Effects of soluble humic acids on the uptake of heavy metals by *Vetiveria zizanioides* (L.) Nash in contaminated mining soils

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INTRODUCTION

In the past, mining wastes were left wherever they might lie in the surroundings of the mine area. Unfortunately, inactive and abandoned mines continue to pollute our environment, reason why these sites should be restored with minimum impact. Phytoextraction is an environmental-friendly and cost-effective technology less harmful than traditional methods that uses metal hyperaccumulator or at least tolerant plants to extract heavy metals from polluted soils. One disadvantage of hyperaccumulator species is their slow growth rate and low biomass production. *Vetiveria zizanioides* (L.) Nash, perennial species adapted to Mediterranean climate has a strong root system which can reach up to 3 m deep, is fast growing, and can survive in sites with high metal levels (Chen *et al.*, 2004). Due to the fact that metals in abandoned mine tailings become strongly bonded to soil solids, humic acids used as chelating agents could increase metal bioavailability (Evangelou *et al.*, 2004; Wilde *et al.*, 2005) and thereby promote higher accumulation in the harvestable parts of the plant. The objective of this study was to examine the performance of humic acid assisted phytoextraction using *Vetiveria zizanioides* (L.) Nash in heavy metals contaminated soils.

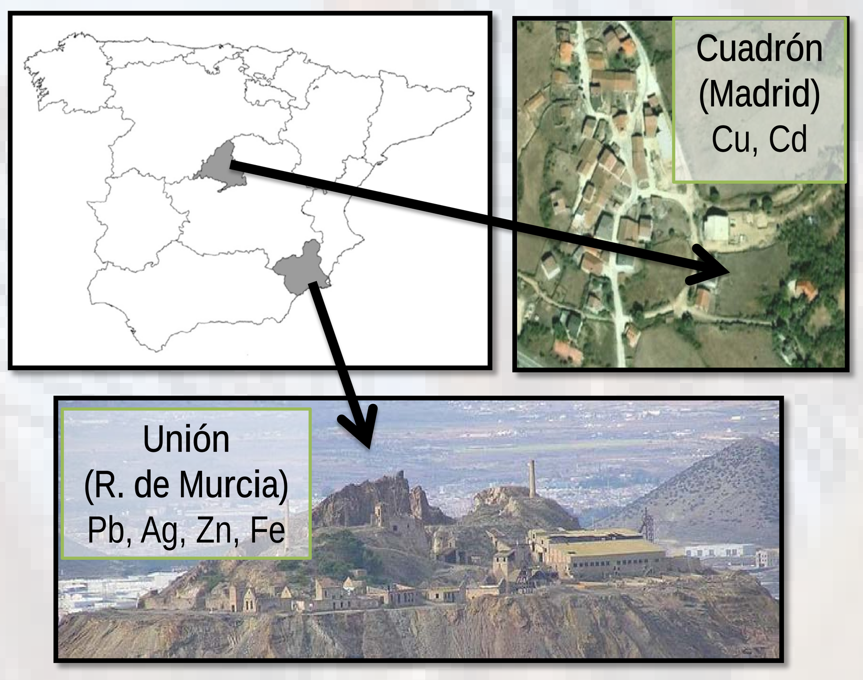
MATERIALS AND METHODS

Soil Samples Location

Abandoned mine (1990) in La Unión (Murcia) (U).

Abandoned mine (1862) in El Cuadrón (Madrid) (C).

Soil samples collected from the top 20 cm. Samples were air-dried and sieved to < 2 mm for analysis.



Soil and plant analysis

CEC (method for acid soils described by Rhoades, 1982).

Soil fractionation of Cd, Cu, Pb, Zn: Ma and Rao, 1999;

Fresh and dry weight of shoots.

Shoots incineration at 450 ºC for 4h and ashes digestion in HNO_3–HCl acid mixture (AOAC, 2000).

Shoots metal concentration by AA (Cd, Cu, Pb, Zn).

Statistical analysis

Data were analyzed using ANOVA. Means were compared using the Tukey's test at a P< 0.5 significance level.

Pot experiment

- Specie: *Vetiveria zizanioides* (L.) Nash.
- Organic amendent: solid humic acid (HA) from american leonardite.
- Treatment: controls (no HA), 0.5, 2 and 10 g HA kg^{-1} soil.
- Harvest: 85 days after planting.

RESULTS

Table 1. Chemical and physical characteristics of soils.

Soil Parameters		Unión	Cuadrón
Soil texture	USDA	Sandy loam	Loamy sand
Organic C	%	0.23	1.08
Organic matter	%	0.39	2.48
pH H_2O extract 1/2.5 (p/v)		4.6	5.6
pH KCl extract 1/2.5 (p/v)		4.4	4.6
Electrical conductivity	$dS \cdot m^{-1}$	2.65	0.031
Cation exchange capacity	$cmol(+) \cdot kg^{-1}$	1.32	4.79

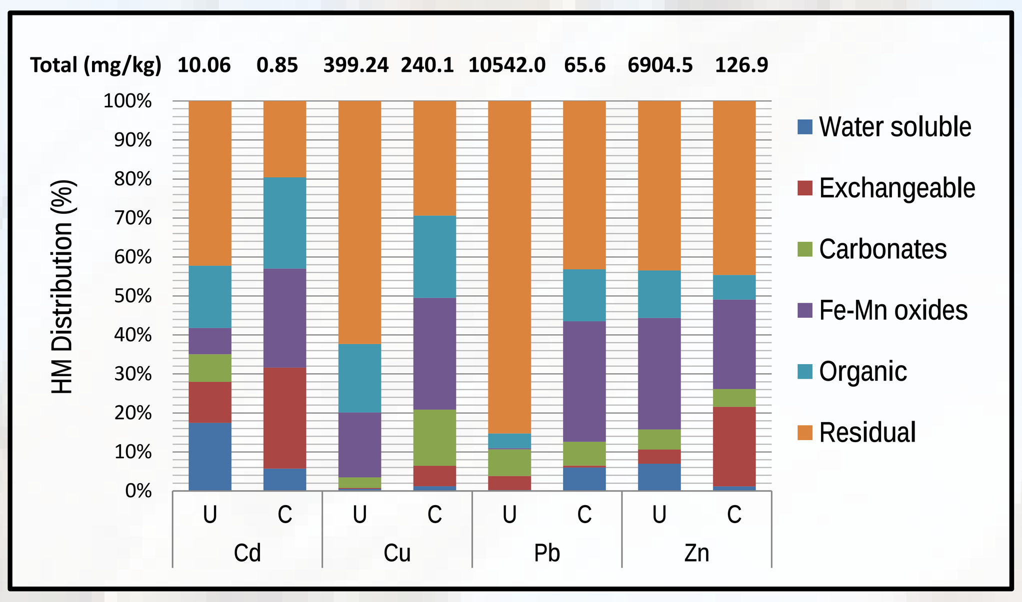


Figure 1. Heavy metal fractionation.

- Soil C, abandoned 150 years ago, showed higher pH, lower EC, finer texture, higher OC content and higher CEC.
- Heavy metal content was much higher in soil U, recently abandoned, and except for Zn, there was a greater metal concentration in the residual fraction of this soil.
- Addition of humic acid resulted in mobilization of metals in soil C. Medium doses (0.5 and 2 g/kg) gave as a result a significant higher amount of metals in the plant tissue.
- Addition of humic acids to soil U, with a very high concentration of metals, resulted in immobilization of the humic acids, evidence by the color of the soil-HA mixture and the clear soil leachates. It also translated into lower plant metal content, particularly Cu and Zn with higher HA doses.
- Addition of humic acid improved plant survival in soil U and decreased in soil C.

Table 2. Heavy metal concentration (mg/kg) in shoots after 84 days in soil U.

HA doses (g/kg)	Cd	Cu	Pb	Zn
0	13.5 ^{a} (±11.4)	27.6 ^{ab} (±10.6)	10.3 ^{a} (±9.2)	317.8 ^{ab} (±200.6)
0.5	3.3 ^{a} (±3.0)	11.8 ^{a} (±1.4)	31.6 ^{b} (±4.3)	1174.6 ^{b} (±643.2)
2	6.5 ^{a} (±5.5)	95.2 ^{b} (±78.2)	9.8 ^{a} (±14.4)	1025.0 ^{ab} (±615.2)
10	3.6 ^{a} (±0.2)	12.3 ^{a} (±1.2)	6.6 ^{a} (±0.6)	245.7 ^{a} (±15.2)

Different letters in the same column represent significant difference according to Tukey's test (P<0.05)

Table 3. Heavy metal concentration (mg/kg) in shoots after 84 days in soil C.

HA doses (g/kg)	Cd	Cu	Pb	Zn
0	0.4 ^{a} (±0.3)	91.0 ^{a} (±36.8)	10.5 ^{ab} (±3.6)	91.0 ^{ab} (±6.5)
0.5	2.4 ^{a} (±1.1)	414.3 ^{ab} (±477.3)	13.5 ^{b} (±3.9)	106.2 ^{b} (±27.2)
2	1.9 ^{a} (±1.0)	573.0 ^{b} (±121.7)	9.0 ^{ab} (±2.2)	68.4 ^{ab} (±15.6)
10	2.3 ^{a} (±2.1)	46.4 ^{a} (±27.3)	4.5 ^{a} (±4.1)	57.6 ^{a} (±27.8)

Different letters in the same column represent significant difference according to Tukey's test (P<0.05)

Table 4. Effect of humic acid doses on plant survival.

	HA doses (g/kg) - Soil U				HA doses (g/kg) - Soil C			
	0	0.5	2	10	0	0.5	2	10
Survival rate (%)	11	80	70	60	90	90	83	43

CONCLUSIONS

Vetiveria zizanioides (L.) Nash survives in soils heavily contaminated with metals.

In soils where mining activity was abandoned more than hundred year ago, addition of humic acid releases unavailable metals making them more available to living organisms.

In soils where activity was recently abandoned, addition of humic acids immobilizes metals increasing the survival rate.

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