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Full Length Article

Lead Phytoremediation Potential of Hydroponically Cultivated Crop Plants

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Abstract

Some plants can be used for phytoextraction in order to remediate lead (Pb) heavy metal-contaminated soils. This study was conducted to investigate the Pb phytoextraction and biomass production potentials of Meriç (sunflower), Trailblazer (switchgrass) and Remzibey-05 (safflower) varieties in hydroponic cultures. Three different plant varieties were tested in Hoagland solution supplemented by 0, 25, 50, 100, 150 mg/kg Pb concentrations. The obtained results showed that these plants could phytoextract Pb heavy metal, the ability of accumulation differed with varieties and concentrations of heavy metal. Meriç's and Remzibey-05's root and shoot biomass were less affected from increasing Pb concentrations than those of Trailblazer's were. When compared to other varieties however, Trailblazer absorbed Pb through its roots more effectively. BCF (bioconcentration factor) and TF (translocation factor) values that were important in accumulation and translocation of heavy metals showed differences among varieties. While high BCF values were recorded for Trailblazer, higher TF values were recorded for Meriç and Remzibey-05 than they were for Trailblazer. These results led that Meriç and Remzibey-05 varieties are good candidates for phytoextraction, because of their high biomass and TF values. Trailblazer might be also utilized for the purpose of phytostabilization because it has both high root Pb content and low TF value.

Keywords: Heavy metal; Lead; Phytoextraction; Phytoremediation

Introduction

One of the most significant types of chemical pollution, resulting in environmental pollution, is the heavy metals. Elmsley (2001) defined heavy metal term as metals that have concentration more than 5 g/cm^3 and are not essential for organisms. Heavy metals as lead, cadmium, chrome, mercury, aluminum, silver and tin are a group of metals whose densities were higher than 5 g/cm^3. These heavy metals are significant environmental pollutants and their presences in atmosphere, water and soil, even in very small concentrations, can cause severe problems for all organisms.

The rapid increase of metal pollution in environment can cause long-term but rapid evolutionary changes in organisms, known as metal tolerance (Steffens, 1990). Plants have both structural and adaptive mechanisms in order to cope with increasing metal concentrations (Vatamaniuk *et al.*, 2000). Plants, which are under the high exposure of metals, change their metabolic processes and gain tolerance to these metals (Cobbett and Goldsbrough, 2002). It was recorded that hyperaccumulator plants could grow and improve in rich metal conditions by developing a tolerance mechanism. They refrained from metal damage by overproduction of cytosolutes that are detoxifying metal ions (Prasad and Strzałka, 1999). These plants accumulate metals in their roots and aboveground organs, and their metal contents are considerably higher than metal level in soil. (Baker and Brooks, 1989), defined metal hyperaccumulator plants as plants subsuming copper, lead, cadmium, chrome, nickel more than 0.1% or subsuming zinc and manganese more than 1% of their own dry mass.

Phytoremediation is an inexpensive, simple yet least harmful method for removing inorganic pollutants from contaminated areas (Shin *et al.*, 2012). Besides, phytoextraction is a phytoremediation method utilizing particular plants for cleaning out inorganic pollutants from contaminated soil and water. In phytoextraction method, plants store pollutants by absorbing them through their roots and transfer them to plants' aboveground parts (Alford *et al.*, 2010). Today, a lot of plant varieties have been used for phytoextraction in remediation of heavy metal polluted areas. The hyper accumulator plants may accumulate the heavy metals in soil organs 100 to 1000 times more than other plant species without showing any toxicity symptoms (Brooks, 1998). Varieties that produce high biomass, have crop easiness and are non-food have been used for this purpose. Perennial grasses are among these plants (Shahandeh and Hossner, 2000; Pulford and Watson, 2003; Arduini *et al.*,

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2006). The plants, which have characteristics such as depth root system and rapid growth rate, have gained importance in phytoremediation. These characteristics are transferred to plants having high accumulation capacities of plants having fast growth and high habitus formation characteristics are a more practical method.

Switchgrass, which is a perennial type of grains, is a candidate plant for phytoextraction due to its agronomic properties. In a great deal of study, switchgrass has been used for phytoremediation of heavy metal contaminated soils (Entry and Watrud, 1998; Reed *et al.*, 1999; Shahandeh and Hossner, 2000). Sunflower, known as a hyperaccumulator, is an annual high biomass producer and rapidly growing plant (Lasat, 2002; Ernst, 2005). Safflower grows rapidly in marginal areas (Dajue and Mündel, 1996). Sayyad *et al.* (2010) reported that safflower could be used to remove heavy metals from soil.

The purpose of this study was to evaluate the potential of Pb phytoremediation by three plants: Meriç (sunflower), Trailblazer (switchgrass) and Remzibey-05 (safflower) in hydroponic culture, to determine the lead uptake by crop plants and to evaluate the potential ability of this species to tolerate and accumulate Pb. At the same time, it is aimed to determine that the different Pb doses whether growth and development in this doses of used plants in this study continued or not and while continuing the growth and development, to determine how much the chosen plants accumulated Pb.

Materials and Methods

Plant Material and Growth Conditions

The experiment was carried out on Meriç sunflower variety, trailblazer switchgrass variety and Remzibey-05 safflower variety grown in a controlled-climate room, in hydroponics with three replicates per variety. Seeds were surface sterilized by immersion in 0.1% (w/v) sodium hypochlorite solution for 15 min. Then, they were planted in viols filled with a sterile perlite moistened with distilled water. Seedlings grew at first 3 weeks and then transplanted to hydroponic culture under sterile conditions.

The uniform seedlings of plants were placed through a perforation in a 1.5 L polypropylene pots containing 1.2 L of 25% strength modified Hoagland solution (Chen *et al.*, 2012). A full-strength Hoagland solution contains 1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 92 µM H_3BO_3, 1.5 µM $ZnSO_4$, 0.6 µM $CuSO_4$, 0.2 µM MoO_3, 18 µM $MnSO_4$, and 3.6 µM $FeSO_4$ (Scheirs *et al.,* 2006). All solutions were adjusted to pH 5.5 ± 0.1. The heavy metal salt used in this study included $Pb(NO_3)_2$. H_2O was separately diluted in deionized water and added into hydroponic plant culture respectively. Treatments were prepared at the concentrations of control, 25, 50, 100 and 150 mg kg^{-1}.

Three seedlings were placed in one pot containing the nutrient solution for a 1 week acclimation. Then, the seedlings were exposed to the Pb-treated solutions for 30 days. Plants were maintained at 25/20°C during the day/night with a relative humidity of 70% and a 16 h photoperiod in controlled-climate room and arranged in a completely randomized design. During 30 days, nutrient solution in the containers was continuously aerated with air pumps and supplied Hoagland solution to maintain the initial volume in all treatments. Controls and treatments were in triplicates for analysis.

At the end of the experiment, each plant was harvested and roots and shoots were washed in running tap water, followed by several rinses in distilled water to remove any perlite particles attached to the plant surfaces. All plant parts were dried in an oven at 70°C for 72 h, and the dry weights were recorded by electronic balance. Then plant samples were wet-digested in HNO_3:$HClO_4$ (6:2 v/v) by Advanced Microwave Digestion System, Ethos Easy and all sample extracts were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (iCAP 6000 SERIES, ICP Spectrometer).

The tolerance index (TI) was expressed on the basis of plant growth parameters and calculated as the following (Das *et al.*, 1997).

$$TI = (\text{Growth parameters}_{Pb})/(\text{Growth parameters}_{control})\times 100$$

The translocation factor (TF) of Pb from root to shoot and bioconcentration factor (BCF) were calculated as follows (Monni *et al.*, 2000; Ali *et al.*, 2002).

$$TF = (Pb_{shoot})/(Pb_{root})$$

$$BCF = (Pb_{shoot\ or\ root})/(Pb_{nutrient\ solution})$$

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 23.0 software (IBM Corp). The data were subjected to ANOVA and the differences between means were determined using the Duncan test.

Results

Biomass

In Trailblazer and Remzibey-05 varieties, the effect of Pb concentrations on shoot biomass, root biomass, and root/shoot ratio was found significant. Meanwhile, in Meriç, the effect of Pb concentrations on shoot biomass and root biomass was found significant however, that effect on root/shoot ratio was found insignificant (Table 1). In Meriç variety, highest shoot biomass (1.4 g/plant) was determined in Pb treatment of 25 mg/kg, while the lowest shoot biomass (0.97 g/plant) was recorded in 100 mg/kg Pb treatment (Fig. 1A). In Trailblazer, the highest shoot biomass value (0.16 g/plant) was determined from both 25 and 100 mg/kg Pb treatments, while the lowest one (0.05 g/plant) was obtained form 150 mg/kg Pb concentration treatment (Fig. 1B). Lastly, in Remzibey-05, higher shoot biomass values were recorded in control and 25 mg/kg Pb concentration

Table 1: Analysis of variance of shoot biomass (g/plant), root biomass (g/plant), root/shoot of Meriç, Trailblazer and Remzibey-05 varieties exposed to different Pb concentration*

Source of variation	df	Shoot biomass (g/plant)		Root biomass (g/plant)		Root/shoot	
		Mean squares	F	Mean squares	F	Mean squares	F
Meriç							
Lead	4	0.070	2.1^{*}	0.003	2.1^{*}	0.000	0.3^{ns}
Error	10	0.040		0.001		0.001	
Trailblazer							
Lead	4	0.008	44.1^{**}	0.002	47.6^{**}	0.290	23.5^{**}
Error	10	0.000		3.750		0.012	
Remzibey-05							
Lead	4	0.440	8.0^{**}	0.040	4.7^{*}	0.008	2.5^{*}
Error	10	0.050		0.008		0.003	

df, degrees of freedom; ns, non-significant; $^{*}P< 0.05$; $^{**}P<0.01$

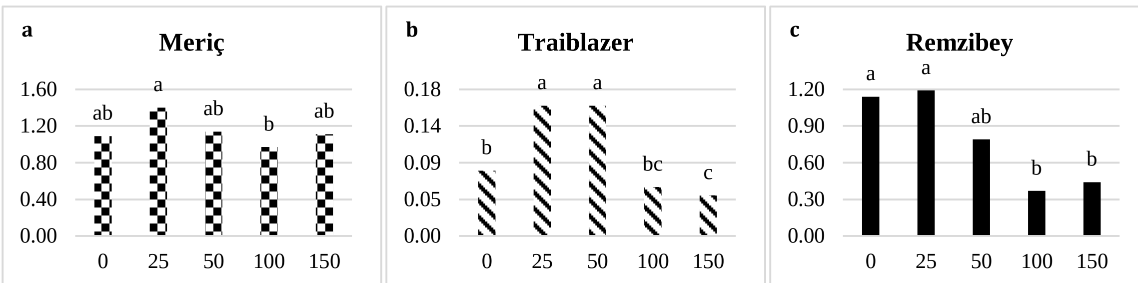

Fig. 1: Shoot biomass (g/plant) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

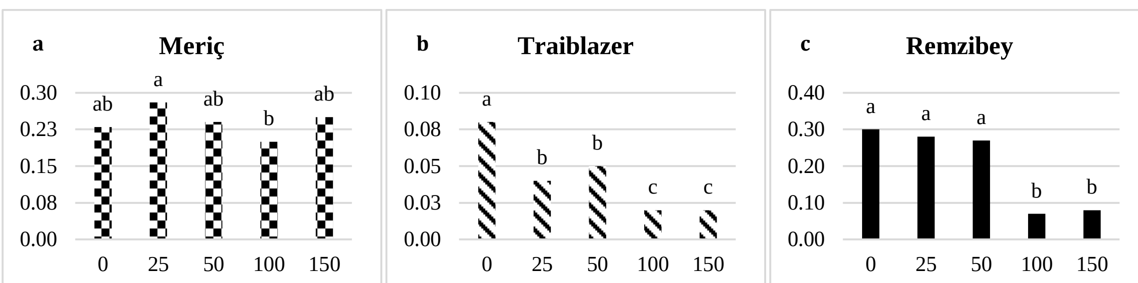

Fig. 2: Root biomass (g/plant) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

treatments (1.15 and 1.19 g/plant, respectively), while, lower shoot biomass values were detected in 100 and 150 mg/kg Pb doses (0.38 and 0.44 g/plant, respectively) (Fig. 1C).

The root biomass values of Meriç variety were ranging between 0.28 and 0.20 g/plant, in essence, the highest root biomass were detected in 25 mg/kg Pb, while the lowest one was detected in 100 mg/kg Pb concentration treatment (Fig. 2A). In Trailblazer, the highest root biomass (0.08 g/plant) was obtained from control treatment, while the lowest value (0.02 g/plant) was recorded in 100 and 150 mg/kg Pb treatments (Fig. 2B). To conclude the highest root biomass values were recorded in control 25 mg/kg, and 50 mg/kg Pb concentration treatments, while the lowest root biomass was obtained from 100 and 150 mg/kg Pb treatments of Remzibey-05 variety (Fig. 2C). Plants showed different reactions to Pb concentrations in terms of root/shoot ratio; with increasing Pb concentrations, root/shoot ratio of Meriç variety did not change, however, a decrease in root/shoot ratio of Trailblazer and Remzibey-05 was observed (Fig. 3A, B and C).

Pb Accumulation and Tolerance Index

The effects of different Pb concentrations on shoot and root tolerance index (TI) of all these plants was found significant (Table 2). It was determined that shoot TI values changed between 128.4 and 89.3% for Meriç (Fig. 4A), between 200.4 and 65% for Trailblazer (Fig. 4B), and between 104.1 and 32.6% for Remzibey-05 (Fig. 4C): Shoot TI values of Meriç showed less response to increasing Pb concentrations with respect to other varieties. Root TI had changed between 123.2 and 88.4% for Meriç (Fig. 5A), between 62.9

Table 2: Analysis of variance of Tolerance index (TI) (%) and Pb content (mg/kg) of Meriç, Trailblazer and Remzibey-05 exposed to different Pb concentration*

Source of variation		TI shoot biomass		TI root biomass		Pb content (mg/kg) shoot		Pb content (mg/kg) root	
	df	Mean squares	F	Mean squares	F	Mean squares	F	Mean squares	F
Meriç									
Lead	3	800.4	2.1^{*}	617.5	3.2^{*}	323.7	171.8^{**}	1786258.4	354.1^{**}
Error	8	367.1		190.6		1.9		5043.8	
Trailblazer									
Lead	3	15919.2	47.5^{**}	939.1	15.6^{**}	3606.7	747.0^{**}	501809028.6	471.1^{**}
Error	8	334.9		60.3		4.8		1065204.1	
Remzibey-05									
Lead	3	3219.6	6.4^{*}	4081.2	3.9^{*}	975.6	652.7^{**}	908460.9	299.7^{**}
Error	8	502.7		1043.4		1.5		3030.9	

df, degrees of freedom; $^{*}P < 0.05$; $^{**}P < 0.01$

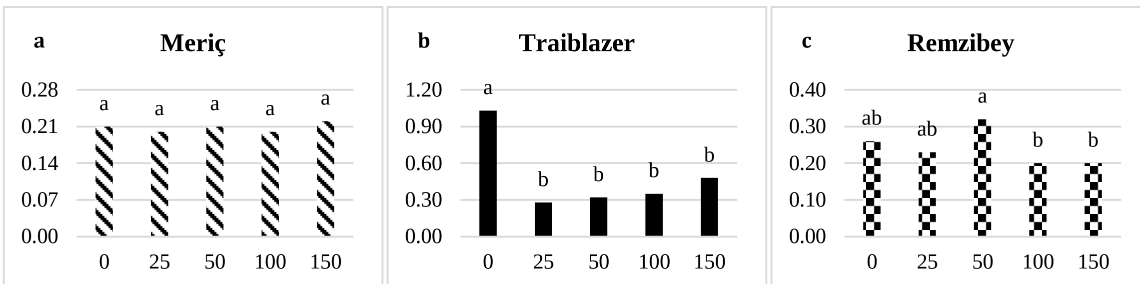

Fig. 3: Root/shoot of plants (**y**) in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

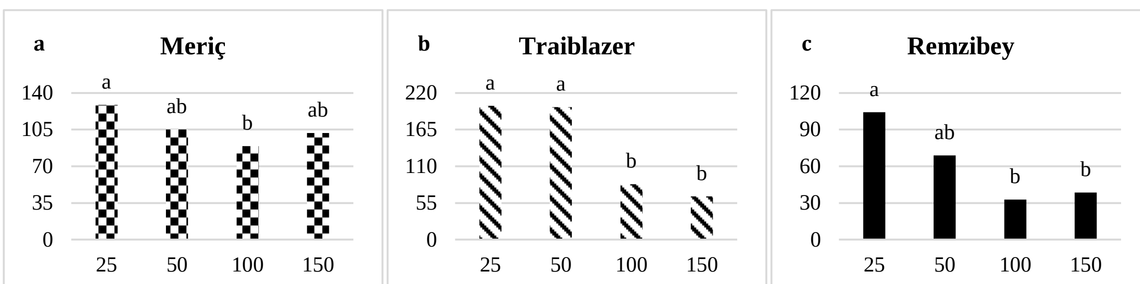

Fig. 4: Shoot TI (%) of plants (**y**) in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

and 29.2% for Trailblazer (Fig. 5B), and between 92.5 and 25.8% for Remzibey-05 (Fig. 5C).

The Pb contents of all varieties in various Pb concentrations were found significant (Table 2). For all varieties, the highest Pb content both in shoot and root were recorded in 150 mg/kg Pb treatment (Fig. 6A, B and C; Fig. 7A, B and C). In all Pb concentrations, the root and shoot Pb content of Trailblazer were found higher than those of other two varieties. The ranks of shoot Pb content of varieties occurred as Trailblazer>Meriç>Remzibey-05, whereas their rank of root Pb content as Trailblazer>Remzibey-05>Meriç.

BCF and TF of Plants in Pb Treatments

The effect of Pb treatments on all plants' root and shoot BCF and TF values was found significant (Table 3). The highest shoot BCF values (28.5 and 29.1%, respectively) were observed on Meriç variety at 25 and 50 mg/kg Pb treatments, while the lowest shoot BCF values (20.6 and 21.1%, respectively) were determined in 100 and 150 mg/kg Pb treatments (Fig. 8A). In Trailblazer treatments, 150 mg/kg Pb treatment led to highest shoot BCF value (62.1%), while the lowest value (47.6%) was obtained from 100 mg/kg Pb treatment (Fig. 8B). The highest shoot BCF values were determined as 29.9 and 30.9%, at 100 and 150 mg/kg Pb concentrations, respectively, whereas the lowest values as 25.9 and 24.6% at 25 and 50 mg/kg Pb concentrations, respectively in Remzibey-05 variety (Fig. 8C). In all varieties, it was established that as the Pb concentration increased, the root BCF values also increased. The root BCF values ranged between 283.1 and 1145.9% at Meriç; between 2759.7 and 18669.9% at Trailblazer; and

Table 3: Analysis of variance of BCF (%) and TF (%) values of Meriç, Trailblazer and Remzibey-05 exposed to different Pb concentration*

Source of variation		BCF (%) shoot		BCF (%) root		TF (%)	
	df	Mean squares	F	Mean squares	F	Mean squares	F
Meriç							
Lead	3	67.7	29.5**	529689.1	185.0**	68.40	153.4**
Error	8	2.1		2863.1		0.50	
Trailblazer							
Lead	3	105.2	6.9*	170870710.6	99.4**	7.90	299.8**
Error	8	15.1		1718787.6		0.03	
Remzibey-05							
Lead	3	27.5	7.2*	153768.9	70.4**	8.00	20.8**
Error	8	3.8		2183.5		0.40	

df: degrees of freedom; ns, non-significant; *P< 0.05; **P<0.01

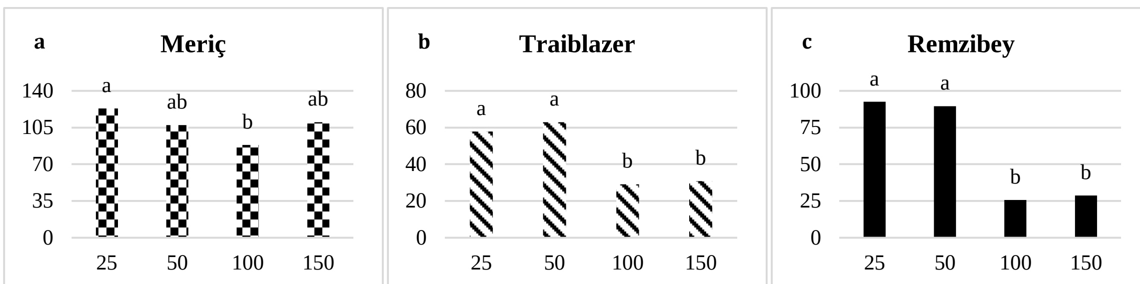

Fig. 5: Root TI (%) of plants (**y**) in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

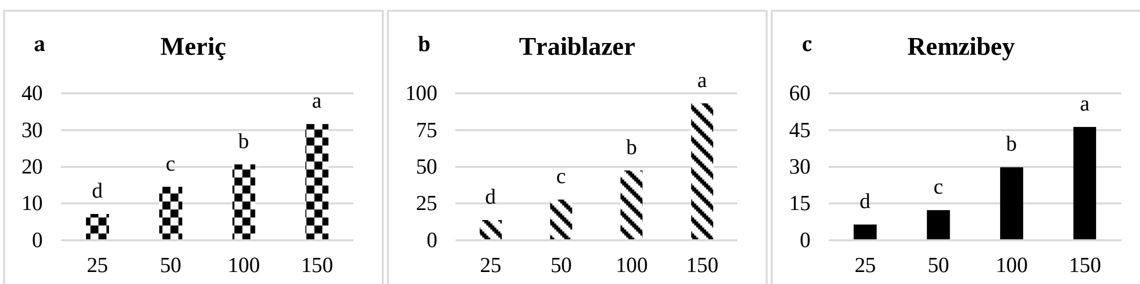

Fig. 6: Shoot Pb content (mg/kg) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

between 375.4 and 890.5% at Remzibey-05 (Fig. 9A, B and C). At Meriç variety, the highest (12.4%) and the lowest (1.9%) TF values were determined from 50 mg/kg and 150 mg/kg Pb treatments, respectively (Fig. 10A). At all varieties, it was observed that the translocation of Pb from roots to shoots decreased as the Pb concentrations increased. At Trailblazer, the highest TF value (4.0%) was obtained in 25 mg/kg Pb treatment, while; the lowest value (0.3%) was obtained in 150 mg/kg Pb treatment (Fig. 10B). While the highest TF (6.9%) was determined in 25 mg/kg Pb treatment for Remzibey-05, other Pb treatments, not having any statistical difference, were found to be in the same Duncan group with TF values ranking between 3.5 through 3.9% (Fig. 10C). Trailblazer variety was the least effective variety; in Pb transfer from root the shoots.

Discussion

Biomass can indirectly refer to plants' tolerance to metal toxicity. Species having high biomass are more suitable for remediation of metal contaminated soils (Papoyan and Kochian, 2004). In this study, Meriç (sunflower) has the highest biomass and its biomass in increasing Pb concentrations had been greater when compared to other plants included in this study. Generally, as the toxic metals in the area increase, plant biomass decreases. It was stated that the effect of the higher concentrations of phytotoxic metals on plant tissues was more hazardous than the effect of the lower concentrations (Niu *et al.*, 2007). In previous studies, it was found that low Pb concentrations as 5, 10, or 25 mg/L had positive stimulator effect on shoot biomass of

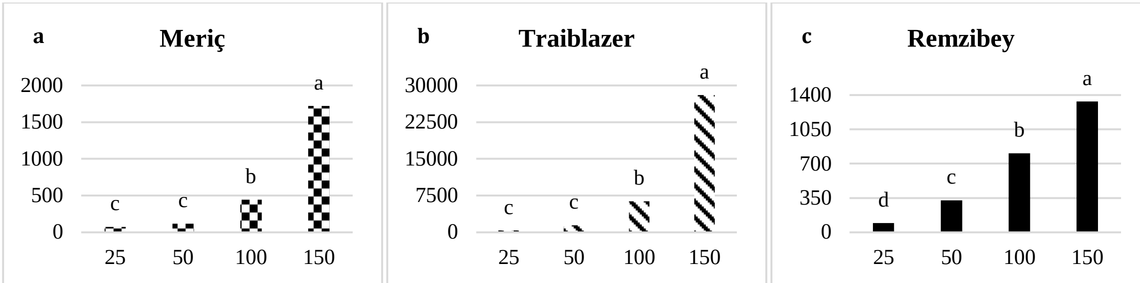

Fig. 7: Root Pb content (mg/kg) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

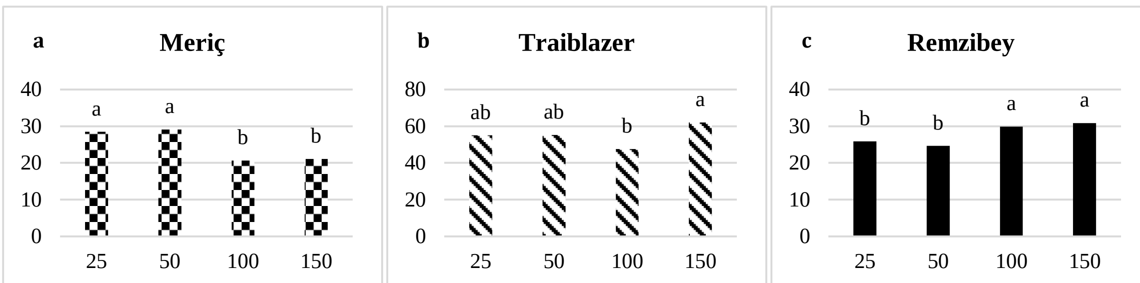

Fig. 8: Shoot BCF values (%) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

some plants (Liu *et al.*, 2000; Amer *et al.*, 2013). In our study, in 25 mg/kg Pb concentrations of Meriç and Remzibey-05 varieties and in 25 and 50 mg/kg Pb treatments of Trailblazer variety, an increase in especially shoot biomass has been found. Piechalak *et al.* (2002) reported that dry weight of *Vicia faba* had increased considerably in 331 mg/L Pb concentration, while *Pisum sativum* and *Phaseolus vulgaris* had not been a considerable difference from the control treatment in dry weight.

The root and shoot tolerance indices of Meriç against increasing Pb concentrations were determined to be higher than that of other varieties. It was found that increasing Pb concentrations affected root TI more than it did shoot TI. Shi and Cai (2009), in their study, stated that the sensitivity to heavy metals is distinct to varieties. Researchers reported that shoot were more sensitive to Cd than roots were. Different plant species can show altered reactions to different pollutants. It was determined that varieties' root Pb contents were higher than their shoot Pb contents. Roots were the first organs to contact with the metal ions in the solution. Metal ions were infiltrated through roots (Piechalak *et al.*, 2002). The continuous contact of roots with solution including metal ions caused increase in Pb content of roots (López-Chuken, 2012). Lead was bind to interchangeable zone ions at the cell wall and it is also accumulated as Pb carbonates in the extracellular areas (Sharma and Dubey, 2005). Pb has a strong binding ability to carboxil groups of galacturonic and glucuronik acids located in the cell wall, consequently, these fact limits the apoplastic transfer of Pb (Rudakova *et al.*, 1988). Manousaki and Kalogerakis (2009), in a study they conducted with *A. halimus*, reported that this plant's root was the main accumulation point of lead.

High BCF values show the plants' potential of accumulating great amount of metals (Chumbley and Unwin, 1982; Cui *et al.*, 2004). Niu *et al.* (2007) reported that BCF values change according to variety and concentration of heavy metals, plants' accumulation ability and their physiological characteristics and environmental conditions. Peer *et al.* (2006) reported that unless plant roots reached the saturation level of Pb, there would not be a transfer to shoot. At the same time, it was stated that as the metal concentrations in solution increases, the Pb transfer from roots to shoots would be more limited (Kabata-Pendias and Pendias, 2001). In over polluted areas, performing phytoextraction using hyperaccumulator plants requires a very long process (Ernst, 1996). Therefore, phytostabilization is a more suitable remediation method for over polluted areas; because metals are immobilized in roots, the aboveground parts of the crops produced could be utilized as hay (Arthur *et al.*, 2005).

Conclusion

Good phytoextraction plants have characteristics of producing high amounts of biomass, accumulating particular metal in high amounts with their developed root systems, and of growing rapidly. These plants well transfer the metals

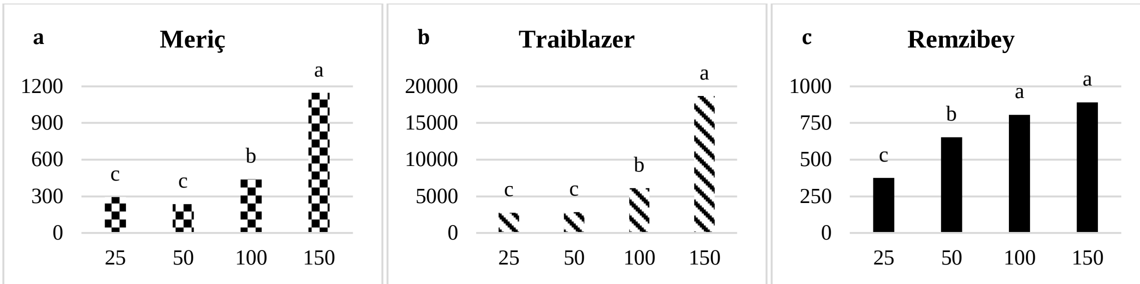

Fig. 9: Root BCF values (%) (**y**) of plants in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

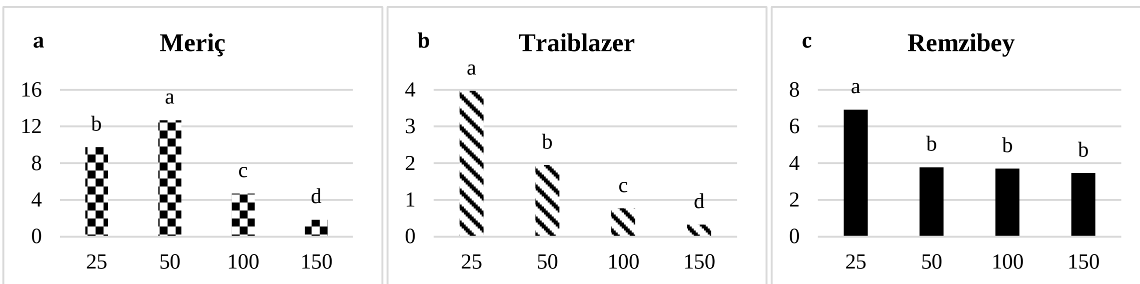

Fig. 10: TF values (%) of plants (**y**) in five Pb treatments (**χ**). Means with different letters within a column represent significant differences between values; Duncan test (P<0.05)

they had absorbed with their roots to aboveground parts. In our study, at Meriç and Remzibey-05 varieties, any decrease in neither root nor shoot biomass had been observed up to 50 mg/kg Pb treatments. For Meriç variety, even at highest level of Pb, there was not seen any significant decreases in growth. Trailblazer variety had absorbed Pb in their root part more than other varieties did. Trailblazer however, showed limited capability in transferring heavy metals from root to shoot, a significant criteria in phytoextraction, when compared to other varieties. In light of these findings, Meriç and Remzibey-05 varieties can be good candidates for phytoextraction in remediating Pb contaminated areas. On the other hand, because Trailblazer was more able to absorb Pb through its roots compared to other varieties, it can be considered as a good variety that would release significant results for studies of phytostabilization. Correspondingly, the performances these varieties in soil conditions should be evaluated according to the obtained results from this study conducted in hydroponic conditions.

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