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The influence of humic acids on the metal bioavailability and phytoextraction efficiency in long-term sludge applied soil

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Abstract

Heavy metal pollution of soils causes many environmental and human health problems. Phytoextraction of heavy metals from contaminated soils has the prospect of being an effective and economic in situ technique. Long-term sludge treated soil enriched with heavy metals (Zn, Cu, Ni, Pb and Cd) was used in the experiment. The influence of exogenous humic acid (HA) on the bioavailability of Zn, Cu, Ni, Pb and Cd from sludge applied soil and heavy metal uptake of tobacco plant was examined in greenhouse experiment. HA were applied to long-term sewage sludge polluted soil at 1 % and 2 %, and the uptake of Zn, Cu, Ni, Pb and Cd into tobacco plant was determined. Soil samples were collected after harvest and total and DTPA-extractable Zn, Cu, Ni, Pb and Cd contents of soil were determined. Diethylenetriaminepentaaceticacid(DTPA)-extractable Zn, Cu, Ni and Pb concentrations and plant uptake of metals increased significantly by HA applications. While HA treatment at 2 % rate to soil increased the heavy metal concentration in the shoot tissue, plant growth was diminished. The results suggest that soil amendments with HA can be considered as an alternative approach to reduce the availability and mobility of heavy metals and to increase phytoextraction efficiency of heavy metal polluted soils.

Keywords*:* Sludge; Humic acids; Phytoremediation; Metal bioavailability; Tobacco

1. Introduction

Soil amendment of sewage sludge is a widespread disposal practice. Sewage sludge contains valuable plant nutrients and organic matter that can improve soil fertility. The phytonutritive capacity of sludge compost has often been demonstrated to be analogous to that of manure [1]. However, sewage sludge often contains potentially toxic elements that can cause soil contamination, phytotoxicity and undesirable residues in plant and animal products [2]. In the long term, the use of sewage sludge can also cause a significant accumulation of Zn, Cu, Pb, Ni and Cd in the soil and plants [3].

Therefore the use of sewage sludge for agricultural purposes in many countries is restricted. At the present time, legislation in different countries limiting the use of sewage sludge in agriculture refers to the total amounts of heavy metals in these wastes and in soils and recommends that soil pH to be maintained at 6 or higher. 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.

Phytoremediation is defined as the use of green plants in removing pollutants from the environment, or in rendering them harmless [5]. It is defined that phytoextraction, the phytoremediation sub-group as use of pollutant-accumulating plants to remove metals or organic pollutants from soil by concentrating them in harvestable parts [6]. 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. Many hyperaccumulator characteristics are found in the tobacco plant, *Nicotiana tabacum* [7].

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 [8].

As an alternative to synthetic chelators widespread 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 [9]. The term humic substances refers to a category of naturally occurring organic materials result from the decomposition of plant and animal residues [10]. Humic acids (HA) contain acidic groups such as carboxyl and phenolic OH functional groups [11]and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals [12]. The aim of this research was to assess the ability of HA on phytoextraction of heavy metals from sludge polluted soil by the use of tobacco plant under greenhouse conditions.

2. Materials and Methods

2.1. Soil charactarisation and analysis

The contaminated soil used in this experiment was sampled from the long-term (5 years or more) sludge amended soil in a representative of the major agricultural areas of Turkey Antalya Aksu. Soils were air dried and passed through a 2 mm sieve before further use. The main analytical characteristics of the experimental contaminated soil and a native soil sampled from same area are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [13].

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 Zn, Cu, Ni, Pb and Cd contents of the soil were digested by the aqua regia method (1:3 HNO_3/HCl, the bioavailable Zn, Cu, Ni, Pb and Cd contents were determined by diethylenetriaminepentaacetic acid (DTPA) extraction method. Zn, Cu, Ni, Pb and Cd were analysed by flame atomic absorption spectrometry (FAAS).

Table 1. The analytical characteristics of the experimental greenhouse soil and unpolluted soil.

Parameters	Soil Characteristics				Limit values in soil ($mg\ kg^{-1}$ dry wt.)
	Native Soil		Polluted soil		
Texture	Loam		Loam		
pH- H_2O (1:5 w/v)	7.03		6.97		
$CaCO_3$, %	8.12		8.12		
Organic carbon, %	0.82		1.52		
Zn, $mg\ kg^{-1}$	88[1]	7.6[2]	238[1]	43[2]	150-300[3]
Cu, $mg\ kg^{-1}$	25	2.7	86	12	50-140
Ni, $mg\ kg^{-1}$	17	0.48	44	1.5	30-75
Pb, $mg\ kg^{-1}$	45	4.0	105	15	50-300
Cd, $mg\ kg^{-1}$	0.15	*	1.2	0.22	1-3

[1]: Total concentrations, [2]: DTPA-extractable concentrations, [3]: Total concentrations at soil 6<pH<7 [13]

*: Below detection limit (< 0.01 $mg\ kg^{-1}$)

2.2. Extraction of humic substances and addition to soils

Humic acids were extracted from leonardite material according to the method as outlined by Stevenson (1994) [14]. 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 $mg\ ml^{-1}$.

Five kilograms of air-dried and sieved soil were filled into plastic pots. A pot-plate was placed under each pot to prevent leaching. To each pot soil the following amounts of basic fertilizer were applied: 300, 200 and 300 $mg\ kg^{-1}$ of N, P and K, respectively. The experiments included the control treatments (no addition of HA). 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 soil was subsequently incubated in the green house for 8 weeks. During these 8 weeks the soil was watered 1-2 times a week with deionised water. Each treatment was performed in five replicates.

2.3. Plant growth and analysis

Tobacco plants (*Nicotiana tabacum L. İzmir*) were used for the pot experiments. Seeds of tobacco plants were germinated in a peat/sand mixture. After the 8-week incubation, seedlings with similar biomass were transferred into the pots. Four seedlings were planted into each pot and were thinned to one plant after 1 week. All tobacco plants were grown under greenhouse environmental conditions. Plants were harvested after 6 weeks of growth. After harvesting, soil samples were collected from each pot for above mentioned analysis.

During harvest, plants were cut short above ground organs. Plant samples were rinsed briefly in deionised water and oven dried at 70 °C to a constant weight. The dry weight was determined and the samples were homogenised in particle size by grounding. After milling, the plant tissue was digested by the aqua regia (1:3 HNO_3/HCl) and Zn, Cu, Ni, Pb and Cd contents were analysed by flame atomic absorption spectrometry (FAAS) under optimised measurement conditions.

2.4. Statistical analysis

A statistical Anova F test was applied to the results and treatment means were compared by the least significant difference test at $P<0.05$.

3. Result and discussion

3.1. Total and DTPA extractable metal contents in the soil

Total and DTPA extractable metal concentrations of experimental soil after tobacco harvesting are presented in Table 1. The heavy metal contents of native soil are well within the accepted normal range of values (Table 1). A comparison of metal contents of polluted soil with that of native soil in the same region showed that the Zn, Cu, Ni, Pb and Cd contents of polluted soil were present in greater concentrations than in the native soil (Table 2). The heavy metal concentrations of polluted soil are above the pollutant limits indicated by the EU (86/278/CEE) [13].

Table 2. Effects of humic acid applications on the total and DTPA extractable concentrations of heavy metals in the polluted greenhouse soil after harvesting

Heavy metal concentration in soil (mg kg^{-1})		Humic acid treatment rates (%)		
		0 (control)	1	2
Zn	Total	238	237	237
	DTPA	43 c	66 b	77 a
Cu	Total	86	85	85
	DTPA	12 b	16 a	16 a
Ni	Total	44	44	44
	DTPA	1.8	2.0	2.0
Pb	Total	105	105	104
	DTPA	15 c	28 b	33 a
Cd	Total	1.2	1.2	1.2
	DTPA	0.22	0.25	0.23

Means followed by the same letter are not statistically different (Duncan tetst, $P\leq0.05$)

The total heavy metal content of the soil, as determined by aqua regia extraction, showed no significant change after harvesting. This may be caused that phytoextraction by tobacco plants was too small due to short vegetation period. Total and DTPA extractable Ni and Cd concentrations showed almost no variation among the HA treatments. The tendency of metal accumulation in the soil was in the following order: Zn>Pb>Cu>Ni>Cd.

In this study, bioavailability of metals was expressed in terms of concentrations extractable with DTPA. As can be seen in Table 1, long-term sludge applications has brought about significant increases in DTPA-extractable Zn, Cu and Pb concentrations in comparison with the native soil. These data also confirm that humic substances added to soil are contributing in keeping the heavy metals in more bioavailable forms [15]. The maximum permissible concentrations of heavy metals in surface soils are normally based on total concentration, although it is the bioavailable metal fraction that posses environmental [16]. Total concentrations of all metals in polluted soil were found above the pollutant limits, but high rates of DTPA-extractable metal fractions could be seen more marked than those of total concentrations. The application of HA caused to a significant increase in the extractability of metals in the soil. The relative increases of Zn, Cu and Pb for HA in comparison with the control were higher for the DTPA-extractable than for the 'total' form. To compare the relative availability of different soil metals, DTPA/Total ratio is used to give available metals as a percentage of total soil metal. Relative availabilities, calculated in this way are given in Figure 1.

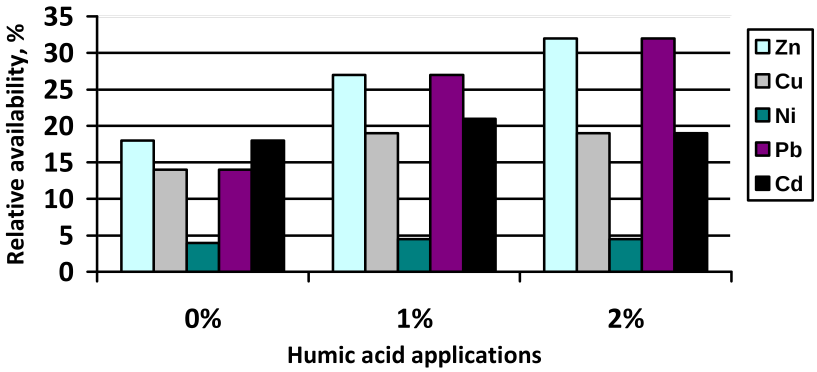

Fig. 1. Effect of humic acid applications to soil on relative availability of heavy metals

Relative metal availability, expressed as DTPA/Total ratios, was greater for Zn, Cu and Pb than the other metals in the greenhouse soil. HA applications increased DTPA-extractable and relative availabilities of metals. Metals in control treatment were the least relative availability. Relative availability of Zn and Pb were the highest in HA treatments.

3.2. Plant growth and metal concentrations

Dry matter yields of the shoots (sum of leaves and stem) and heavy metal concentrations of tobacco plant are shown in Table 3. Humic acid applications have led to a significant decrease in plant yield. However plants treated with humic acid at 1 % and 2 % levels showed no toxicity symptoms such as chlorosis and necrosis at vegetation period. Biomass production in plants was significantly higher for control than humic acid treatments.

The addition of humic acids to soil increased the Zn, Cu, Pb and Cd content of tobacco plants from sludge polluted soil. Similar results was obtained in previous studies [17]. Only Ni contents of plants were not changed, this may possibly be caused by lower availability of Ni as seen in Table 2. The plants growing in the control treatment (no HA treatment), tobacco plants received a lower Zn, Cu, Ni and Pb amounts due to their lower bioavailability in the soil and thus may be revealed higher biomass than that of HA treated plants . The advantage of the HA applications is that a larger amount of Zn, Cu, Ni and Pb could be extracted by the plants in a shorter period of time. Similar results on the enhancement of the bioavailability and mobility of heavy metals in soil by humic substances were also reported [18,19].

Table 3. Effect of humic acid application to polluted greenhouse soil on the dry matter yield and heavy metal concentration in the shoots of tobacco plant

	Humic acid treatment dosage (%)		
	0 (control)	1	2
Dry matter yield (g)	86 a	77 b	72 b
Zn (mg kg-^{1})	37 c	52 b	77 a
Cu (mg kg-^{1})	16 c	23 b	33 a
Ni (mg kg-^{1})	2.4	2.5	2.6
Pb (mg kg-^{1})	3.1 b	5.5 a	5.6 a
Cd (mg kg-^{1})	0.7 c	1.34 b	1.44 a

Means followed by the same letter are not statistically different ($P \leq 0.05$)

4. Conclusion

The results of the present study indicated that soil application of HA increased DTPA-extractable levels of Zn, Cu, Ni, Pb and Cd in the soil, and HA applications were significantly increased these metals in tobacco plant compared to the control treatment. Results appear that verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with sludge; 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.

The positive effect of the HA applications is that a larger amount of metals can be extracted by the plants in a shorter period of time. To use this advantage, selected phytoremediation plants have to be tolerate to high toxic metal concentrations or to have hyperaccumulator properties. Thus, a combination of using natural chelators and a hyperaccumulator plant would significantly increase the phytoextraction efficiency.

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Literature

[1] Roe, N.E., Stoffela, P.J., Bryan, H.H. Utilization of MSW compost and other organic mulches on commercial vegetable crops. *Compost Sci. Utilization*, 1(3):73-84, 1993.

[2] Alloway, B.J., Jackson, A.P. The behaviour of heavy metals in sewage sludge-amended soil. *Sci. Total Environ.*, 100, 151-176, 1991.

[3] Mulchi, C.L., Adamu, C.A., Bell, P.F., Chaney, R.L. Residual heavy metal concentrations in sludge-amended coastal plain soils. I. Comparison of extractans. Commun. *Soil Sci. Plant Anal.*, 22(9/10):919-941, 1991.

[4] Planquart, P., Bonin, G., Prone, A., Massiani, C. Distribution movement and plant availability of trace metals in soils amended with sewage sludge composts: application to low loadings. *The Science of the Total Environment,* 241:161-179, 1999.

[5] Raskin, I., Smith, R.D., Salt, D.E. Phytoremediation of metals: using plants to remove pollutants from the environment. *Curr. Opin. Biotechnol.*, 1997. 8, 221-226.

[6] Salt, D.E., Smith, R.D., Raskin, I. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1998,49, 643-668.

[7] Romkens, P., Bouwman, L., Japenga, J.,Draaisma, C. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environmental Pollution*, 2002, 116, 109-121.

[8] Barona, A., Aranguiz, I., Elias, A. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. *Environ. Pol.*, 2001, 113, 79-85.

[9] Senesi, N. The fractal approach to the study of humic substances. In: Senesi N, Miano TM (eds) *Humic substances in the global environment and implications on human health*. Elsevier, Amsterdam, The Netherlands, 3-41, 1994.

[10] MacCarthy, P. The principles of humic substances. *Soil Science*, 2001, 166, 738-751.

[11] Hofrichter, M., Steinbüchel, A. Biopolymers, vol. 1, *Lignin, Humic Substances and Coal*. Wiley Europe-VCH, Weinheim, New York, 2001.

[12] Lagier, T., Feuillade, G., Matejka, G. Interactions between copper and organic macromolecules: determination of conditional complexation constants. *Agronomie*, 2000, 20, 537- 546.

[13] C.E.C. Council of the European Communities. Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/CEE*). Official Journal of the European Communities*, L181, 6-12, 1986.

[14] Stevenson, F.J. Humus chemistry: Genesis, composition and reaction. (second ed.), Wiley, New York, 1994.

[15] Pitchell, J., Kuroiva, K., Sawyer, H.T. Distrubution of Pb, Cd, and Ba in soils and plants of two contaminated soils. *Enviromental Pollution*, 1999, 110, 171-178.

[16] Wallace, A., Wallace, G.A. A possible flaw in EPA's 1993 new sludge rule due to heavy metal interactions. *Commun. Soil Sci. Plant Anal*. 25, 129-135, 1994.

[17] Li, Z., Shuman, L.M. Heavy metal movement in metal contaminated soil profiles. *Soil Sci.*, 1996, 161, 656-666.

[18] Evangelou, V.P., Marsi, M. Composition and metal ion complexation behaviour of humic fractions derived from corn tissue. *Plant Soil*, 2001, 229, 13-24.

[19] Halim, M., Conte, P., Piccolo, A. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere*, 2003, 52, 265-275.