International Association of Scientists
www.iasnetedu.com

Available online at www.jobiost.com
IJBLS 2023; 2(3):340-348

International Journal of BioLife Sciences

Original paper

Phytoextraction Potential and Physiological and Biochemical Changes of Sunflower (Helianthus annuus) Plant in Metal Contaminated Soil

Bülent Topcuoğlu*

Akdeniz University Sustainable Agriculture Department, 07058 Antalya Türkiye

***Received**: 18 September 2023* ***Revised**: 25 September 2023* ***Accepted**: 30 September 2023*

Abstract

Background and aim: Heavy metals are major environmental pollutants due to toxicity and non-biodegradability. Sunflower phytoextraction is a cost-effective method for remediating metal-contaminated soils. Sunflowers, adaptable and fast-growing, show promise, but there's limited knowledge on the targeted metals and the plant's response to heavy metal stress. This study aims to uncover sunflower's phytoextraction capacity and its reaction to soil heavy metal stress.

Materials and methods: In a greenhouse experiment, Sunflower plants were grown by applying heavy metals (Zn, Cu, Ni, Pb and Cd) to the soil in order to determine the phytoextraction capacity and the physiological and biochemical changes occurring within the plant related to heavy metal applications. Soil and plant analysis were carried out in the experiment. Plant metal phytoremediation parameters and plant biochemical parameters were evaluated.

Results: Heavy metals applied to the soil in increasing amounts increased the heavy metal contents in the plant. Sunflower plant especially absorbed Pb and Cd metals at relatively high levels. Among the metals applied to the soil, Cd was determined at the highest level in the plant. The highest metal transfer factor and phytoextraction efficiency were determined for Zn, Cu and Cd metals. The dry matter amount of the plant did not change significantly with metal applications. Sunflower plants grown in soil where high levels of heavy metals were applied produced high biomass during the 5-week vegetation period after germination and did not show phytotoxicity, but a significant decrease in chlorophyll content was noted. Regarding the heavy metals applied to the soil, the amounts of proline, polyphenols and flavonoids in the plant were increased, and the highest values were determined in Pb and Cd applications.

Conclusion: Research findings have shown that the Sunflower plant is well adapted to heavy metal stress conditions and can be used as an effective phytoextraction plant.

Keywords: *Heavy metals, Phytoremediation, Sunflower, Phytoxicity*

***Corresponding author:** Bülent Topcuoğlu, Akdeniz University Sustainable Agriculture Department, 07058 Antalya Türkiye.
E-mail address: btoglu@akdeniz.edu.tr

Introduction

Heavy metals are among the most important pollutants affecting the environment due to their toxicity, large emission sources, non-biodegradable properties and accumulation behavior. Heavy metal pollution in agricultural soils is one of the most important environmental and public health problems and has significant harmful effects on human health and ecosystems [1]. Biocides, various organic and mineral fertilizers, various industrial emissions and air pollutants commonly used in agricultural soils are among the common sources of heavy metals and are the main sources of soil pollution.

In recent years, phytoextraction technique has been widely accepted as an effective alternative to sophisticated and expensive technologies in the remediation of soils contaminated with metals. In phytoextraction studies, hyperaccumulator plants that have high metal absorption capacity and produce high biomass in a short time are preferred. However, the fact that many plants that have been identified as hyperaccumulators so far generally accumulate only limited elements, tend to grow slowly, have low biomass, and are compatible with different climates and ecologies are the main difficulties in the selection of phytoextraction plants [2], [3]. The main strategy of phytoextraction is to identify plants with high biomass and high metal accumulation properties from nature. Sunflower (Heliantus annuus) plant is a plant species that can live in different ecological regions with its many varieties, can create high biomass vegetatively in a short time, and can adapt to difficult soil and climate conditions. In recent years, high hyperaccumulator properties of Sunflower plant has been stated in many scientific reports [4], [5], [6], [7], [8].

Exposure of plants to heavy metals can lead to many negative effects that hinder metabolic activities [9]. Plants produce reactive oxygen species in their cells when exposed to heavy metal stress. Some phenolic compounds produced within the plant in response to metal stress can reduce the toxic effects of heavy metals by increasing plant tolerance and help protect plants from oxidative stress [10], [11]. Phenolic compounds are classified as beneficial antioxidants that can relieve oxidative stress in plants exposed to stress factors [12]. Flavonoids enhance the process of metal chelation, which can reduce the level of harmful hydroxyl radicals in plant cells and perform a protective function under stress conditions by neutralizing radicals before they can damage cells. In plants, proline is an amino acid that is generally synthesized by plants under stress conditions and is considered an indicator of metabolic disorder in plants [13]. Therefore, the concentration of proline and phenolic compounds in plant tissues is considered a good indicator that allows researchers to estimate the range of tolerance to stress factors occurring in plants [14].

The metal accumulation feature of the Sunflower plant and its adaptation to survive in wide climatic and geographical regions are considered as a useful material that can be used to understand the physiological mechanisms related to resistance to abiotic stresses [7], [8]. There is still a lack of information about the types of metals that phytoextraction plants remove from the soil and how heavy metals affect the physiology and biochemistry of these plants. In this study, it was aimed to reveal the phytoextraction capacity of the Sunflower plant and the response of the Sunflower plant to the stress caused by heavy metals in the soil.

Material and Methods

- *Experimental Studies*

The experiment was carried out as a control in a naturally ventilated glass greenhouse. Sunflower seeds were disinfected with 5% sodium hypochlorite solution for a few minutes for sterilization and then rinsed with distilled water before planting in the soil. Red Mediterranean soil was used in the experiment. The soil was air dried, sieved to 2 mm and mixed with perlite at a ratio of 30 percent and 20 percent peat to provide appropriate physical conditions in the potting medium. The analytical characteristics of the test soil are presented in Table 1. The physical and chemical properties of the greenhouse soil mixture examined before the experiment are within the range of normal agricultural values, and heavy metal concentrations are below the levels specified by the European Union [15].

Basic N-P-K fertilization was applied to the experimental soil at the rates of 80, 40 and 70 mg kg-1 N (as NH_4NO_3), P (as KH_2PO_4) and K (as K_2SO_4). Heavy metals (Zn, Cu, Ni, Pb and Cd) were added to the experimental soil as metallic salt solutions (in the forms of $ZnSO_4$, $CuSO_4$, $NiSO_4$, $PbSO_4$, $CdSO_4$, respectively), as shown in Table 2. Metal concentrations were applied above the European Union pollutant limit values and in geometrically increasing rates. Soil and metals were incubated with water at field capacity for at least 1 months before experimentation.

The experiment was conducted in a randomized block factorial design with 5 repetitions and 5 levels of heavy metals. Sunflower seeds were germinated in peat+perlite substrate mixture. Then, 3 seedlings from each plant were planted in each pot containing 10 kg of air dried soil. During the experiment, plants were irrigated regularly in field capacity water tension and processed according to common agrotechnical principles. After 60 days of growth, all plants were harvested from the soil surface. Plant samples (aboveground plant parts) were dried in a airforced oven at 60 ºC, ground with a shaking mortar and digested in aqua regia (1:3 HNO3/HCl). Total metal concentrations were analyzed by using ICP-MS under optimized measurement conditions, and values were adjusted for oven-dried (12 h at 105 °C) material.

Table 1: The analytical characteristics of the experimental soil before applications.

Parameters		Metal limits in soil,mg kg^{-1} dry wt [15]
Texture	Loam	
pH- H_2O (1:5 w/v)	7,52	
$CaCO_3$, %	6,45	
Organic matter, %	5,2	
Clay,%	7,7	
CEC, cmol kg^{-1}	22,3	
EC, dS m^{-1} 25°C	0,74	
Total N, %	1,15	
P (ex), mg kg^{-1}	6,8	
K (ex), mg kg^{-1}	88	
Ca (ex), mg kg^{-1}	754	
Mg (ex), mg kg^{-1}	118	
Zn, mg kg^{-1}	58.2[1]	150-300
Cu, mg kg^{-1}	8,2	50-140
Ni, mg kg^{-1}	6,7	30-75
Pb, mg kg^{-1}	15,6	50-300
Cd, mg kg^{-1}	0,01	1-3

1:Total concentrations

Table 2. Heavy metal treatment levels of experiment.

Metals	Metal treatments, mg kg^{-1}				
	Control	1	2	3	4
Zn	0	300	600	1200	2400
Cu	0	140	280	560	1120
Ni	0	75	150	300	600
Pb	0	300	600	1200	2400
Cd	0	3	6	12	24

- *Plant Metal Evaluation Parameters*

Heavy Metal Transfer (or Bioconcentration) Factor: The TF of metals in the soil to the aboveground part of the plants was defined as the ratio of the heavy metal concentration in the plants to that in the soil [16].

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows [17] :

$$TF = \frac{C\ plant}{C\ soil} \quad (1)$$

where: C_{Plant} is heavy metal concentration in plant tissue, mg kg-1 dry weight; and C_{Soil} is heavy metal concentration in soil, mg kg-1 dry weight.

Theoretical total metal uptake was calculated using Eq. 2, as follows [18]:

$$Metal\ uptake\ (mg\ pot^{-1}) = C\ x\ W\ x\ n \quad (2)$$

where: C is heavy metal concentration in plant tissue, mg kg^{-1} dry weight; and W is plant dry weight kg $plant^{-1}$ and n is number of plant

Theoretical phytoextraction efficiency (%) of harvested plants was calculated using Eq. 3, as follows [12]:

$$Phytoextraction\ efficiency\ (\%) = \frac{Cp\ x\ W\ x\ n}{Cs\ x\ 10\ kg\ pot-1} \quad (3)$$

where: Cp is heavy metal concentration in plant tissue, mg kg^{-1} dry weight; and W is plant dry weight kg pot^{-1}; n is number of plant; Cs is metal concentration of soil mg kg^{-1}

- *Plant Physiological and Biochemical Evaluation Parameters*

Total chlorophyll content were analyzed by acetone extraction method [19] and total chlorophyll content was reported as mg kg^{-1} on a fresh weight material. The content of proline is determined according to the method of Bates et al. [20] and the proline contents are expressed mg g^{-1} of dry weight. Total polyphenol content in plant was determined according to the method of FolinCiocalteu phenol reagent and contents expressed as mg EAG g^{-1} dry weight [21]. The flavonoid content in plant was determined by the aluminum trichloride method [22] and the flavonoid contents were expressed as mg EQ g^{-1} dry weight.

- *Statistical Analysis*

ANOVA test ($p \leq 0.05$) calculated using the statistical package SPSS23 for Windows program were applied to compare the differences in parameters

Results and Discussion

- *Plant Growth and Heavy Metal Concentration of Plants*

There was no significant change in the dry matter values of the Sunflower plant with heavy metal applications, and no phytotoxicity was observed in the applications (Table 3). This shows that plants are well adapted to stress conditions, even up to eight times the maximum soil metal concentration limits. The concentrations of Zn, Cu, Ni, Pb and Cd in the Sunflower plant have also increased by inreasing metal applications. The lowest values in the relative change of metals compared to the control application were determined in Zn and Cu metals. Compared to the control treatment, Cd metal gave by far the highest relative change with increasing metal concentrations (Figure 1). Although each of the heavy metals was applied up to 8 times starting from the threshold limit value, the presence of Cd metal in relatively higher concentrations in the plant compared to the control application shows that the Sunflower plant effectively accumulates Cd metal and is a high Cd hyperaccumulator. This indicates that Sunflower plant has adapted to accumulate heavy metals without any physiological disorder in natural conditions.

Table 3. Dry matter (g pot^{-1}) and heavy metal concentration (mg kg^{-1}) of Sunflower plant.

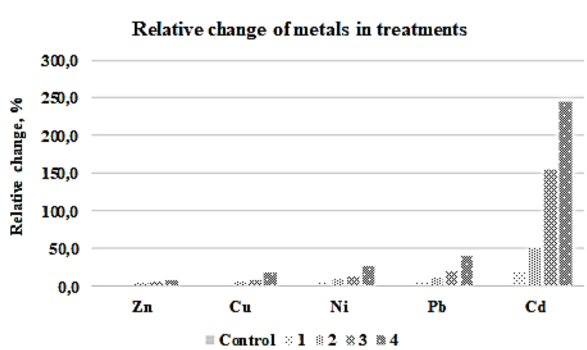
Treatments	Dry matter	Zn	Cu	Ni	Pb	Cd
Control	124,5	24,5	8,82	0,658	2,215	0,006
1	133,4	88,5	11,24	3,524	14,56	1,251
2	126,5	124,6	14,56	6,254	25,68	3,254
3	125,4	176,1	20,35	9,125	45,66	10,254
4	120,2	218,3	26,89	16,951	88,62	16,215

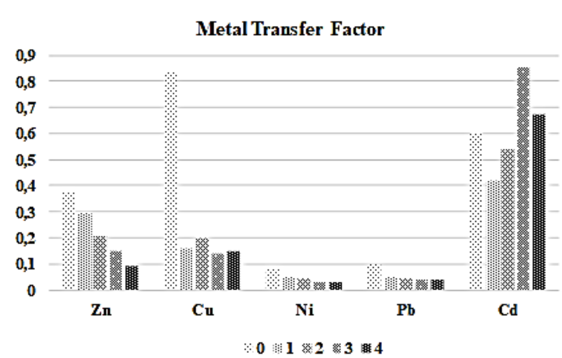
- *Changes in Phytoremediation Parameters in Metal Treatments*

Metal transfer factor (TF) of Sunflower plant was decreased by increasing amounts of metal treatments with the exception of Cd, (Figure 1). Metal TF of Zn, Cu and Cd in Sunflower plant was determined at the higher rates. The lowest TF values were determined in Ni and Pb applications and the highest TF value in Cd applications. These data show that the Sunflower plant contains high amounts of Zn, Cu and Cd metals in metal absorption from the soil.

Metal uptake (MU) amount of Sunflower plant were increased by the treatments. Metal uptake of Sunflower was found higher for Zn, Cu and Pb metals (Figure 1). Total metal uptake amount was determined highest for Zn metal in plant. Metal uptake rate of Zn and Cd were increased about 8-10 fold by the treatments compared to control. In all treatments metal uptake amount was determined for metals in Zn>Cu>Pb>Ni>Cd order.

Phytoextraction (PE) rates of plant were decreased by the treatments (Figure 1). In terms of phytoextraction efficiency, Sunflower plant gave higher values for Zn, Cu and Cd. At control treatment Cd metal has the highest rate of PE value. In all treatments Cd has the highest PE values. This indicates that Sunflower has the ability of phytoextraction especially for Cd metal in soil remediation. Similar results were reported that Sunflower plant has the potential to allow cultivation of Zn, Cu and Cd contaminated farmland to assist in lowering total Cd content of soil [7], [18], [23].





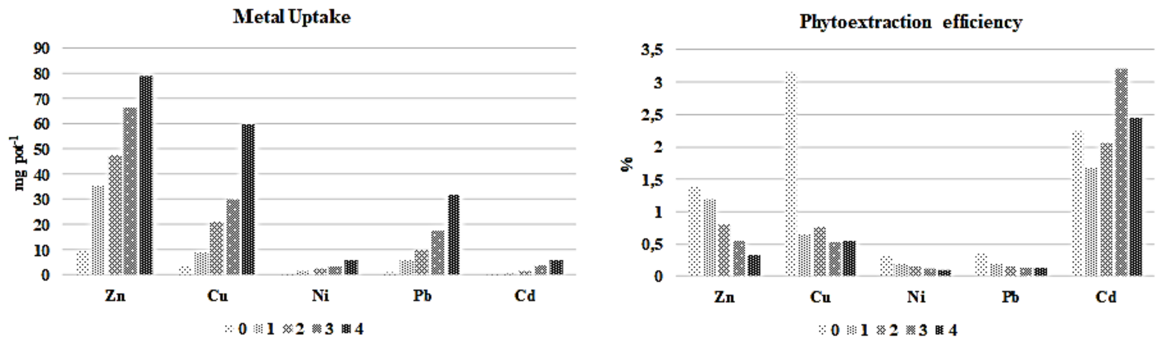


Figure 1. Relative change, metal transfer factor, metal uptake and phytoextraction efficiency of Sunflower plant in metal treatments

- *Changes in Plant Biochemical Parameters in Metal Treatments*

Metal applications to the soil caused a significant decrease in the chlorophyll content of the Sunflower plant, with the exception of Cu metal (Figure 2). Compared to the control application, the lowest chlorophyll values were obtained in the highest metal application. The lowest values in total chlorophyll content were determined in Cd application. Low total chlorophyll values may be an important indicator of the plant's exposure to metal stress and the toxicity of metal applications on the plant. It is stated that exposure of plants to metals may cause deformation of chloroplast structures [24], and metals may also inhibit some enzymes involved in the Calvin cycle [25].

Heavy metals applied to the soil increased the total proline content of the Sunflower plant (Figure 2). The proline content of the Sunflower plant has increased in proportion to the increasing applications of heavy metals. The highest proline values were obtained in Pb and Cd applications. The data are compatible with the results obtained on the synthesis of proline in plants under heavy metal stress [26], [27], [28], [29], [30].

Heavy metals applied to the soil increased the total polyphenol content of the Sunflower plant (Figure 2). The polyphenol content in the plant generally have increased by increased metal applications to the soil. The increase in the polyphenol content of the plant by metal applications was more evident in Ni and Cd metal applications. The findings support the hypothesis that plants exposed to abiotic stress metabolically synthesize high levels of polyphenols.

As well as the polyphenol content, heavy metals applied to the soil increased the total flavonoid content of the Sunflower plant (Figure 2). With the partial exception of some applications of Zn and Cu metals, the flavonoid content in the plant generally increased due to increased metal applications to the soil. The increase in the flavonoid content of the plant with metal applications was more evident in Pb and Cd metal applications. Flavonoid content of Sunflower plant showed higher values in Pb and Cd applications. In Pb applications to the soil, unlike the polyphenol content, higher flavonoid content was obtained in the plant compared to Zn and Cu metals. The high flavonoid content in Cd metal applications is parallel to the high TF value of the Sunflower plant in Cd uptake from the soil.

The increase in the amount of proline, total polyphenols and flavonoids of the Sunflower plant due to heavy metal applications supports the thesis that the production of these substances may be a defense mechanism of the plant against heavy metal stress. The high level of polyphenol accumulation in Sunflower plants under heavy metal stress is consistent with studies conducted on different plants [10], [11].

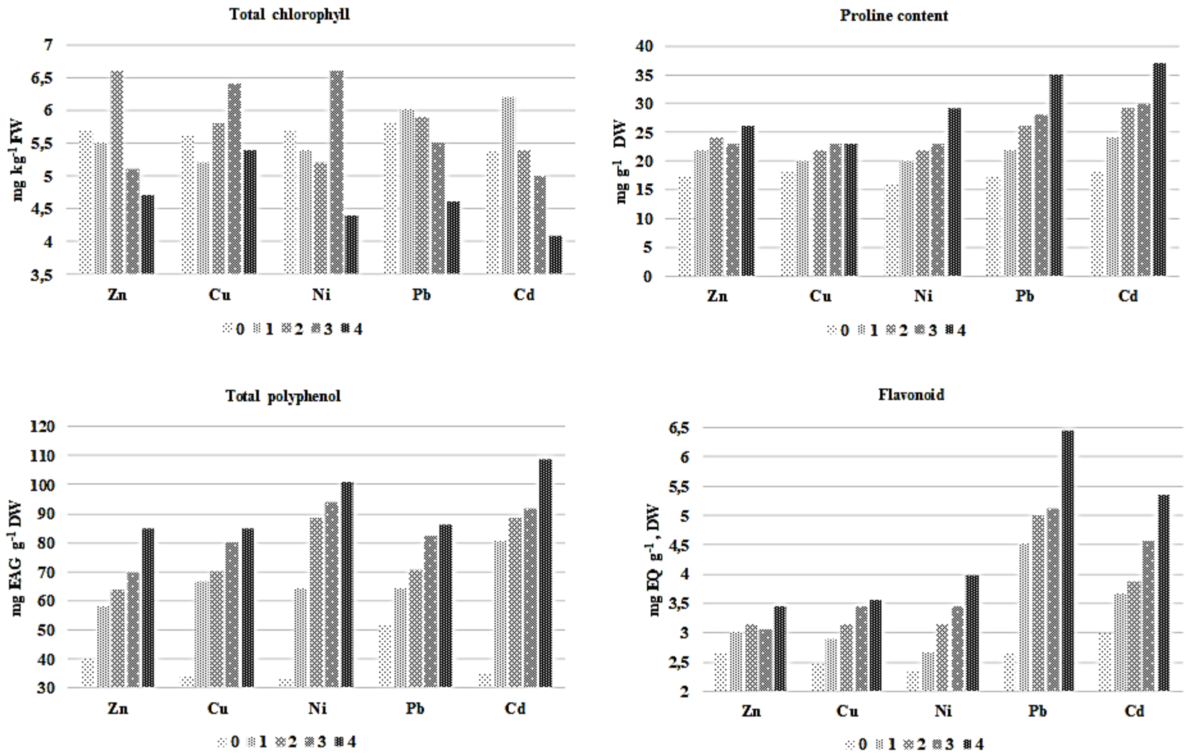


Figure 2. Total chlorophyll, proline, total phenol and flavanoid contents of Sunflower plant in metal treatments

Conclusion

The metal contents in the aboveground organs of the plant increased by increasing levels of metal applications. The Sunflower plant showed relatively high levels of phytoextraction activity, especially on Zn, Cu and Cd metals. Sunflower plants grown in soil treated with high levels of heavy metals produced a high biomass in the short vegetation period and although total chlorophyll values decreased, plant did not show phytotoxicity. It is understood that in the presence of metal stress in Sunflower plants, plants resist stress conditions by synthesizing total polyphenols and flavonoids with the proline as an amino acid. Research findings have shown that the Sunflower plant is well adapted to heavy metal stress conditions with its high phenolic substance production. The high total proline, polyphenol and flavonoid values of the Sunflower plant when exposed to high metals, its ability to survive and develop in metal toxicity and its high phytoextraction activity show that this plant can be used as an alternative and promising plant in phytoextraction applications.

Acknowledgment

This study was supported by Akdeniz University. The author would like to thank the Technical Sciences Vocational School Directorate for their valuable technical and administrative support.

Conflict of interests

The authors declare that there are no competing interests.

Reference

[1]. Ruilian YU, Xing Y, Yuanhui ZH, Gongren HU, Xianglin TU. Heavy metal pollution in intertidal sediments from Quanzhou Bay, China. Journal of Environmental Sciences. 2008;20(6):664-9.
[2]. Chen Y, Li X, Shen Z. Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. Chemosphere. 2004;57(3):187-96.
[3]. Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N. Phytoextraction and phytoexcretion of Cd by the leaves of Tamarix smyrnensis growing on contaminated non-saline and saline soils. Environmental Research. 2008;106(3):326-32.
[4]. Zehra A, Sahito ZA, Tong W, Tang L, Hamid Y, Khan MB, Ali Z, Naqvi B, Yang X. Assessment of sunflower germplasm for phytoremediation of lead-polluted soil and production of seed oil and seed meal for human and animal consumption. Journal of Environmental Sciences. 2020;87:24-38.
[5]. Oh K, Li T, Cheng HY, Hu XF, Lin Q, Xie YH. A primary study on assessment of phytoremediation potential of biofuel crops in heavy metal contaminated soil. Applied Mechanics and Materials. 2013;295:1135-8.
[6]. Forte J, Mutiti S. Phytoremediation potential of Helianthus annuus and Hydrangea paniculata in copper and lead-contaminated soil. Water, Air, & Soil Pollution. 2017;228:1-11.
[7]. Francis E. Phytoextraction potentials of Sunflower (Helianthus annuus L.) Asteraceae on contaminated soils of abandoned dumpsites. International Journal of Scientific & Engineering Research. 2017;8(1):1751-157.
[8]. Nasser S, Soad E, Fatma E. Phytoremediation of lead and cadmium contaminated soils using sunflower plant. Journal of Stress Physiology & Biochemistry. 2014;10(1):122-34.
[9]. Erdei S, Hegedus A, Hauptmann G, Szali J, Horvath G. Heavy metal induced physiological changes in the antioxidative response system. Acta biologica szegediensis. 2002;46(3-4):89-90.
[10]. Ghori NH, Ghori T, Hayat MQ, Imadi SR, Gul A, Altay V, Ozturk M. Heavy metal stress and responses in plants. International journal of environmental science and technology. 2019;16:1807-28.
[11]. Saitta M, Curto SL, Salvo F, Di Bella G, Dugo G. Gas chromatographic–tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils. Analytica Chimica Acta. 2002;466(2):335-44.
[12]. Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology. 2002;177(1):67-80.
[13]. Szabados THE, Savoure A. Proline: a multif unctional amino acid. Trends in Plant Science. 2009;15(2):89- 97.
[14]. Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules. 2019;24(13):2452.
[15]. C.E.C. (Council of the European Communities). Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. 1986.
[16]. Dutta S, Mitra M, Agarwal P, Mahapatra K, De S, Sett U, Roy S. Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. Plant signaling & behavior. 2018;13(8):e1460048.
[17]. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in environmental science.

2014;2:53.
[18]. Kumar A, Prasad MNV, Sytar O. Lead toxicity, defense strategies and associated indicative biomarkers in Talinum triangulare grown hydroponically. Chemosphere. 2012;89(9):1056-65.
[19]. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. InMethods in enzymology. 1987;148:350-82. Academic Press.
[20]. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-7.
[21]. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965;16(3):144-58.
[22]. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry. 1999;64(4):555-9.
[23]. Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA. Phytoremediation potentials of sunflowers (Tithonia diversifolia and Helianthus annuus) for metals in soils contaminated with zinc and lead nitrates. Water, air, and soil pollution. 2010;207:195-201.
[24]. Stefanov K, Seizova K, Popova I, Petkov V, Kimenov G, Popov S. Effect of lead ions on the phospholipid composition in leaves of Zea mays and Phaseolus vulgaris. Journal of plant physiology. 1995;147(2):243-6.
[25]. Vallee BL, Ulmer DD. Biochemical Effects of Mercury, Cadmium, and Lead. Annual Review of Biochemistry. 1972;41(1):91-128.
[26]. Ikram K, Abdelhakim RY, Topcuoglu B, Badiaa O, Houria T. Accumulation of polyphenols and flavonoids in Atriplex canescens (Pursh) Nutt stressed by heavy metals (zinc, lead and cadmium). Malays. J. Fundam. Appl. Sci. 2020;16:334-7.
[27]. Thiery l, Leprince AS, Lefebvre D, Ghars MA, Debarbieux E, Savoure A. Phospholipase D is a negative regulator of proline biosynthesis in Arctbidopsis thaliana. Journal of Biological Chemistry. 2004;279(15):14812-8.
[28]. Balestrasse KB, Gallego SM, Benavides MP, Tomaro ML. Polyamines and proline are affected by cadmium stress in nodules and roots of soybean plants. Plant and Soil. 2005;270:343-53.
[29]. Badiaa O, Yssaad HAR, Topcuoglu B. Effect of heavy metals (copper and zinc) on proline, polyphenols and flavonoids content of tomato (lycopersicon esculentum mill.). Plant Archives (09725210). 2020;20(2).
[30]. Topcuoğlu B. Effects of biochar applications on tomato plant under cadmium metal stress and on soil cadmium characteristics. Journal of Biological Studies. 2022;5(3 (Special Issue)):320-32.