The Effects of Humic Acid on the Phytoremediation Efficiency in the Sludge Applied Soil

Bülent Topcuoğlu

Abstract**—** A greenhouse pot experiment was carried out to research the effects of humic acids (HA) on the phytoremediation efficiency of Atriplex canescens (Pursh) Nutt in sludge applied soil. In a factorial experimental design, Atriplex canescens plant was grown in soil treated with sewage sludge and HA. Plant metal (Zn, Cu, Ni, Pb and Cd) concentrations, metal uptake, metal transfer factor and phytopremediation efficiency were determined. Sewage sludge treatments increased the metal concentration in *Atriplex canescens* plants. Humic acid applications increased plant shoot and root biomass, metal accumulation, metal transfer factor, metal uptake and metal phytoextraction efficiency in *Atriplex canescens* plants. *Atriplex canescens* showed a high phytoextraction efficiency especially for Cd and Zn. The results showed that *Atriplex canescens* plant had particularly high Cd and Zn phytoextraction efficiency.

Keywords**—** Sludge; Humic acid; Phytoremediation; Atriplex canescens.

I. INTRODUCTION

Heavy Metals are recognized as one of the most important contaminants in agricultural soils due to their toxic effects, widespread sources, non-biodegradable properties and increasing accumulation in agricultural fields. Sewage sludge can contain significant levels of heavy metals depending on their emission sources, which significantly limits their use as an organic fertilizer material in agricultural soils.

In recent years, as an alternative to traditional technologies for soil remediation, the importance of phytoremediation technique for effective and economical removal of heavy metals from soil has been emphasized. [1]. *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 soil remediation, erosion control, revegetation of mine sites and other harsh environments [2].

The bioavailability of metals in soil is affected by numerous factors, such as cation exchange capacity, pH values of the soil, excess amounts of fertilizers, and chelators. The term humic substances refers to a category of naturally occurring organic materials result from the decomposition of plant and animal residues [3]. Humic acids (HA) contain acidic groups such as carboxyl and phenolic OH functional groups [9] and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals and can be used in phytoremediation [4].

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The aim of this research was to assess the ability of HA on bioavailability and phytoextraction of heavy metals from sludge applied soil by the use of Atriplex canescens plant under greenhouse conditions.

II. MATERIALS AND METHOD

A. Soil Charactarization and Analysis

In this experiment an uncontaminated soil was sampled from a red mediterranean soil, representative of the major agricultural areas of Turkey Antalya Aksu. Experimental soil was air dried, siewed by 2 mm then mixed by perlite at the rate of 30 percent and 20 % peat to maintain slight texture in the pot medium. The main analytical characteristics of the experimental soil material (pot medium) are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [5].

TABLE I: THE ANALYTICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL BEFORE APPLICATIONS

Parameters	
Texture Grade	Loam
pH- H_2O (1:5 w/v)	7.15
$CaCO_3$, %	1,25
Organic matter, %	5,32
Clay,%	6,5
CEC, cmol kg^{-1}	24,3
EC, dS m^{-1} 25°C	0,65
Total Zn, mg kg^{-1}	47,3 (150-300)*
Total Cu, mg kg^{-1}	14,2 (50-140)*
Total Ni, mg kg^{-1}	5,2 (30-75)*
Total Pb, mg kg^{-1}	11,2 (50-300)*
Total Cd, mg kg^{-1}	< 0,001 (1-3)*

*: Metal limits in soil, mg kg^{-1} dry wt [6]

Soil texture was determined by the hydrometer method, the soil pH was measured by the $CaCl_2$ method, organic matter content, as determined by the Walkley-Black method, $CaCO_3$ was determined by scheibler calcimeter, the total Zn, Cu, Ni, Pb and Cd contents of the soil were digested by the aqua regia method (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.

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

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metal concentrations are below the levels indicated by the EU [12].

B. Preparation of Humic Substances and Addition to soils

Leonardite is a low-rank coal with significant amounts of humic materials, mainly humic acids. Leonardite was treated with an aqueous solution of 0.5 M NaOH (1:5 w:v). The residue was further extracted two more times for 1 h by the same extraction solution. The supernatants were filtered through glass wool, combined, and brought to pH 1 with concentrated HCl and the precipitated HA allowed settling for 24 h. The precipitate was separated from the soluble fraction (fulvic acids) by centrifugation at 4000 rpm for 20 min, and washed 2-3 times with deionised water at a ratio of 1:3. The washed precipitate was transferred into a round bottom flask, freezed and lyophilised. The freeze-dried HA was suspended in water and then dissolved to pH 7 by adding 0.5 N NaOH stepwise. The humic acid solution was brought to volume in order to reach a final HA concentration of 25 mg ml^{-1}[7].

C. Experimental Design

A factorial experiment was conducted in randomized complete block design including 2 levels of humic acid and 4 levels of sewage sludge with 5 replications. Ten kilograms of air-dried and sieved soil were filled into plastic pots. A pot-plate was placed under each pot to prevent leaching. Basic N-P-K fertilization was applied to experimental soil at the rate of 50, 25 and 50 mg kg^{-1} of N (as NH_4NO_3), P (as KH_2PO_4) and K (as K_2SO_4) respectively to support plant growth.

HA were added in a solution form in order to raise the soil organic carbon by 0 (control, no HA added) and 1 % by weight. A uniform application was obtained by homogenization of the soil. The soil was subsequently incubated in the greenhouse for 8 weeks before experiment. During these 8 weeks the soil was irrigated 1-2 times a week with deionised water to maintain field capacitiy of water.

TABLE II. SLUDGE APPLICATION LEVELS OF EXPERIMENT

Treatments	Sludge applications, g pot^{-1}
Control	0
1	160
2	320
3	640

D. Plant growth and analysis

The seed of *Atriplex canescens* (Pursh) Nutt were obtained from the region of El Bayedh, Algeria. Seeds were disinfected by sodium hypochlorite solution of 5 % during a few minutes and then rinsed in the distilled water before sowing to soil. The Seeds were germinated in peat+perlite substrate mixture. Then, 3 seedlings of each plant were transplanted in every pot containing 10 kg soil. All *Atriplex canescens* (Pursh) Nutt plants were grown under greenhouse environmental conditions. During the experiment, the plants were watered regularly and treated according to common agrotechnical principles. After 60 days of growth all plants were harvested. Shoots and roots of plants samples were rinsed briefly in deionised water and 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).

After harvesting, soil samples were collected from each pot for above mentioned analysis. Total metal concentrations were both in plant and soil samples analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

E. Evaluation parameters and statistical Analysis

Heavy Metal Transfer (or Bioconcentration) Factor:

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows [8]:

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

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 [9]:

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

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

Phytoextraction efficiency (*PE*): PE value of harvested plants (shoot and root) was calculated using Eq. 3, as follows [10]:

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

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}

Statistical Analysis: 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.

III. RESULTS AND DISCUSSION

A. Plant growth and heavy metal concentration of plants

The effect of sludge treatments on the growth, shoot and root dry matter (DM) of*Atriplex canescens* plants was found to be statistically significant and also no phytotoxicity symptoms were observed with heavy metal treatments. (Figure 1). Shoot and root dry matter values of*Atriplex canescens* increased with the application of sewage sludge to the soil and the highest dry matter values were obtained at 640 g pot^{-1} treatment level.

Total metal concentrations with the exception of Pb both in the shoots and roots of plant were increased by the increasing amounts of sludge treatments (Figure 2, Figure 3). Heavy metal concentration of *Atriplex canescens* plant was determined higher in humic acid treatment than control (no humic acid application) treatment.

In both treatments Zn was relatively the highest accumulating metal. Metals accumulated both in shoots and roots in all treatments were followed as Zn>Cu>Pb>Ni>Cd. Metal concentrations of *Atriplex canescens* in the root tissues was found higher than that of shoots. Some reports indicate that metals deposited by Atriplex are mostly distributed in root tissues [11].

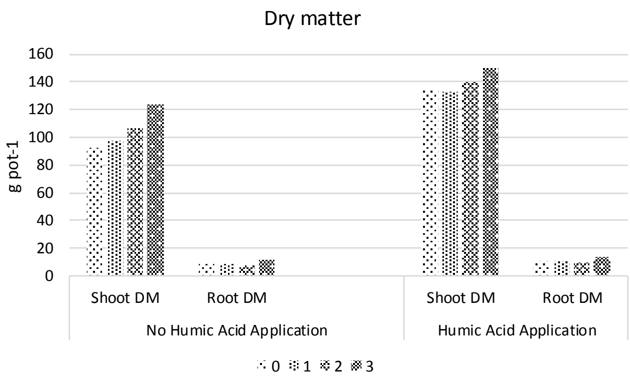

Fig. 1. Shoot and root dry matter of Atriplex canescens plant in sludge and humic acid treatments.

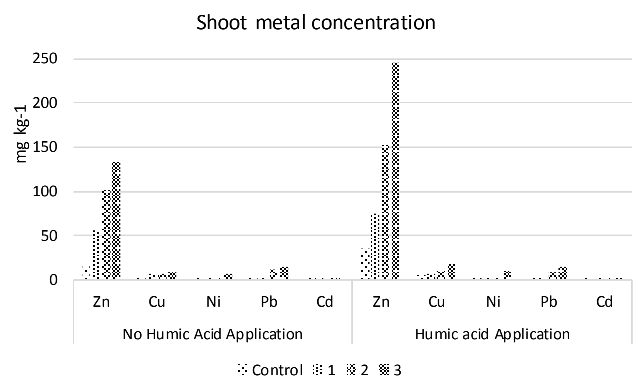

Fig. 2. Shoot heavy metal concentration of Atriplex canescens plant in sludge and humic acid treatments.

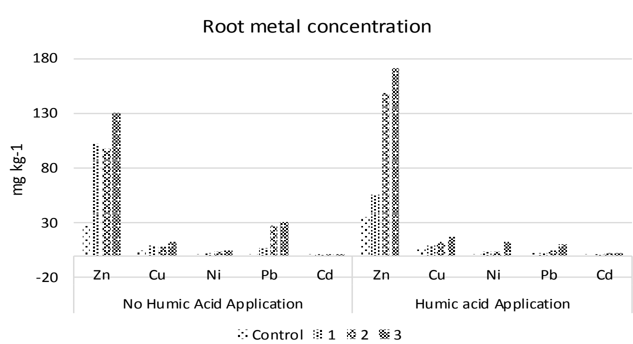

Fig. 3. Root heavy metal concentration of Atriplex canescens plant in sludge and humic acid treatments.

B. Metal transfer factor (TF) of plants

TF value of *Atriplex canescens* in both treatments were decreased by the increasing amounts of sludge treatments (Figure 4 and Figure 5). TF value of *Atriplex canescens* were determined higher in humic acid applications. TF value of *Atriplex canescens* was determined at the highest rate for Cd followed by Zn. TF value of metals in *Atriplex canescens* was followed Cd>Zn>Cu>Pb>Ni order. These results indicate that *Atriplex canescens* has a significant ability to accumulate Cd and that humic acid applications promote metal transfer to the plant by increasing the metal availability.

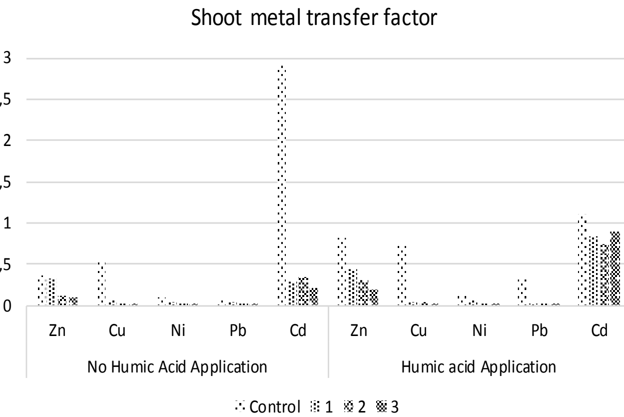

Fig. 4. Shoot metal transfer factor of Atriplex canescens plant in sludge and humic acid treatments.

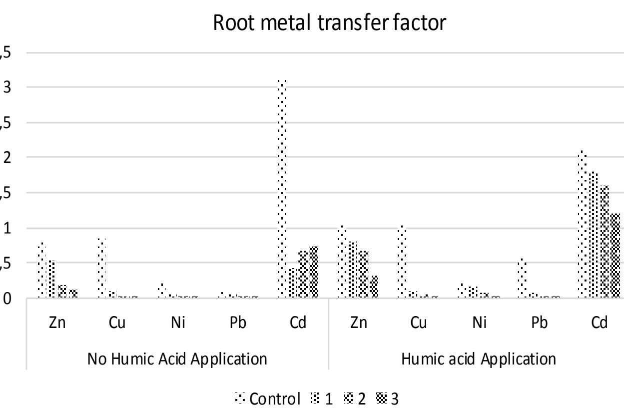

Fig. 5. Root metal transfer factor of Atriplex canescens plant in sludge and humic acid treatments.

C. Metal uptake of plants

Metal uptake (MU) amount of plants were increased by the increasing amounts of sludge applications. Metal uptake of Atriplex canescens treated with humic acid was found higher than control treatments (Figure 6 and Figure 7). Total metal uptake amount was determined as highest for Zn metal in both humic acid tretaments. Metal uptake rate of Zn was increased about 2 fold by the treatments compared to control. In all treatments metal uptake amount was determined for metals in Zn>Pb>Cu>Ni>Cd order.

D. Phytoextraction efficiency (PE) of plants

PE values of *Atriplex canescens* plant were decreased by the applications of increasing amounts of sludge (Figure 8). Cd metal has the highest rate of PE value in both control and humic acid treatments. PE values was determined at the higher rates for humic acid. This indicates that humic acid affect the ability of phytoextraction for all examined metals, especially for Cd and Zn metals in soil remediation.

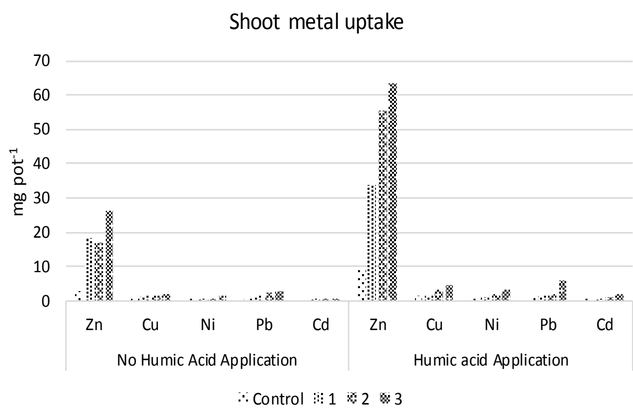

Fig. 6. Shoot metal uptake of Atriplex canescens plant in sludge and humic acid treatments.

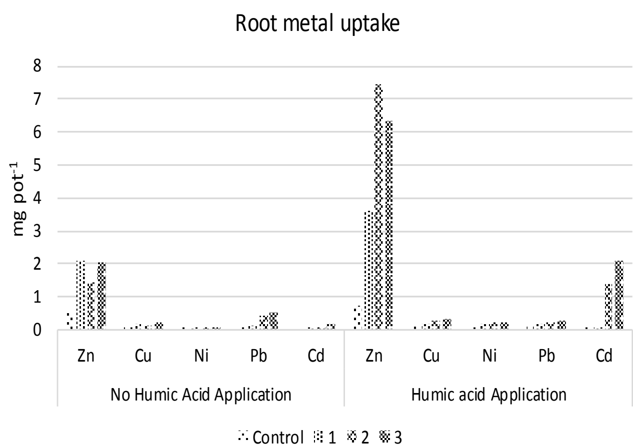

Fig. 7. Root metal uptake of Atriplex canescens plant in sludge and humic acid treatments.

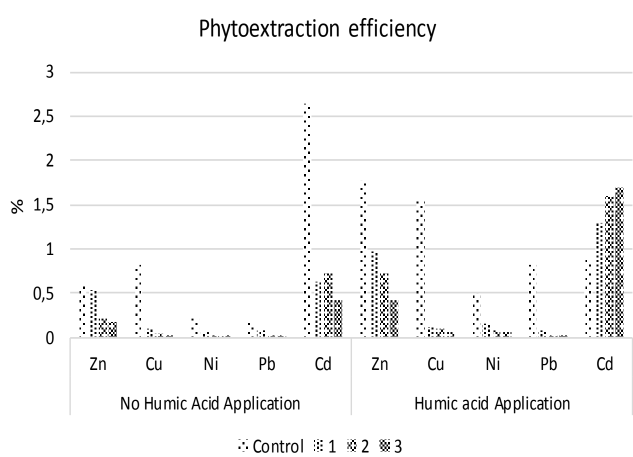

Fig. 8. Phytoextraction efficiency of Atriplex canescens plant in sludge and humic acid treatments.

IV. CONCLUSION

Results showed that *Atriplex canescens* plant has the ability of high metal accumulation, metal transfer from soil and especially high Cd and Zn phytoextraction efficiency. Sewage sludge applications increased metal concentration in *Atriplex canescens* plants. Humic acid applications have increased metal transfer factor, metal uptake and metal phytoextraction efficiency in *Atriplex canescens* plants. The results showed that humic acid applications can be used as an effective practice in phytoremediation studies to improve phytoremediation efficiency in sludge contaminated soils.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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