Effects of Arbuscular Mycorrhiza on Pb Phytoextraction Capacity of Buckwheat in Contaminated Soil

Ayşen Akay and Zeynep Görkem Doğaroğlu

***Abstract*—There are many factor that affect the phytoextraction capacity such as plant species, soil nature and microbial activity, and environmental conditions. In this study, the effects of presence and absence of AMF on Pb pyhtoextraction capacity of two types of buckwheat (Güneş and Aktaş) was determined. Buckwheat plants were grown for 112 days under greenhouse conditions after the seeds were sown. 500 pieces' mycorrhiza spore was applied to per pots and plants were exposed to 0, 200, 400 and 800 mg Pb/kg during 90 days under AM (+) and AM (-) conditions. The plant height, shoot weight, flower weight and grain weight were measured in all pots to determine the effect of VAM on plants growth. Results showed that Pb accumulation in biomass and grain significantly increased at increasing Pb concentration in the absence of AM. Besides that, the Pb uptake was more in Güneş than Aktaş. The value of the active enrichment equation changed between 4.74 and 13.71. Also, after the pot experiments, the average remaining Pb content in soil was 72.79 mg/kg for Aktaş and 78.31 mg/kg for Güneş.**

***Keywords*— Buckwheat, Glomus mosseae, Lead, Mycorrhiza, Phytoremediation.**

I. INTRODUCTION

The presence of heavy metals in soil is one of the most important problem in the aquatic, terrestrial and atmospheric environment, which are negatively effects in ecosystems. Among the heavy metals, lead (Pb) that known as one of the most important toxic metal, has adverse effects in soil-plant systems even at low concentrations. It was reported in the last decade, the consistently increasing amount of lead in topsoil for different terrestrial environment [1]. The presence of Pb in soil commonly results from different anthropogenic activities, such as mining, smelting, sewage sludge, production of agricultural chemicals (e.g. fertilizers and pesticides) [2,3].

Decontamination of Pb contaminated soil can be carried out by different physical, chemical or biological cleaning processes. The physical and chemical processes are expensive, not ecofriendly, may cause visual pollution, and also destroy the soil nature and integrity, as opposite of biological processes [2,4-6], also these physicochemical processes are not completely safe and satisfactory [7]. Besides that, the biological processes can allow for recovery of metals from soil. The phytoextraction mechanism includes to absorb the heavy metals from soil and thus obtained contaminated soil by plants. The plants nature has a mechanism that the uptake and accumulate the nutrients, and this mechanism depends on the availability of metals, firstly [7]. For this reason, lead must be soluble and exchangeable for plants to successfully phytoextraction [8]. There are many factor that effect the phytoextraction capacity such as choosing ideal plant, soil nature (e.g. pH, texture), environmental conditions (e.g. climate), and may the organic or inorganic additives (e.g. EDTA, citric acid). Wallace et al. (1977) [9] and Freitas et al (2013) [10] reported that the presence of EDTA and citric acid respectively, increased Pb uptake. The bioavailability of Pb is generally influenced from microorganisms in soil rhizosphere. These microorganisms can enhance the availability of heavy metals for plants via changing heavy metal speciation and interaction of metal/organic matter [3]. There are different types of mycorrhizae that vary with the capacity of accessibility to organic and inorganic matters in soils. Arbuscular mycorrhiza (AM), one of these mycorrhizae, is formed by Glomeromycota species. This fungus species lives with plants which formed AM, as a mutualist. The plants, can form AM, if inorganic N and P nutrients are abundant in soils [11]. The plants species as many as 95% in the world are in mycorrhizal relationship with fungi, and it is known that the plants life depends on to this interaction. Thus, this symbiotic life between fungi and plants, is very important to plants life. When AM fungi (AMF) interact with an accumulator plant, they may transform the toxic heavy metals in plants root, grown in contaminated soil, to nontoxic forms [12], besides that they may help to plants for uptake of some nutrients (e.g. P, K, NO3-, Zn) [13].

Buckwheat is a plant that produces high-yield biomass and can adapt to large areas in the world [14]. Pirzadah et al (2015) suggested that buckwheat plant is a good model plant for phytoremediation processes due to having both external and internal resistance mechanisms [15]. Some researchers proved that the buckwheat excrete oxalic acid from their roots, and thus they show good resistance to toxic effects of heavy metals such as Al, and Pb [15-18]. However, the buckwheat plants have really good Pb phytoremediation capacity, this plant has not received the attention it deserves.

In many studies, it has been proved that the heavy metals not only accumulated in the roots of buckwheat but also they can translocate from roots to green parts of plants. In a study conducted with buckwheat, it was found that 4000 mg Pb/kg DW was accumulated, and this accumulation was not only in the roots but at the same time it was transported to ground surface part [19]. In another study with buckwheat, the Pb content and

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accumulation in shoots was reported as 1456.5 mg/kg DW and 233.0 mg/m2, respectively [20]. To evaluate the environmental risks of irrigating crops with treated wastewater, a study was undertaken to quantify heavy metal uptake by 4-week old buckwheat plants during 18 days of irrigation with 8 different Cu and Zn solutions under two transpiration rates. At the end of the study, researchers reported that the buckwheat roots contained the greatest levels of Cu and Zn, indicating their role in moderating heavy metal uptake [21].

In this study, it was evaluated that the effects of presence and absence of AMF on Pb uptake and Pb accumulation rates in grain and green parts of the two types of buckwheat plants.

II. Material and Methods

A. Experimental Setup

Pots experiments were conducted with soil collected from 0-30 cm depth at Selcuk University Agricultural Faculty Experiment area in Konya-Turkey. After fine stream sand and soil were mixed as the rate of 1:2 (W/W), the soil samples were passed through a 4 mm sieve. The pots were filled with 650 g sieved soil samples. The soil used in the experiments was analysed physically and chemically (sand, silt, clay and textural class) according to Bouyoucos (1951) [22]. Besides that, the other parameters of soil were determined via the Olsen sodium bicarbonate method [23], organic matter (%) [24], calcium carbonate by Scheibler calcimeter method [25], available Fe, Cu, Zn, Mn and Pb were determined as described by Lindsay and Norvell (1978) [26].

The extracted elements were analysed by AAS(Perkin Elmer Analyst 700 Model). The soil samples were characterized as soil classification as shown in Table I. Lead nitrate ($Pb(NO_3)_2$) was used as Pb source and it was added to the pots at different concentration of Pb (0, 200, 400, and 800 mg/kg). The pots were inoculated with mycorrhiza spores at a depth of 5 cm from the surface and these pots were represented as M(+) and the pots with absence of mycorrhiza were represented as M(-). 500 pieces' mycorrhiza spore (*Glomus intraradices*) was applied to per pots. After the 5 buckwheat seeds were sown in every pots, the pots were fertilized with 20 mg K_2O/kg in the form of K_2SO_4 and 32 mg P_2O_5/kg in the form of TSP. Two types of buckwheat seeds (*Fagopyrum esculentum* Güneş and Aktaş) were used which obtained from Bahri Dagdas International Agricultural Research Institute. Buckwheat seeds were sown and grown for 112 days under greenhouse conditions. During the experimental time, pots were watered daily with ultrapure water.

B. Observation of Plants Growth and Harvesting

The plant height, shoot weight, flower weight and grain weight were measured in all pots to determine the effect of vesicular-arbuscular mycorrhizal (VAM) on plant growth. For that plant height (cm) was measured from the soil surface to top of the plant. Then the plants were removed carefully from pots, they were cleaned and cut from the roots. Plants shoots, flower and grain were weighed.

C. Determination of VAM infection and counting spore

After harvesting, collected root samples were washed carefully with deionizer water and preserved in ethyl alcohol, as described by Koske and Gemma (1989) [27]. The percentage of root colonization was calculated by the gridline intersect method and, when the amount of roots was low, by the slide method. The percentage of AM colonization was calculated and examined under a stereo microscope at 40X magnification [28].

D. Lead content in plant and soil

0.2 g plant sample was dissolved in HNO3 on a hot plate. The samples were filtered and analyzed for Pb content in plants by AAS. In this study, all of the results are expressed on a dry weight basis. By the way, total Pb concentration of soil was determined by AAS after digestion with a mixture of HNO3/HClO4(1:4 v/v) [29].

E. Phytoextraction capacity

The phytoextraction capacity was found by multiplying the total amount of Pb (mg/kg) removed from the soil by the plant over-the-surface, surface part and grain and by the amount of soil around 250,000 kg in 1 da area. Enrichment coefficient (EC) is used to determine the degree of heavy metal accumulation of plants growing in contaminated soil [30,31]. Enrichment coefficient was determined as in Equation 1.

"Enrichment Coefficient " ("EC")"=Pb in Plants Shoot/Pb in Soil " (1)

Where the heavy metal element concentration (DM: dry matter) in plant above ground part expressed as Pb in plants shoot and the heavy metal element concentration (extractable in DTPA) (DM) in soil expressed as Pb in soil.

F. Statistical analysis

The data were statistically analysed using Minitab 16 and 18.

III. Result and Discussion

A. Physical and Chemical properties and Elemental Content of soil

The physical and chemical properties of soil are shown in Table I, that the soil sample is classified as loamy sand based on United States Department of Agriculture (USDA). The soil used in experiments contains very low organic substance percentage, sufficient P, Cu, Mn, and high amounts of Fe and Zn. It was known that the antagonistic effects between Zn and Pb inhibit the translocation of these both elements from root to other parts of plants and Pb-P interaction was reported that it may cause the insoluble P-formation in plants tissue and/or soil [1]. The total Pb content in the soil was 751.5 mg/kg. Kabata-Pendias and Pendias (1984) stated that that the toxic level of Pb for plants was not easy to specify but the threshold concentrations of Pb was given by many investigators in the range of 100-500 mg/kg [32]. The pH value and electrical conductivity were 8.79 as alkaline and 122.3, respectively.

TABLE I: THE PHYSICAL AND CHEMICAL PROPERTIES OF SOIL USED IN EXPERIMENTS

	Texture Class	$CaCO_3$ (%)	pH	Electrical Conductivity (µS/cm)	Organic Matter (%)	Field Capacity (%)
Soil Properties	Loam sand	23.93	8.79	122.3	0.87	27.34
	Fe (mg/kg)	**Cu (mg/kg)**	**Mn (mg/kg)**	**Zn (mg/kg)**	**P (mg/kg)**	**Total Pb (mg/kg)**
	13.53	2.99	18.32	2.78	18.40	751.5

B. Mycorrhizal colonization and its effects on plants biomass

There were no significant differences in plants height, hyphae enter the cells, mycorrhizal infection rate and flower-grain weight at the increasing Pb concentrations which were determined depending on buckwheat species and presence or absence of AM (Table II and III).

TABLE II: THE EFFECT OF DIFFERENT PB CONCENTRATIONS TREATMENT ON THE GROWTH OF AKTAŞ AND GÜNEŞ BUCKWHEAT TYPES

Plant Varieties	The Doses of Lead (mg/kg)	Plant Height (cm)	Hyphae Enter the Cells (%)	Mycorrhiza Infection Rates (%)	Plant Stem Weight (g)	Flower and Grain Weight (g)
AKTAŞ	0	29,9	25	28	7,61 B	0,21
	200	28,9	37	30	9,30 AB	0,30
	400	38,9	58	37	11,21 A	0,36
	800	29,8	68	27	9,50 AB	0,37
GÜNEŞ	0	34,0	40	52	8,69 B	0,28
	200	35,5	42	37	12,24 A	0,40
	400	37,2	53	48	12,85 A	0,34
	800	38,2	52	27	12,14 A	0,28
LSD Value P<0.01		ns-ns	ns-ns	ns-ns	ns-P<0.01	ns-ns

**Capital letters; It shows the statistical differences in the varieties

TABLE III: THE EFFECT OF MYCORRHIZAE ON THE GROWTH OF AKTAŞ AND GÜNEŞ BUCKWHEAT TYPES IN PB-CONTAMINATED SOIL

Plant Species	Mycorrhiza Application	Plant Height (cm)	Hyphae Enter the Cells (%)	Mycorrhiza Infection Rates (%)	Plant Stem Weight (g)	Flower and Grain Weight (g)
AKTAŞ	(+)	29,3	78	52	7,88 B	0,26
AKTAŞ	(-)	34,5	16	9	10,93 A	0,36
GÜNEŞ	(+)	34,7	79 A	56 A	11,19	0,31
GÜNEŞ	(-)	37,7	14 B	26 B	11,77	0,34
LSD Value P<0.05		ns-ns	ns-P<0.01	ns-P<0.01	ns-P<0.01	ns-ns

**Capital letters; It shows the statistical differences in the varieties

The effect of different lead concentrations in mycorrhiza inoculated buckwheat cultivars on the DTPA-Pb content remaining in the soil, Pb content in grain-flower and stem, the amount of Pb removed from the soil by grain and plant stem, phytoextraction capacity and active enrichment equality value are presented in Table IV. The examination table showed that, the DTPA-Pb content remaining in the soil after trial increased with the increased lead doses. These values ranged from 16.8 to 133.2 mg/kg. In the soil samples taken respectively from each pot following the pot trial, available Pb content was found to be 72.79 mg/kg on average in Aktaş cultivar, whereas it was 78.31 mg/kg in Güneş cultivar. Also, there were observed statistical differences between the interaction of mycorrhizal and Pb in Aktaş cultivar (P<0.01). On the other hand, Pb content of plant stem, Pb content of flower and grain, the amount of removed Pb from soil by plant stem and grain, phytoextraction capacity and also active enrichment equality was significantly changes depending on Pb concentrations on buckwheat varieties and presence or absence of AM (P<0.01) (Table IV).

TABLE IV: THE EFFECT OF DIFFERENT LEAD CONCENTRATIONS IN MYCORRHIZA INOCULATED BUCKWHEAT CULTIVARS ON PB CONTENT IN GRAIN- FLOWER AND STEM, THE AMOUNT OF PB REMOVED FROM THE SOIL BY GRAIN (RSG) AND PLANT STEM (RSS) (MG/KG SOIL), PHYTOEXTRACTION CAPACITY (KG/DA) AND ACTIVE ENRICHMENT EQUALITY

Plant Varieties	Mycorrhiza Application	The Doses of Lead (mg/kg)	DTPA Pb in Soil (mg/kg)	Flower and Grain Pb (mg/kg)	Plant Stem Pb (mg/kg)	RSG Pb (mg/kg soil)	RSS Pb (mg/kg soil)	Phytoextraction Capacity (kg/da)	Active Enrichment Equality
AKTAŞ	(+)	0	18,1 F	0,00 E h	248 F l	0,00 B	2,38 D	0,59 E	13,71 A
		200	55,5 E	0,31D efg	338 E ıj	0,23 B	4,38 CD	1,15 CDE	6,09 B
		400	86,3 CD	0,85 B cd	435 CD fg	0,39 B	6,59 BC	1,74 BCD	5,09 B
		800	110,6 AB	1,60 A b	631 B c	0,40 B	6,81 BC	1,80 BC	5,72 B
	(-)	0	16,8 F	0,00 E h	195 F m	0,00 B	2,68 CD	0,67 DE	11,68 A
		200	65,4 DE	0,58 C de	402 D gh	0,26 B	6,29 BCD	1,64 BCDE	6,16 B
		400	100,6 BC	0,94 B c	489 C e	0,64 B	9,49 B	2,53 B	4,86 B
		800	129,1 A	1,81 A b	753 A b	1,44 A	13,85 A	3,82 A	5,89 B
GÜNEŞ	(+)	0	21,2	0,00 h	283 F kl	0	3,68 E	0,92 E	13,36 A
		200	58,8	0,49 DE ef	365 E hı	0,24	6,94 CDE	1,79 CDE	6,21 BC
		400	98,8	0,87 CD cd	464 D ef	0,59	9,09 BCD	2,42 BCD	4,74 C
		800	124,5	1,73 B b	730 B b	0,75	12,51 B	3,31 B	5,86 BC
	(-)	0	27,7	0,17 EF gh	306 F jk	0,08	4,17 DE	1,063 E	11,19 A
		200	55,8	0,25 EF fgh	322 EF jk	0,19	6,01 DE	1,55 DE	5,90 BC
		400	106,5	1,07 C c	561 C d	0,40	11,20 BC	2,90 BC	5,26 BC
		800	133,2	2,24 A a	992 A a	0,95	20,05 A	5,253 A	7,48 B
LSD Value P<0.01(varieties)			ns	0,292-P<0.05	39,43-P<0.01	ns-ns	ns-P<0.01	ns-P<0.01	ns-P<0.01

* Lower case letters show the statistical differences between varieties **Capital letters; It shows the statistical differences in the varieties

Soil factors such as soil texture, pH, cation exchange, as well as plant factors such as root surface area, root exudates, transport rate of mycorrhizas affect plant Pb uptake [33]. Pb accumulation depends on the type of the plant, cultivars, plant organ, concentration of lead, and the presence of other ions in the environment. Pb transport is more effective in plant cultivars with high shoot/root Pb concentration. It was reported that the accumulation of Pb in *Thlaspi rotundifolium* plant is 130-8200 mg Pb/kg [34]. Plants with slow growth and low biomass are not suitable for phytoextraction of Pb from the contaminated soil [33]. When the soils contain high, potentially toxic amounts of heavy metals, mycorrhizal formation usually induces lower concentrations of these metals in the aerial part of the plant and consequently has a beneficial effect on plant growth [35,36]. In a study, it was found that buckwheat could accumulate 1500 mg kg-1 DW of Pb in leaves and removed about 200 mg of Pb per m^2 from the soil [20]. In another study, it was determined that buckwheat accumulated 4000 mg Pb/kg [19]. It was observed that buckwheat accumulated some heavy metals within itself in various previous phytoremediation studies, but there is not enough information about the translocation of heavy metals to the grain. In this study, in order to control the transition of Pb to the grain, buckwheat was grown in soil contaminated with Pb at different concentrations. Pb content in flower-grain (mg/kg) increased statistically significant with applied lead doses between both varieties and also within the varieties (P<0.01 and P<0.05). Values are between 0.00-2.24 mg/kg. Mycorrhizal inoculation again decreased grain's Pb content.

A similar situation was also true for plant stem Pb content. Stem Pb content was between 195-992 mg/kg, which was considerably higher than that of the grain-flower component. There was a significant difference between the species also in terms of plant stem Pb content (P<0.01). While average Pb content was 436 mg/kg in Aktaş species, it was 503 mg/kg in Güneş species. Average Pb content was 437 mg/kg in mycorrhizae-inoculated plants, whereas it was 503 mg/kg in plants that were not subjected to mycorrhizal inoculation. Stem Pb content showed a statistically significant increase with increasing doses of lead. The highest average Pb content was observed as 992 mg/kg in the concentration of 800 mg Pb/kg at Güneş variety. Besides, lead content was higher in applications without mycorrhizal inoculation compared to the others. In this case, it can be said that plant growth and development measured through phenological observations were found to be better in mycorrhizae-inoculated plants compared to non-mycorrhizal plants due to the fact that mycorrhizal inoculation decreased the Pb intake in these plants and the plants became more resistant. In Japan, the Pb content in BW was 0.36 mg/kg on average [37]; for various plants, the Pb content in the grass at the roadside was changed to 67-950 mg/kg in Sweden [38]. The total Pb concentration of the wheat plant grown in dirty soil having 861 and 2400 mg/kg Pb content was 1660-1820 mg/kg DW in the body part [39].

The content of lead taken from the soil by the grain and the surface part of plants increased with increasing Pb doses (P<0.01) (Tablo IV). The Pb taken from the soil was less in mycorrhiza inoculation doses compared to not inoculated ones; mycorrhiza inoculation negatively affected this situation. In other words, plants having mycorrhiza inoculation removed less Pb from the soil compared to those without mycorrhiza. In addition, Pb intake from the soil in Güneş cultivar was more compared to Aktaş cultivar; while the amount of Pb taken from the soil (RSS) was 13.85 mg/kg in the case of 800 mg Pb/kg soil application in the Aktaş cultivar, the Pb uptake in Güneş cultivar was 20.05 mg/kg in the same application.

The phytoextraction capacity was also higher in Güneş cultivar than those in Aktaş buckwheat cultivar. Phytoextraction capacity in Güneş cultivar was 0.92 and 1.06 kg Pb/da in control application and 5.25 kg/da at the concentration of 800 mg Pb/kg in the treatment of AM(-). In Aktaş cultivar, it was 0.59 and 0.67 kg Pb/da in control application while the highest value was 3.82 kg/da in the case of at concentration of 800 mg Pb/kg in the

treatment of AM(-). This value showed a significant increase with increasing lead doses (P<0.01) (Table IV).

Enrichment coefficient is used as an important criterion to assess the degree of accumulation of metals in the roots and shoots of the plants with respect to their concentration in the growing medium. The value of the active enrichment equation, i.e. the proportion of the lead in the form that can be taken from the soil with the plant body, changed between 4.74 and 13.71. The rate of activation, which is high in those with mycorrhizal inoculation and control applications, decreased with increasing doses of Pb, but the values were not found to be statistically significant (Table IV). This rate was significantly reduced with increasing doses of Pb (P<0.01) (Table IV-V).

TABLE V. THE EFFECT OF DIFFERENT CONCENTRATIONS OF PB ON BUCKWHEAT ON PB CONTENT FROM THE SOIL BY STEM, PHYTOEXTRACTION CAPACITY AND ACTIVE ENRICHMENT EQUILIBRIUM

Concentrations of Lead (mg/kg)	RSS Pb (mg/kg soil)	Phytoextraction Capacity (kg Pb/da)	Active Enrichment Equilibrium
0	0,43 c	0,81 d	12,48 a
200	0,62 b	1,53 c	6,09 b
400	0,79 a	2,40 b	4,99 c
800	0,86 a	3,55 a	6,24 b
LSD Value P<0.01	0,147	0,47	0,968

In a study conducted by Kumar et al (2013) in order to determine the heavy metal accumulation potential of twelve native weed, all the plants were found to have an enrichment coefficient greater than 1, which reflects their high metal accumulation potential [31]. EC was ranged from 1.105 (Cd in *S. nigrum*) to 609.157 (Cr in *T. procumbens*) and from 0.866 (Cd in *S. xanthocarpum*) to 1130.372 (Cr in *T. procumbens*) in roots and shoots of studied plant cultivars, respectively.

IV. CONCLUSION

Significant differences were observed between the buckwheat cultivars in terms of the examined characteristics. When the general mean values were taken into account, the Pb content taken from the soil by plant stem was higher in Güneş cultivar than Aktas cultivar. This value was 13.85 mg Pb/kg in the Aktaş cultivar at concentration of 800 mg Pb/kg whereas it was 20.05 mg Pb/kg at the same concentration in Güneş cultivar. Also it was 6.81 mg Pb/kg in the Aktaş cultivar and 12.51 mg Pb/kg in Güneş cultivar at the concentration of 800 mg /kg Pb with inoculated mycorrhiza. It was determined that the amount of Pb taken from the soil by the plant stem was far higher than Pb which taken from soil by the grain. Although AM inoculation positively affects the development of the plant, it has a negative effect on plant Pb uptake. AM inoculation decreased the amount of Pb taken from the soil by the plant stem and the grain. The plant's root spreads over a larger area in the root zone, also with the effect of the hyphae that enter the root zone of the plant and utilizes the plant nutrient elements, and thus the plant becomes selective and decreases Pb uptake. In both buckwheat cultivars, it was observed that AM hyphae entry was through the roots and inoculation occurred. Therefore, it was concluded that the buckwheat was a plant that could receive mycorrhizal inoculation. By the way, the content of Pb in the grain was also very low and it changed between 0.00-2.24 mg/kg DM.

Consequently, the buckwheat plant is the candidate hyperaccumulator plant for Pb, however, the AM inoculation was not suitable to enhance the phytoremediation capacity of buckwheat.

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