# The Effects of Humic Acids on the Phytoremediation Efficiency of Atriplex canescens (Pursh) Nutt Grown in Heavy Metal Contaminated Soil

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**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 as a hopeful phytoremediation plant. In a factorial experimental design, HA were applied to metals applied soils at 0 % (control) and 2 %, and the metal (Zn, Cu, Ni, Pb and Cd ) concentrations, metal uptake, metal transfer factor of Atriplex canescens plants and phytpremediation efficiency was determined. Results showed that Atriplex canescens plants are well adapted to high level of heavy metal stress conditions and has the ability of high metal accumulation, metal transfer from soil and especially high Cd phytoextraction efficiency. Humic acid applications increased plant shoot and root biomass, metal accumulation, metal transfer factor, metal uptake and metal phytoextraction efficiency in Atriplex canescens plants. Results showed that humic acid applications could be used in phytoremediation studies to increase phytoremediation efficiency in contaminated soils*

**Keywords:** *Sludge; Humic acid; Phytoremediation; Atriplex canescens*

## 1. 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. A metal polluted soil can directly be used for agricultural purposes by successful phytoremediation. All plants have the potential to extract metals from soil, but some plants termed hyperaccumulators have shown the ability to extract, accumulate and tolerate high levels of heavy metals. In the phytoremediation studies natural hyperaccumulator plants with exceptional metal 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 soil remediation, erosion control, revegetation of mine sites and other harsh environments [5].

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. These may all be manipulated to improve heavy metal phy¬toextraction. Although phytoremediation has revealed great potential and synthetic chelators have shown positive effects in enhancing heavy metal extraction through phytoremediation, a vast num¬ber of negative side-effects was revealed and need therefore exist for low cost-effective and environmental friendly materials as an alternative to synthetic chelators [6].

As an alternative to synthetic chelators wide¬spread natural sources, found in soils, natural waters, sea sediment plants, lignite, oxidized bituminous coal, leonardite and gyttja sediments such as humic substances, could be used [7]. The term humic substances refers to a category of naturally occurring organic materials result from the decomposition of plant and animal residues [8]. Humic acids (HA) contain acidic groups such as carboxyl and phenolic OH functional groups [9] and, therefore, provide or¬ganic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals [10].

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

# 2. Materials and Method

## 2.1. Soil charactarization and analysis

The contaminated soil used in this experiment 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 mixtered by perlite at the rate of 30 percent and 20 % peat to maintain slighty texture in the pot medium. The main analytical characteristics of the experimental soil are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [11].

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

| Parameters | |
|---|---|
| Texture Grade | Loam |
| pH- $H_2O$ (1:5 w/v) | 7.22 |
| $CaCO_3$, % | 4,45 |
| Organic matter, % | 4,25 |
| Clay,% | 10,2 |
| CEC, cmol $kg^{-1}$ | 18,7 |
| EC, dS $m^{-1}$ 25°C | 0,71 |
| Total N, % | 0,105 |
| P (ex), mg $kg^{-1}$ | 14,2 |
| K (ex), mg $kg^{-1}$ | 78 |
| Ca (ex), mg $kg^{-1}$ | 741 |
| Mg (ex), mg $kg^{-1}$ | 132 |
| Total Zn, mg $kg^{-1}$ | 51,2 (150-300)* |
| Total Cu, mg $kg^{-1}$ | 8,5 (50-140)* |
| Total Ni, mg $kg^{-1}$ | 6,9 (30-75)* |
| Total Pb, mg $kg^{-1}$ | 14,8 (50-300)* |
| Total Cd, mg $kg^{-1}$ | 0,01 (1-3)* |

*: Metal limits in soil, mg $kg^{-1}$ dry wt [12]

Soil texture was determined by the hydrometer method, the soil pH was measured by the CaCl2 method, organic matter content, as determined by the Walkley-Black method, CaCO3 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 HNO3/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 metal concentrations are below the levels indicated by the EU [12].

## 2.2. Extraction 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 solu¬tion 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$^{-1}$[13].

## 2.3. Experimental Design

A factorial experiment was conducted in randomized complete block design including 2 levels of humic acid and 5 levels of heavy metals 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 100, 50 and 100 mg kg-1 of N (as NH4NO3), P (as KH2PO4) and K (as K2SO4). Heavy metals Zn, Cu, Ni, Pb and Cd were added to experimental soil as metalic salt solutions (as Zn(NO3)2, CuSO4, Ni(NO3)2, Pb(NO3)2, Cd(NO3)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 [12] .

HA were added in a solution form in order to raise the soil organic carbon by 1 % and 2 % by weight. A uniform application was obtained by homogenization of the soil. The experiments also included the control treatments (no addition of HA). The soil was subsequently incubated in the green house for 8 weeks before experiment. During these 8 weeks the soil was watered 1-2 times a week with deionised water to maintain field capacitiy of water. . Each treatment was performed in five replicates.

TABLE II. 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 |

## 2.4. 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. After harvesting, soil samples were collected from each pot for above mentioned analysis.

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 HNO3/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.

## 2.5. 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 shoots and roots of the plants was defined as the ratio of the heavy metal concentration in the plants to that in the soil [14].

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows [15]:

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

where: CPlant is heavy metal concentration in plant tissue, mg kg$^{-1}$ dry weight; and CSoil is heavy metal concentration in soil, mg $kg^{-1}$ dry weight.

Theoretical total metal uptake was calculated using Eq. 2, as follows [16]:

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

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 (shoot and root) was calculated using Eq. 3, as follows [17]:

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

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 ($p \leqslant 0.05$) 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.

# 3. Results and Discussion

## 3.1. Plant growth and heavy metal concentration of plants

No statistical differences were determined on the growth and shoot and root dry matter (DM) of Atriplex canescens plants, and also no phytotoxicity symptoms were observed by the treatments of heavy metals (Table 3a, Table 3b, Table 4a and Table 4b). Atriplex canescens 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 [18]. Total metal concentrations both in the shoots and roots of plant were increased by the increasing amounts of metal treatments. Heavy metal concentration of Atriplex canescens plant was determined higher in humic acid treatment than control treatment. In both treatment Cd was relatively the highest accumulating metal. Metals accumulated both in shoots and roots in control treatments (no humic acid) were followed as Zn>Pb>Cu>Ni>Cd, but in humic acid treatments this order were followed as Zn>Pb>Cu>Cd>Ni. Metal concentrations of Atriplex canescens in the root tissues was found higher than that of shoots.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. [19].

TABLE IIIA. Shoot Dry Matter (G Pot-1) And Heavy Metal Concentration (Mg Kg-1) Of Atriplex Canescens Plant In No Ha Treatments

| Treatments | DM | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control | 115,6 | 18,5 | 4,5 | 0,77 | 0,98 | 0,029 |
| 1 | 122,3 | 114,2 | 8,5 | 2,89 | 8,5 | 0,88 |
| 2 | 133,4 | 98,5 | 7,6 | 2,63 | 14,5 | 3,54 |
| 3 | 129,5 | 156,2 | 10,2 | 8,54 | 16,6 | 4,45 |
| 4 | 138,5 | 185,6 | 11,2 | 7,54 | 34,6 | 7,41 |

TABLE IIIB. Root Dry Matter (G $Pot^{-1}$) And Heavy Metal Concentration (Mg $Kg^{-1}$) Of Atriplex Canescens Plant In No Ha Treatments

| Treatments | DM | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control | 10,55 | 32,4 | 5,3 | 1,02 | 1,12 | 0,051 |
| 1 | 11,05 | 146,5 | 10,4 | 3,05 | 8,52 | 0,98 |
| 2 | 9,56 | 115,4 | 9,85 | 3,56 | 32,56 | 5,23 |
| 3 | 11,45 | 136,2 | 15,4 | 5,62 | 36,2 | 11,25 |
| 4 | 9,86 | 286,2 | 18,3 | 11,23 | 40,1 | 14,2 |

TABLE IVA. Shoot Dry Matter (G $Pot^{-1}$) And Heavy Metal Concentration (Mg $Kg^{-1}$) Of Of Atriplex Canescens Plant In Ha Treatments

| Treatments | DM | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control | 167,4 | 41,8 | 6,12 | 0,88 | 5,23 | 0,095 |
| 1 | 166,2 | 155,6 | 7,45 | 5,56 | 7,45 | 6,62 |
| 2 | 174,5 | 245,3 | 14,56 | 8,88 | 8,96 | 7,56 |
| 3 | 168,9 | 288,4 | 21,36 | 14,62 | 25,9 | 17,85 |
| 4 | 170,2 | 554,3 | 23,65 | 18,9 | 34,52 | 19,63 |

TABLE IVB. Root Dry Matter (G $Pot^{-1}$) And Heavy Metal Concentration (Mg $Kg^{-1}$) Of Of Atriplex Canescens Plant In Ha Treatments

| Treatments | DM | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control | 13,05 | 41,6 | 6,36 | 1,02 | 6,56 | 0,124 |
| 1 | 13,00 | 212,5 | 10,5 | 10,5 | 9,96 | 6,25 |
| 2 | 13,86 | 412,6 | 15,4 | 11,8 | 12,51 | 13,45 |
| 3 | 12,51 | 388,5 | 20,1 | 12,5 | 15,45 | 18,56 |
| 4 | 13,09 | 615,2 | 32,5 | 18,8 | 28,4 | 24,11 |

## 3.2. Metal transfer factor (TF) of plants

TF of *Atriplex canescens* in both treatments were decreased by the increasing amounts of treatments (Table 5a, table 5b, Table 6a and Table 6b). TF of *Atriplex canescens* were determined higher in humic acid applications. TF of *Atriplex canescens* was determined at the highest rate for Cd. This indiactes that both plant have adapted to accumulate heavy metals without any physiological disorder in natural conditions. TF of metals in *Atriplex canescens* was followed Cd>Zn>Cu>Ni>Pb order. This results also show the remerkable ability of Cd accumulation in *Atriplex canescens* plant and also effects of humic acid applications on metal availability and metal transfer to plant.

TABLE VA. Shoot Metal Transfer Factor Of Atriplex Canescens Plant In No Ha Treatments

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,36 | 0,53 | 0,11 | 0,07 | 2,90 |
| 1 | 0,33 | 0,06 | 0,04 | 0,04 | 0,29 |
| 2 | 0,12 | 0,02 | 0,01 | 0,01 | 0,35 |
| 3 | 0,10 | 0,01 | 0,02 | 0,01 | 0,22 |
| 4 | 0,06 | 0,01 | 0,01 | 0,01 | 0,25 |

TABLE VB. Root Metal Transfer Factor Of Atriplex Canescens Plant In No Ha Treatments

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,63 | 0,66 | 0,15 | 0,08 | 5,10 |
| 1 | 0,42 | 0,07 | 0,04 | 0,04 | 0,33 |
| 2 | 0,14 | 0,03 | 0,01 | 0,03 | 0,52 |
| 3 | 0,09 | 0,02 | 0,01 | 0,02 | 0,56 |
| 4 | 0,09 | 0,01 | 0,01 | 0,01 | 0,47 |

TABLE VIA. Shoot Metal Transfer Factor Of Atriplex Canescens Plant In Ha Treatments

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,82 | 0,72 | 0,13 | 0,35 | 9,50 |
| 1 | 0,44 | 0,05 | 0,07 | 0,03 | 2,20 |
| 2 | 0,31 | 0,04 | 0,03 | 0,01 | 0,75 |
| 3 | 0,19 | 0,03 | 0,03 | 0,01 | 0,89 |
| 4 | 0,18 | 0,02 | 0,02 | 0,01 | 0,65 |

TABLE VIB. Root Metal Transfer Factor Of Atriplex Canescens Plant In Ha Treatments

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,81 | 0,80 | 0,15 | 0,44 | 12,40 |
| 1 | 0,61 | 0,07 | 0,13 | 0,05 | 2,08 |
| 2 | 0,51 | 0,04 | 0,05 | 0,01 | 1,33 |
| 3 | 0,25 | 0,03 | 0,02 | 0,01 | 0,93 |
| 4 | 0,20 | 0,02 | 0,02 | 0,01 | 0,80 |

## 3.3. Metal uptake of plants

Metal uptake (MU) amount of plants were increased by the increasing amounts of metal applications. Metal uptake of Atriplex canescens treated with humic acid was found higher than control treatments (Table 7a, Table 7b, Table 8a and Table 8b). Total metal uptake amount was determined as highest for Zn metal in both humic acid tretaments. Metal uptake rate of Zn and Cd were increased about 2 to 4 fold by the treatments compared to control. In all treatments metal uptake amount was determined for metals in Zn>Pb>Cu>Cd>Ni order.

TABLE VIIA. Shoot Metal Uptake Of Plants In No Ha Treatments, Mg $Pot^{-1}$

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 2,14 | 0,52 | 0,09 | 0,11 | 0,00 |
| 1 | 13,97 | 1,04 | 0,35 | 1,04 | 0,11 |
| 2 | 13,14 | 1,01 | 0,35 | 1,93 | 0,47 |
| 3 | 20,23 | 1,32 | 1,11 | 2,15 | 0,58 |
| 4 | 25,71 | 1,55 | 1,04 | 4,79 | 1,03 |

TABLE VIIB. Root Metal Uptake Of Plants In No Ha Treatments, Mg $Pot^{-1}$

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,41 | 0,06 | 0,01 | 0,01 | 0,00 |
| 1 | 1,62 | 0,11 | 0,03 | 0,09 | 0,01 |
| 2 | 1,10 | 0,09 | 0,03 | 0,31 | 0,05 |
| 3 | 1,56 | 0,18 | 0,06 | 0,41 | 0,13 |
| 4 | 2,82 | 0,18 | 0,11 | 0,40 | 0,14 |

TABLE VIIIA. Shoot Metal Uptake Of Plants In Ha Treatments, Mg $Pot^{-1}$

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 7,00 | 1,02 | 0,15 | 0,88 | 0,02 |
| 1 | 25,86 | 1,24 | 0,92 | 1,24 | 1,10 |
| 2 | 42,80 | 2,54 | 1,55 | 1,56 | 1,32 |
| 3 | 48,71 | 3,61 | 2,47 | 4,37 | 3,01 |
| 4 | 94,34 | 4,03 | 3,22 | 5,88 | 3,34 |

TABLE VIIIB. Root Metal Uptake Of Plants In Ha Treatments, Mg $Pot^{-1}$

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,54 | 0,08 | 0,01 | 0,09 | 0,00 |
| 1 | 2,76 | 0,14 | 0,14 | 0,13 | 0,08 |
| 2 | 5,72 | 0,21 | 0,16 | 0,17 | 0,19 |
| 3 | 4,86 | 0,25 | 0,16 | 0,19 | 0,23 |
| 4 | 8,05 | 0,43 | 0,25 | 0,37 | 0,32 |

### 3.4. Phytoextraction efficiency (PE) of plants

PE rates of *Atriplex canescens* plant were decreased by the applications of increasing amounts of metals (Table 8 and Table 9). At control treatment Cd metal has the highest rate of PE value in both humic acid tretaments. In all treatments Cd has the highest PE values. PE values 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 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 [20].

TABLE IX. Phytoextraction Efficiency Of Atriplex Canescens Plant In Control Treatments, %

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 0,497 | 0,678 | 0,178 | 0,140 | 14,181 |
| 1 | 0,444 | 0,078 | 0,047 | 0,063 | 0,524 |
| 2 | 0,178 | 0,031 | 0,016 | 0,023 | 0,600 |
| 3 | 0,140 | 0,021 | 0,024 | 0,013 | 0,358 |
| 4 | 0,093 | 0,012 | 0,014 | 0,016 | 0,342 |

TABLE X. Phytoextraction Efficiency Of Atriplex Canescens Plant In Humic Acid Tretaments, %

| Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Control | 1,473 | 1,303 | 0,411 | 0,679 | 97,153 |
| 1 | 0,815 | 0,093 | 0,133 | 0,066 | 4,275 |
| 2 | 0,606 | 0,077 | 0,066 | 0,017 | 1,550 |
| 3 | 0,345 | 0,054 | 0,054 | 0,024 | 1,664 |
| 4 | 0,336 | 0,032 | 0,042 | 0,019 | 1,113 |

## 4. Conclusion

Results showed that *Atriplex canescens* plants are well adapted to high levels of heavy metal stress conditions and has the ability of high metal accumulation, metal transfer from soil and especially high Cd phytoextraction efficiency. Humic acid applications increased metal accumulation, metal transfer factor, metal uptake and metal phytoextraction efficiency in *Atriplex canescens* plants. Results showed that humic acid applications could be used in phytoremediation studies to increase phytoremediation efficiency in contaminated soils.

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