M (2014). Phytoremediation of Pb-contaminated soils using synthetic chelates. in Chapter 14, Hakeem K, Sabir M, Ozturk M & Mermut AR (Eds.). *Soil remediation and plants: prospects and challenges*. Academic Press.

Ullmann A, Brauner N, Vazana S, Katz Z, Goikhman R, Seemann B, Marom H, Gozin M (2013). New biodegradable organic-soluble chelating agents for simultaneous removal of heavy metals and organic pollutants from contaminated media. J.Hazard. Mater. 260: 676-88. DOI: 10.1016/j.jhazmat.2013.06.027

Wuana RA, Okieimen FE (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology, Volume 2011, Article ID 402647, 20 pages, DOI: 10.5402/2011/402647