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Phytoremediation potential of Atriplex canescens (Pursh) Nutt and Nicotiana tabacum grown in heavy metal contaminated soil

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Abstract
In the greenhouse experiment Atriplex canescens (Pursh) Nutt and tobacco (Nicotiana tabacum) plants were treated with heavy metals to estimate extraction capacity of heavy metals from soil. In the greenhouse experiment increasing levels of heavy metals (till to ten fold of limit values of Zn, Cu, Ni, Pb and Cd) were applied experimental soil and saltbush and tobacco plant were grown. Dry matter amounts of both plant were not changed and no phytotoxicity was observed by the metal applications even at the highest levels. Results showed that increasing applications of heavy metals to experimental soil led to increase both in Atriplex canescens and tobacco plants. Heavy metal concentration, heavy metal bioconcentration factor and metal uptake amount both in Atriplex canescens and tobacco were increased by the increasing applications of heavy metals to experimental soil. All heavy metal concentration, heavy metal bioconcentration factor and metal uptake amount of tobaco plant were found higher than Atriplex canescens plant. Phytoextraction efficiency rate both in Atriplex canescens and tobacco were decreased by increasing applications of heavy metals. Phytoextraction efficiency rate of Cd element both in Atriplex canescens and tobacco was found the highest in control treatment. Results showed that both plant could be used in phytoremediation studies with their high biomass and high metal bioconcentration factor properties.
Keywords: *Atriplex canescens, Tobacco, Heavy Metals, Phytoremediation*

Introduction

Heavy metals are of considerable concern due to their toxicity, wide sources, non-biodegradable properties and accumulative behaviours [1]. Heavy metal pollution of agricultural soil is one of the most serious environmental problems and has significant detrimental effects on human health. Due to intensive use of agrochemicals in agricultural soils, some industrial activities heavy metals are become to common pollutants in agricultural soils and adjacent environment. Although some engineering techniques may efficiently be used to clean up the contaminated soils, most of them are expensive and sophisticated technologies, and they used for small scale contaminated areas [2].

Recent years, as an alternative to sophisticated traditional technologies for soil remediation phytoremediation has been highlighted for the efficient and economic removal of heavy metals from soil. In the phytoremediation studies natural hyperaccumulator plants with exceptional metal

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accumulating capacity, and high-biomass plants accumulating relatively high amounts of the metals are used. However, there are some difficulties for natural plants such as that hyperaccumulator plants are usually accumulate only a specific element, tended to grow slowly and to have a low biomass [3, 4]. The main strategy for the phytoremediation is to detect plants from nature those have a high biomass and metal hyperaccumulating properties.

Atriplex canescens are halophyte species and adapted excess saline soil conditions in arid regions. Fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.)) has been especially recommended for revegetation of mine sites and
other harsh environments [5]. On the other hand it is reported that many hyperaccumulator characteristics with high biomass were found in the tobacco plant, *Nicotiana tabacum* [6].
The aim of this study was to estimate and to compare phytoremediation efficiency of Atriplex canescens and Tobacco as phytoremediation plant in greenhouse controlled conditions.

Materials and Methods

A greenhouse pot experiment was carried out to research and compare the possibility of using Atriplex canescens (Pursh) Nutt and Nicotiana tabacum as phytoremediation. The seed of *Atriplex canescens* (Pursh) Nutt were obtained from the region of El Bayedh, Algeria. Tobacco seeds (Nicotiana tabacum L. Çelikhan var.) which cultivated commanly in southeast of Turkey were obtained from of Adıyaman region, Turkey. Seeds were disinfected by sodium hypochlorite solution of 5 % during a few minutes and then rinsed in the distilled water before sowing to soil.

A red mediterranean soil was used in the experiment. Soil was air dryed , siewed by 2 mm then mixtered by perlite at the rate of 30 percent and 20 % peat to maintain slighty texture in the pot medium. Analytical characteristics of experimental soil was presented in Table 1.

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

Parameters		Metal limits in soil, mg kg^{-1} dry wt [7]
Texture	Loam	
pH- H_2O (1:5 w/v)	7.25	
$CaCO_3$, %	5,26	
Organic matter, %	8.25	
Clay,%	6,5	
CEC, cmol kg^{-1}	18,4	
EC, dS m^{-1} 25°C	0,65	
Total N, %	1.28	
P (ex), mg kg^{-1}	8.7	
K (ex), mg kg^{-1}	74	
Ca (ex), mg kg^{-1}	658	
Mg (ex), mg kg^{-1}	124	
Zn, mg kg^{-1}	65.2[1]	150-300
Cu, mg kg^{-1}	10,5	50-140
Ni, mg kg^{-1}	7,8	30-75
Pb, mg kg^{-1}	22,6	50-300
Cd, mg kg^{-1}	0,01	1-3

[1]:Total concentrations,

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Physical and chemical characteristics of greenhouse soil mixture studied before the experiment are well within the accepted normal range of agronomic values, and the heavy metal concentrations are below the levels indicated by the EU [7].

Basic N-P-K fertilization was applied to experimental soil at the rate of 100, 50 and 100 kg ha^{-1} of 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 experimental soil as metalic salt solutions (as $Zn(NO_3)_2$, $CuSO_4$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$, respetively) as in Table 2. Metal concentrations were designed to maintain beginning from maximum till to 10 fold of maximum metal limits of European Union [7]. Soil and metals were incubated field capacity of water at least 3 months before experiment.

Table 2. Heavy metal treatment levels of experiment

Metals	Metal treatments, mg kg^{-1}				
	Control	1	2	3	4
Zn	0	300	750	1500	3000
Cu	0	140	350	700	1400
Ni	0	75	250	500	750
Pb	0	300	1000	2000	3000
Cd	0	3	10	20	30

A factorial experiment was conducted in randomized complete block design including 2 plants and 5 levels of heavy metals with 5 replications. The Seeds were germinated in peat+perlite substrate mixture. Then, 3 seedlings of each plant were transplanted in every pot containing 10 kg soil. During the experiment, the plants were watered regularly and treated according to common agrotechnical principles. After 60 days of growth all plants were harvested from soil surface. Plant samples were dried at 60 ºC in a forced-air oven, ground with agitate mortar and then digested in aqua regia (1:3 HNO_3/HCl). Total metal concentrations were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

Evaluation parameters and statistical analysis

Heavy Metal Transfer (or Bioconcentration) Factor: oil-to-plant transfer is one of the key components of human exposure to metals through food chain. Heavy metal transfer factor (TF) is a parameter used to describe the transfer of heavy metals from soil to plant body. The TF of metals in the soil to the aerial part of the plants was defined as the ratio of the heavy metal concentration in the plants to that in the soil [8].

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows [9]:

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

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 [10]:

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

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

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Theoretical phytoextraction efficiency (%) of harvested plants was calculated using Eq. 3, as follows [11]:

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

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}

One-way ANOVA test ($p \leq 0.05$) calculated using the statistical package SPSS-16 for Windows program were applied to compare the differences in heavy metal concentrations in crops and in evaluation parameters.

Results and Discussion

Plant growth and heavy metal concentration of plants: Both plants growth were not changed, and also no phytotoxicity was observed by the treatments (Table 3 and Table 4). This shows that both plants are well adapted to stress conditions, even to ten fold of maximum soil metal concentration limits. It is reported that Atriplex species have an excellent tolerance to drought and salinity and therefore these species are good candidates for phytodesalination and phytoremediation of soils [12, 13]. However, Zn, Cu, Ni, Pb and Cd concentration in both plants were increased by the increasing amounts of metal treatments. Heavy metal concentration of Tobacco plant was determined higher in all treatments than Atriplex canescens. In both plant Cd was relatively the highest accumulating metal compared to control treatments.

However, some reports indicated that metals accumulated by Atriplex were mostly distributed in root tissues, and the increased concentration of heavy metals in soil led to increases in heavy metal shoot and root concentrations of Ni, Cu, Pb and Zn in plants as compared to those grown on unpolluted soil. [14].

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

Treatments	Dry matter	Zn	Cu	Ni	Pb	Cd
Control	131,4	27,37	3,213	0,842	1,535	0,416
1	137,5	130,84	4,063	2,687	10,864	1,034
2	125,6	104,32	7,595	2,704	21,893	6,503
3	132,5	216,39	9,017	9,436	31,118	5,620
4	129,4	490,31	16,011	9,454	28,511	8,717

Table 4. Dry matter (g pot^{-1}) and heavy metal concentration (mg kg^{-1}) of tobacco plant

Treatments	Dry matter	Zn	Cu	Ni	Pb	Cd
Control	152,3	37,73	5,357	0,559	3,142	1,050
1	157,6	177,05	6,678	6,456	8,709	5,811
2	165,4	385,09	11,679	10,529	11,857	12,363
3	148,4	383,23	18,319	11,050	17,577	20,371
4	150,2	517,21	20,820	20,910	33,218	23,446

Metal transfer factor (TF) of plants: Metal TF of both plants were increased by increasing amounts of treatments (Table 5 and Table 6). Metal TF of Tobacco plant were determined higher in all treatments than Atriplex canescens. Metal TF of Cd was determined at the highest rate for Cd. Tobacco plant has the hishest TF rate than Atriplex canescens. This indiactes that both plant have adapted to accumulate heavy metals without any physiological disorder in natural conditions.

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Table 5. Metal transfer factor of atriplex canescens plant

Treatments	Zn	Cu	Ni	Pb	Cd
Control	0,35	0,23	0,07	0,07	20,85
1	1,68	0,29	0,22	0,49	51,72
2	1,34	0,54	0,23	1,00	325,15
3	2,77	0,64	0,79	1,41	281,00
4	6,29	1,14	0,79	1,30	458,85

Table 6. Metal transfer factor of tobacco plant

Treatments	Zn	Cu	Ni	Pb	Cd
Control	0,48	0,38	0,05	0,14	52,55
1	2,27	0,48	0,54	0,40	290,50
2	4,94	0,83	0,88	0,54	618,10
3	4,91	1,31	0,92	0,80	1018,55
4	6,63	1,49	1,74	1,51	1172,35

Metal uptake of plants

Metal uptake amount of both plants were increased by the treatments. Metal uptake of Tobacco was found higher than Atriplex canescens (Table 7). Total metal uptake amount was determined highest for Zn metal in both plant. Metal uptake rate of Zn and Cd were increased about 20 fold by the treatments compared to control. In all treatments metal uptake amount was determined for metals in Zn>Pb>Cu>Ni>Cd order.

Table 7. Metal uptake of atriplex canescens and tobacco plants, mg pot^{-1}

Treatments	Zn		Cu		Ni		Pb		Cd	
	Atriplex	Tobacco	Atriplex	Tobacco	Atriplex	Tobacco	Atriplex	Tobacco	Atriplex	Tobacco
Control	3,597	5,746	0,422	0,816	0,111	0,085	0,202	0,479	0,055	0,160
1	17,992	27,903	0,559	1,052	0,369	1,017	1,494	1,373	0,142	0,916
2	13,103	63,695	0,954	1,932	0,340	1,741	2,750	1,961	0,817	2,045
3	28,672	56,871	1,195	2,719	1,250	1,640	4,123	2,608	0,745	3,023
4	63,447	77,686	2,072	3,127	1,223	3,141	3,689	4,989	1,128	3,522

Phytoextraction efficiency (PE) of plants

PE rates of both plant were decreased by the treatments (Table 8 and Table 9). At control treatment Cd metal has the highest rate of PE value in both plant. In all treatments Cd has the highest PE values. PE values determined at the higher rates for Tobacco. This indicates that Tobacco and Atriplex canescens has the ability of phytoextraction especially for Cd metal in soil remediation. Recent reports suggest that halophyte species could be more suitable for heavy metal extraction than glycophytes most frequently used so far [15]. Similar results were reported that tobacco plant has the potential to allow cultivation of Cd contaminated farmland to assist in lowering total Cd content of soil [10].

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Table 8. Phytoextraction efficiency of atriplex canescens plant, %

Treatments	Zn	Cu	Ni	Pb	Cd
Control	0,535	0,350	0,107	0,097	31,678
1	0,546	0,042	0,049	0,053	0,540
2	0,208	0,035	0,017	0,035	1,073
3	0,204	0,019	0,027	0,023	0,417
4	0,239	0,017	0,019	0,014	0,436

Table 9. Phytoextraction efficiency of tobacco plant, %

Treatments	Zn	Cu	Ni	Pb	Cd
Control	0,737	0,583	0,071	0,199	79,958
1	0,738	0,068	0,117	0,042	3,032
2	0,769	0,053	0,066	0,019	2,041
3	0,360	0,038	0,032	0,013	1,510
4	0,252	0,022	0,041	0,016	1,173

Conclusion

Results showed that both Atriplex canescens and Tobaco plants are well adapted to heavy metal stress conditions. Both of plants has the ability of high metal accumulation, metal transfer from soil and especially high Cd phytoextraction efficiency. Tobacco plants has the higher values of metal concentrations, metal transfer factor, metal uptake and metal phytoextraction efficiency. However, due to well adaptation of atriplex canescens to saline soil conditions, this plant also could be used in phytoremediation studies in excessively contaminated soils.

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