

Fractionation of Heavy Metals in the Metal Contaminated Soils Amended with Humic Acids

Bülent Topcuoğlu

Abstract— A greenhouse pot experiment was carried out to study the effects of humic acids (HA) on the heavy metal bioavailability and metal fractionation of metal treated soil. An uncontaminated calcareous soil was treated with heavy metals (Zn, Cu, Ni, Pb and Cd) and humic acid was applied to these metals applied soils at 0 % (control) and 2 %, rates. After 8 weeks of incubation, atriplex canences, a phytoremediation plants were cultured on these soils and at the end of experiment the DTPA extractable heavy metals and metal fractions of the soils were analysed.

Metal treatments increased DTPA extractable metals and water soluble, exchangeable metal contents. Soil application of HA increased DTPA-extractable/bioavailable levels of Zn, Cu, Ni, Pb and Cd in the soil compared to the in all treatments. Also HA application increased mainly organic bound and water soluble and acid soluble metal fractions, and increased mobility of all metals. Results showed that humic acid applications could be used in phytoremediation studies to increase phytoremediation efficiency in contaminated soils.

Keywords— Bioavailability, Fractionation, Humic acid, Soil Metals, Mobility

I. 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].

The maximum permissible concentrations of heavy metals in contaminated soils are normally based on total concentration, although it is the bioavailable metal fraction that poses environmental concern [3]. Nevertheless, these criteria are insufficient since mobility, environmental diffusion and bioavailability largely depend on soil physico-chemical characteristics and, likewise, on trace metal chemical forms [4]. From an environmental point of view, the evaluation and forecast of food contamination is related to the bioavailable

fraction of heavy metals in soil.

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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 phytoextraction. Although phytoremediation has revealed great potential and synthetic chelators have shown positive effects in enhancing heavy metal extraction through phytoremediation, a vast number of negative side-effects was revealed and need therefore exist for low cost-effective and environmental friendly materials as an alternative to synthetic chelators [5].

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 [6]. The term humic substances refers to a category of naturally occurring organic materials result from the decomposition of plant and animal residues [7]. Humic acids (HA) contain acidic groups such as carboxyl and phenolic OH functional groups [8] and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals [9].

The aim of this research was to assess the ability of HA on the bioavailability and metal fractionation of heavy metals (Zn, Cu, Ni, Pb and Cd) from metal polluted soil under greenhouse controlled conditions.

II. MATERIALS AND METHODS

A. Soil characterization 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. Greenhouse soil samples were taken at a depth of 0 to 20 cm and these were air-dried, sieved (< 2 mm) and stored in polyethylene bags sealed awaiting analysis. Electrical conductivity (EC) and pH were measured a soil: water ratio of 1:2. cation exchange capacity (CEC) was determined by 0.1 M NH_4OAc extraction; CaCO_3 content was determined by the calcimeter; organic carbon was measured by wet oxidation; and texture was determined by Bouyoucos hydrometer method. For the

determination of 'total' heavy metal concentrations, soil were digested in aqua regia (1:3 HNO₃/HCl) according to the international standard [8]. Bioavailable fractions of metals were extracted from soil with diethylenetriaminepentaacetic acid-CaCl₂-triethanolamine adjusted to pH 7.3 (DTPA) procedure [10]. The main analytical characteristics of the unpolluted experimental soil are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [10].

TABLE I
THE ANALYTICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL
BEFORE APPLICATIONS

Parameters	
Texture Grade	Loam
pH- H ₂ O (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 [10]

Sequential extraction method [11] was applied to soil samples to identify metal fractions. The heavy metal sequential extraction procedure had the following steps:

F1. 1 M MgCl₂ (1:8 w/v, pH 7) for 1 h at room temperature; metals in soil solution and in exchangeable forms.

F2. 1 M NaOAc (1:8 w/v, pH 5) for 5 h at room temperature; metals mainly in the carbonate fraction.

F3. 0,04M NH₂OH/HCl in 25 % (v/v)HOAc (1: 20 w/v) for 6 h at 96 °C ; metals associated with Fe and Mn oxides.

F4. 3 ml 0,02 M HNO₃+5 ml 30 % H₂O₂ (pH 2) for 3 h at 85 °C; metals associated with organic matter.

F5. HNO₃-HCl digestion; residual fraction.

For the determination of total, bioavailable and sequential extracted metal concentrations, soil samples were digested in aqua regia (1:3 HNO₃/HCl) and HClO₄ according to the international standard [8]. Zn, Cu, Ni, Pb and Cd concentrations of greenhouse soil samples were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

B. 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 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, freeze-dried 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[12].

C. Experimental Design

A factorial experiment was conducted in randomized complete block design including 2 levels of humic acid (control treatment and % 2 humic acid treatment) and 2 levels of heavy metals (control treatment and 300, 140, 75, 300 and 3 ppm concentration of Zn, Cu, Ni, Pb and Cd, respectively) 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 metallic salt solutions (as Zn(NO₃)₂, CuSO₄, Ni(NO₃)₂, Pb(NO₃)₂, Cd(NO₃)₂, respectively). Metal concentrations were designed to maintain maximum metal limits of European Union [10]. A uniform application was obtained by homogenization of the soil. 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 capacity of water. After that a phytoremediation plant, *Atriplex canescens* (Pursh) Nutt were grown at 2 months. After harvesting, soil samples were collected from each pot for above mentioned analysis.

D. Metal Mobility Factor

Due to some metal forms are strongly bound to soil components than those extracted in F1 and F2, the mobility of metals in soil samples may be evaluated on the basis of absolute and relative content of fractions weakly bound to soil component. Mobility of soil metal was computed based on the metal fractions theoretically mobile fractions in sequential extraction procedure, where the metals are softly bound to the solid phases. Relative index of metal mobility was calculated as a 'mobility factor' (MF) on the basis of the following equation:

$$MF: \frac{(F_1 + F_2 + F_3)}{(F_1 + F_2 + F_3 + F_4 + F_5)} \times 100$$

This equation is largely describes the potential mobility of metals [13]

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 soils and in evaluation parameters.

III. RESULTS AND DISCUSSION

A. DTPA extractable metal contents of soil

Before the treatments experimental soil have generally slightly alkaline reaction, moderate CEC, high EC values and highly calcareous. Physical and chemical characteristics of greenhouse soil are well within the accepted normal range of

agronomic values, and the heavy metal concentrations are below the levels indicated by the EU [10]. DTPA extractable metal concentrations of experimental soil after humic acid and metal treatments are presented in Figure 1 and Figure 2.

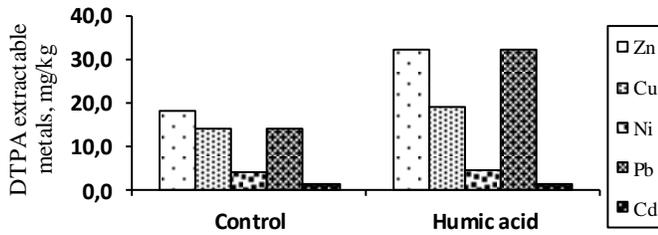


Fig. 1. DTPA extractable metal concentration in control soil and humic acid treated soil

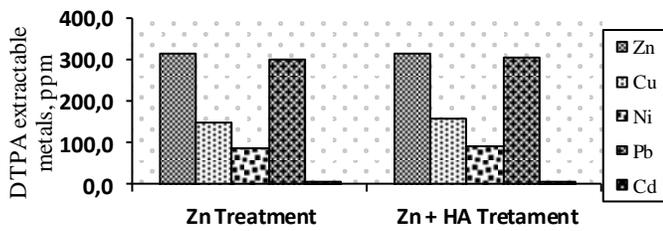


Fig. 2. DTPA extractable metal concentrations of soil in metal and metal+humic acid treatments

In this study, bioavailability of metals was expressed in terms of concentrations extractable with DTPA and means metals can be easily taken by the plants. As can be seen in Figure 1 and Figure 2, humic acid applications has brought about significant increases in DTPA-extractable Zn, Cu, Ni, Pb and Cd concentrations both in control and metal treated soil. These data also confirm that humic substances added to soil are contributing in keeping the heavy metals in more bioavailable forms (Pitchell et al., 1999). Metal applications to the soil increased the amount of DTPA-extractable metals considerably. The tendency of DTPA extractable metal accumulation in soil was in the following order: Zn>Pb>Cu>Ni>Cd.

B. Soil Metal Fractionation

Concentrations of Zn, Cd, Ni, Pb and Cd in metal fractions of soil were given in Figures 3-11. Distribution of metals in control treatment showed that the greatest percentage of all metals was present in the residual fraction. The residual phase represents metals largely embedded in the crystal lattice of the soil fraction and should not be available for remobilization except under very harsh conditions [13]. In control treatment the distribution of metals in greenhouse soil samples generally followed the order below for the metals studied.

- Zn: F1<F2<F3<F4<F5
- Cu: F1<F2<F3<F4<F5
- Ni: F1<F2<F1<F4<F5
- Pb: F1<F3<F1<F4<F5
- Cd: F1<F2<F4<F3<F5

Humic acid applications considerably increased heavy metal concentrations in soil fractions both in control and metal treated soil with the exception of F5 fraction. Also Soil metal applications applications shaply increased heavy metal concentrations in soil fractions with the exception of F5 fraction. Metals in F1, F2 and F4 fractions were markedly increased by both metal and humic acid applications. The exchangeable (F1) and acid-extractable fractions (F2) are considered to be easily soluble and available. Changes in F3 fraction with humic acid applications were very less. In metal treatments, the distribution of metals in soil samples generally followed the order below.

- Zn: F4<F2<F1<F5<F3
- Cu: F4<F1<F2<F5<F3
- Ni: F4<F2<F1<F5<F3
- Pb: F2<F1<F4<F5<F3
- Cd: F1<F2<F4<F5<F3

Although there was a consideration that humic acid-rich materials can be useful amendments for soil remediation involving stabilisation in calcareous soil [14]; these results show that humic acid has an ability to make soil metals in soluble or exchangeable form. As can be expected soil metal applications increased F1 and F2 metal fractions due to the metal applications were in soluble metal salts forms. At the same time F4 fraction of metals that represents organic matter bound metals were increased by humic acid applications.

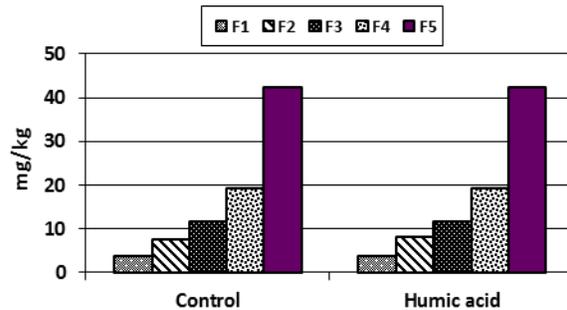


Fig. 3. Distrubution of Zinc fractions in the control and humic acid treated soil.

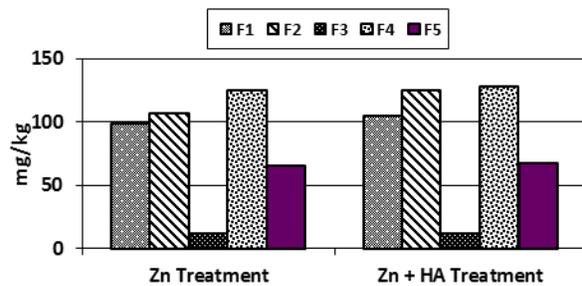


Fig. 4. Distrubution of Zinc fractions in the Zinc treated and Zn + humic acid treated soil.

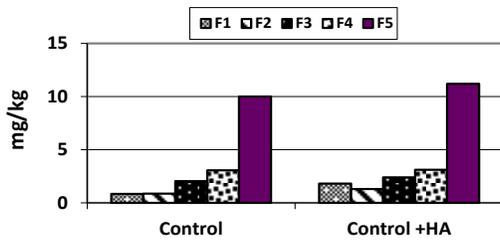


Fig. 5. Distrubution of Copper fractions in the control and humic acid treated soil.

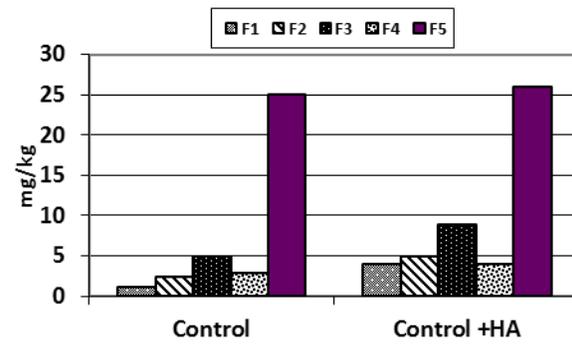


Fig. 9. Distrubution of Lead fractions in the control and humic acid treated soil.

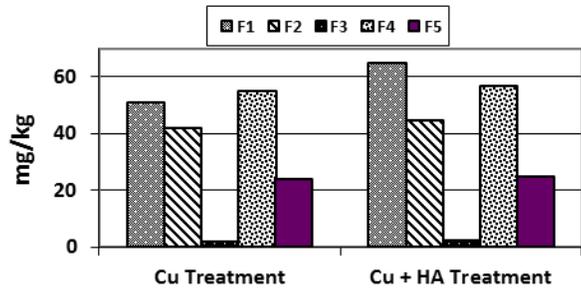


Fig. 6. Distrubution of Copper fractions in the Copper treated and Copper + humic acid treated soil.

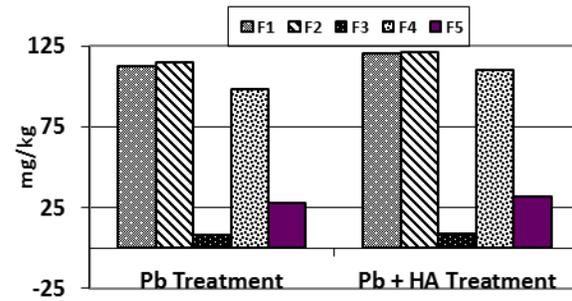


Fig. 10. Distrubution of Lead fractions in the Lead treated and Lead + humic acid treated soil.

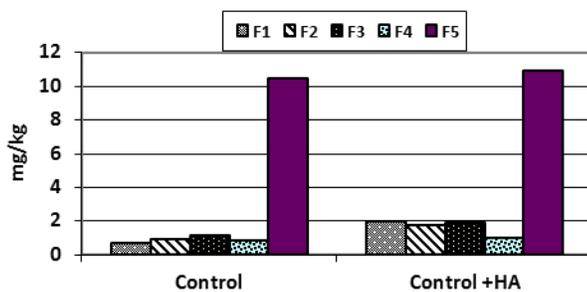


Fig. 7. Distrubution of Nickel fractions in the control and humic acid treated soil.

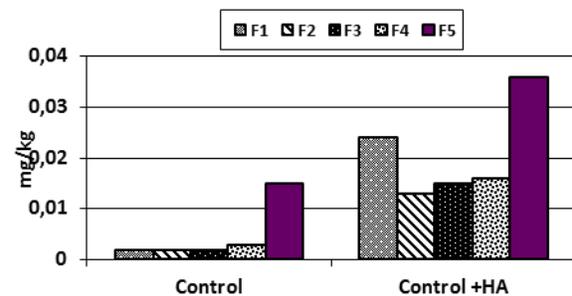


Fig. 11. Distrubution of Cadmium fractions in the control and humic acid treated soil.

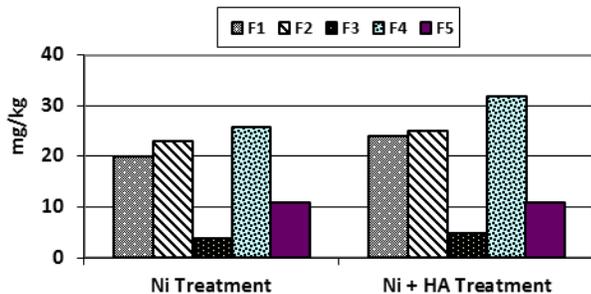


Fig. 8. Distrubution of Nickel fractions in the Nickel treated and Nickel + humic acid treated soil.

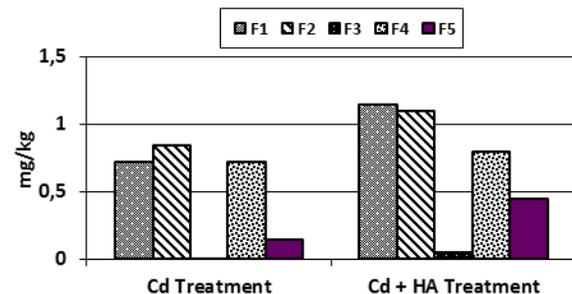


Fig. 12. Distrubution of Cadmium fractions in the Cadmium treated and Cadmium + humic acid treated soil.

C. Mobility of Metals

This equation largely describes the potential mobility of metals; expressed as mobility factor (MF) and the high MF values have been interpreted as symptoms of relatively high lability and biological availability of heavy metals in soils (15). The MF values in control soil were considerably higher for Zn and Cd. Humic acid applications increased mobility factor value for all metals examined especially for Pb and Cd (Figure 13). Both in metal treatments and metal+humic acid treatments no statistically important relation with the exception of Cd was recorded for mobility factor of metals. This may be caused by the using of soluble salts of metals in treatments. The mobility of metals in humic acid and metal treated soils may be assessed on the basis of absolute and relative content of fractions weakly bound to soil components. The results of the present study suggest that the mobility of metals declines in the following order:

Control soil: Zn>Cd>Pb>Cu>Ni
 Humic acid treated soil: Cd>Pb>Ni>Cu>Zn
 Metal treated soil: Cd>Pb>Ni>Cu>Zn
 Metal +humic acid treated soil: Cd>Pb>Ni>Cu>Zn

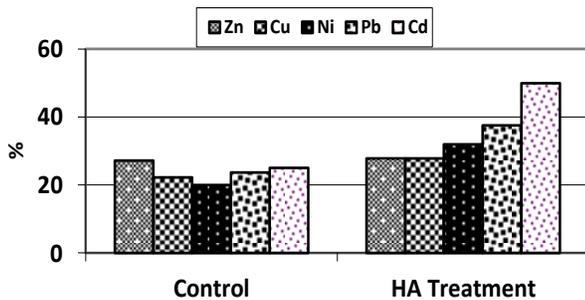


Fig.13. Metal mobility factors of control and humic acid applied soil.

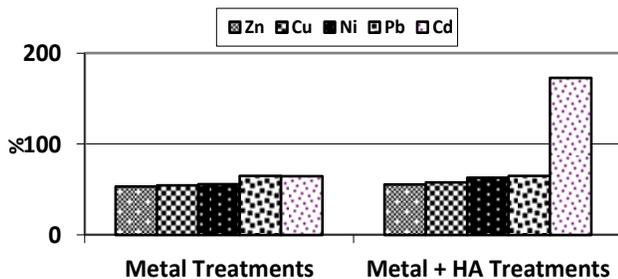


Fig.14. Metal mobility factors of soils treated with heavy metals and humic acid

IV. CONCLUSION

The results of the present study indicates that soil application of HA increased DTPA-extractable/bioavailable levels of Zn, Cu, Ni, Pb and Cd in the soil compared to the in all treatments. Also HA application increased mainly organic bound and water and acid soluble metal fractions, and increased mobility of all metals. Results appear that verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with metals; and potential environmental availability of metals may be controlled by soil amendments with exogenous humic substances. Thus in case metal pollution of soil is a cause of diminished plant growth and yield, the

possibility to increase metal availability to plants may represent an advantage when contaminated soil should be reclaimed by phytoextraction process. Results showed that humic acid applications could be used in phytoremediation studies to increase phytoremediation efficiency in contaminated soils.

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