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PHYTOEXTRACTION OF HEAVY METALS FROM MINE SOILS USING HYPERACCUMULATOR PLANTS

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Abstract: Phytoextraction is an environmental-friendly and cost-effective technology that uses metal hyperaccumulator plants to remove heavy metals from soils. The metals are absorbed by the roots, transported and accumulated in the aerial parts of the plants, which can be harvested and eliminated. The aim of this work was to study some hyperaccumulator species that could be useful to decontaminate mine soils and also to investigate the bioavailability and uptake of these metals by plants with the addition of organic amendments. Pot experiments were performed with soil samples collected from two mining areas in the north of Madrid, where there was an intense mining activity more than 50 years ago. Three species (*Thlaspi arvense*, *Brassica juncea* and *Atriplex halimus*) were grown under controlled conditions in pots filled with contaminated soils mixed with 0 Mg, 30 Mg and 60 Mg per hectare of two different organic amendments: a commercial compost made of pine bark, peat and wood fiber and other made of horse and sheep manure and wood fiber. Plants were harvested at the end of their crop cycle and were digested in order to measure metal concentration (Zn, Cu and Cd) in roots and shoots. Highest plant metal concentration was observed in pots treated with pine bark amendment and with pure soil due to an increase in metal bioavailability with decreasing pH. Also in those treatments the total plant biomass was lower, even some plants could not germinate. On the contrary, there was a lower metal concentration in plant tissues of pots with manure because its higher pH whereas plant growth was significantly larger so there was an incresing amount of metals removed from soil by plants. Comparing the three species results indicate a higher total metal uptake in *A. halimus* than *B. juncea* and *T. arvense*. In conclusion, results show that pH affects metal bioavailability and uptake by hyperaccumulator plants. Addition of organic amendments could be a successful technique for stabilization of metals in contaminated soils.
Key Words: Hyperaccumulator, Phytoextraction, Mine soil, Organic amendment, *Thlaspi arvense, Brassica juncea*

1. INTRODUCTION

Metal mining and smelting activities are important sources of heavy metals in the environment, resulting in considerable soil contamination. The accumulation of these metals in soil can result in a decrease in soil microbial activity, biodiversity and soil fertility, crop yield losses, and even damage on animal and human health through the food chain. Various *in situ* and *ex situ* techniques have been employed to remediate soils but most of them are expensive or unsuitable.

Phytoremediation, defined as the use of green plants to remediate contaminated soils or waters, is a cost-effective and environmental-friendly strategy, which can compliment or replace conventional approaches. There are several specific subsets of metal phytoremediation being developed. One of them is phytoextraction, in which high biomass metal-accumulating plants and appropriate soil amendments are used to transport and concentrate metals from the soil into the above-ground shoots, which are harvested with conventional agricultural methods (Raskin et al., 1997). One approach for phytoextraction is to use hyperaccumulator plants, which are plants capable of accumulating more than 100 times larger concentrations of metals than normal plants (Brooks et al., 1977).

Several studies have been made using Indian mustard (*Brassica juncea*), an oilseed crop tolerant of the Mediterranean climate and capable of substantial heavy metal accumulation in its above-ground parts (Kumar et al., 1995; Blaylock et al., 1997). On the other hand, species belonging to the genus *Atriplex* may be of special interest because of their high biomass production associated with a deep root system able to cope with the poor structure and xeric characteristics of several polluted substrates. In this study Mediterranean saltbush (*Atriplex halimus*) was used, which is present as a natural invading shrub in several mining areas of Northern Africa and Sothern Europe. Also a non-accumulator species was used to compare results, *Thlaspi arvense*, a Brassicaceae related to *Thlaspi caerulescens*, another known hyperaccumulator.

Disadvantages of hyperaccumulator plants are that they have a small biomass, usually are only tolerant to one or two metals and are sensitive to climate conditions. A low-cost option could be stabilization using organic matter. Organic amendments can decrease the bioavailability of heavy metals in soil by adsorption and by forming stable complexes with humic substances (Shuman, 1999), thus permitting the re-establishment of vegetation on contaminated sites. This organic matter can re-distribute heavy metals from soluble and exchangeable forms to fractions associated with organic matter or carbonates and the residual fraction. Also, the use of organic wastes as source of organic matter for agricultural or ecological benefit is a way of recycling them. Effects of organic matter on metal fractionation in soil are pH-dependant. Walker et al. (2004) suggested that changes in soil pH and the presence of phosphorous and inorganic salts in organic amendments could contribute more to the change in metal fractionation in soil when such amendments were applied than the nature and the humification degree of the organic matter.

The objectives of this study were to investigate the effect of two organic amendments (pine bark compost and manure compost) on metal concentration in plants and on fractionation of metals in an acid and sandy mine soil from Madrid through pot experiments. Several hyperaccumulator species were grown and compared evaluating such plants performance in relation with the different chemical fractions of metals.

2. MATERIAL AND METHODS

Two heavy metal contaminated soils from the north of Madrid (Spain) were selected for this study. The first one ("Garganta") is situated at the village Garganta de los Montes close to an abandoned copper mine. The second ("Cuadron") is situated in El Cuadron where there is an old blend mine. Soils were collected from the top 20 cm and samples were air-dried and sieved to < 2 mm for analysis. In the samples different properties were determined (Table 1). pH and Electric Conductivity (EC) in distilled water (1:2.5), Total Organic Carbon (TOC) by Loss On Ignition method, Water soluble Carbon (WSC) by Ciavatta et al. (1991) and Benito (2002), Cation Exchange Capacity (CEC) by barium chloride method, total heavy metal content (Cu, Zn and Cd) extracted in aqua regia and texture. These two soils are acid, sandy and poor in organic matter. Two organic amendments were added to these soils: a compost made of sheep and horse manure with wood fiber ("Manure"), and a compost of pine bark, wood fiber and peat ("Pine bark"). Main properties of these amendments are also shown in Table 1. Ten different treatments were prepared with mixtures of each soil with one of the amendments and the doses applied were 0, 30 and 60 Mg ha^{-1} of dry organic matter. Thus, treatments with Garganta soil were: soil not amended (G0), soil with 30 Mg ha^{-1} of manure compost (G30M), soil with 60 Mg ha^{-1} of manure (G60M), soil with 30 Mg ha^{-1} of pine bark compost (G30P) and soil with 60 Mg ha^{-1} of manure (G60P); and treatments with Cuadron soils were: C0, C30M, C60M, C30P and C60P.

In this study three species were used: *Brassica juncea*, *Atriplex halimus* and *Thlaspi arvense*. Plants were grown in 0,7 L polyethylene pots filled with 700 g of soil and amendment mixtures mentioned above. The base of the pots was covered with a metallic mesh and with a 2-3 cm layer of gravel. Pots were placed at a greenhouse with four replicates per treatment and for each species one control treatment with pine bark, wood fiber and peat but without contaminated soil was used. A total of 44 pots per species were prepared.

B. juncea and *T. arvense* seeds were stored 12 days at 3±2ºC and 7 days at 10-25ºC. *B. juncea* seeds were scarified in order to promote its germination. Eight seeds of these species were sown in the pots and plants were harvested 110 days after sowing. Seedlings of *A. halimus* were prepared in root boxes with perlite and environmental control and later one seedling was transplanted into each pot. *A. halimus* plants were harvested 393 days after transplanting.

Plants were watered with a nutrient solution containing 17 g L^{-1} $Ca(NO_3)_2$; 0,50 g L^{-1} KNO_3; 0,16 g L^{-1} $H_2PO_4NH_4$ and 0,20 g L^{-1} NH_4NO_3. This solution was added manually every one or two days (30-60 ml) to keep the water content near to field capacity but avoiding leaching. Temperature and insolation were also controlled at the greenhouse.

Table 1. Physical and chemical properties of soils, amendments and different mixtures

		pH	EC (dS m^{-1})	TOC (%)	WSC (g kg^{-1})	CEC (cmol$_+$ kg^{-1})	Total Cu (mg kg^{-1})	Total Zn (mg kg^{-1})	Total Cd (mg kg^{-1})	Texture USDA
Garganta	G0	6.2	0.08	1.52	1.41	4.74	801.2	238.4	6.5	Sand
	G30M	6.5	0.51	1.88	8.78	5.28	879.9	315.8	7.6	
	G60M	6.8	0.94	2.53	18.61	5.99	939.9	336.4	8.2	
	G30P	5.9	0.11	1.92	3.51	5.30	1130.9	363.3	8.6	
	G60P	5.6	0.17	2.61	4.61	5.68	957.4	325.1	8.5	
Cuadron	C0	5.5	0.10	2.39	2.51	4.79	251.4	146.2	3.0	Sand
	C30M	5.8	0.58	2.73	1.64	5.67	264.5	156.6	3.3	
	C60M	6.1	0.99	3.29	2.29	6.16	290.0	167.0	3.2	
	C30P	5.4	0.16	2.81	5.68	4.87	252.9	141.8	3.2	
	C60P	5.3	0.16	2.80	6.78	5.89	268.5	157.2	3.3	
Manure		9.4	4.95	46.95						
Pine bark		5.7	0.40	78.96						

After harvest plants were cut at ground level and stems, leaves and inflorescences of each plant were separated and weighed for fresh weight determination. Also plant height and inflorescence height were measured. Roots were rinsed with deionised water and roots and aerial organs were dried in an oven at 65ºC for 48h. Dry weight was measured and material was ground gathering together stems, leaves and inflorescences.

For each vegetable sample, digestion of ground dry matter was accomplished by a dry ashing procedure (Tüzen, 2003) at 450ºC for 4h and dissolving ashes in HNO_3 (25 % v/v) in order to measure metal concentration in plant tissues (shoots and roots).

A sequential extractions procedure was applied to fractionate Zn, Cu and Cd presented in pots after plant harvest, following the methodology of Tessier et al. (1979) and Lena and Rao (1997), designed to separate heavy metals into six operationally defined fractions: Water Soluble, Exchangeable, Carbonate-bound, Fe-Mn Oxides-bound, Organic-bound and residual. Water Soluble and Exchangeable were summed and data was presented as Water Soluble + Exchangeable.

Heavy metal concentration in solutions was measured by Atomic Absorption Spectrophotometry (AAS). All reagents used were of analytical grade or better. Double deionised water was used for all dilutions and all the plastic and glassware were cleaned by soaking in dilute HNO_3.

Statistical treatment of experimental data was performed using SPSS 14.0. Means were compared by a one-way analysis of variance (ANOVA) with Duncan's test ($P \leq 0.05$) and in order to assess relationships between metal fractions and metal concentration in plant tissues Pearson's correlation coefficients (r) were obtained by a two-tailed test. Standard deviations were calculated to determine means variability between replicates.

3. RESULTS AND DISCUSSION

Plant growth presented different values in the applied treatments as indicates shoot and root dry weight (figure 1), and in all cases was lower than growth obtained in control pots. Plants grown in pine bark mixtures and in not amended soils did not result in a high biomass production. Even, all *T. arvense* plants (G0, G30P, G60P, C0, C30P and G60P) and some of *B. juncea* (C30P and almost every pot of G30P) did not germinate in such treatments or died few days after the germination. However, manure treatments reached higher yields and every plant of *B. juncea* and some of *T. arvense* (only in Garganta treatments) presented flowering at the end of the crop. The higher growth was probably due to a lower metal bioavailability and therefore a low metal stress, specially for the non-accumulator *T. arvense*, and also because the improvement of soil fertility with these amendments. Wu et al. (2004) observed that N and P application produced a increasing yield in *B. juncea* that resulted in a higher Cu uptake, in spite of a decrease in Cu concentration.

A.halimus developed more biomass production in all treatments than *B. juncea* and *T. Arvense*. The last was the species that presented the lowest growth, specially in Garganta treatments where the high Cu content of this soil probably induced Cu stress in this plant. In all cases dry weight of root material was much less than the weight of aerial parts but in most cases both followed the same pattern.

The metal concentration in different parts of the plants was related to the amendment applied as it is shown in figure 2, 3 and 4. Highest plant metal concentration (Zn, Cu) was observed in pots treated with pine bark amendment and with pure soil. On the contrary, there was a lower metal concentration in plant tissues of pots with manure due to a decrease in metal bioavailability caused by an increasing pH with the addition of an alkaline amendment such as horse and sheep manure (pH 9.4) to an acid soil. The lower concentration is emphasized with an increasing dose of manure amendment. Organic matter contributed by this amendment and increasing pH affects the adsorption sites of soils and reduce metal uptake by plants. On the other hand, metal concentration was not significantly different with the addition of pine bark amendment compared to not amended soils.

Cu concentration in plant tissues was higher with Garganta soil (copper mine) while there was more Zn content in plants with Cuadron soil (zinc blende mine), except in G60P where Zn concentration in roots of *B. juncea* (14429 mg kg^{-1}) was extremely high.

Cu concentration in roots was generally higher than the concentration in aerial organs of the plants, whereas Zn in shoots was much higher than root concentration in some treatments of *A. halimus* (C0 and C30P) and *T. arvense* (G30M and G60M).

Generally there was a greater Zn concentration in shoots and roots of *B. juncea* and *T. arvense* than *A. halimus* but this last had a higher shoot Cu concentration. Ebbs and Kochian (1997) showed that *Brassica* spp. were more effective at removing Zn from a nutrient solution than Cu and that the removal of each metal was reduced in presence of both.

Cd concentration in plant tissues was also measured but it was in most cases below detection limits (< 0,02 mg L^{-1}).

In spite of the higher concentration of metals in plants grown in pine bark amendment and in pure soil the total uptake by plants (Figure 2, 3 and 4) was in most cases lower in these treatments due to the lesser growth reached. Results suggest that manure amendments not only stabilize metals in mine soils but also allow more plant growth and consequentially an increasing amount of metals removed from soil.

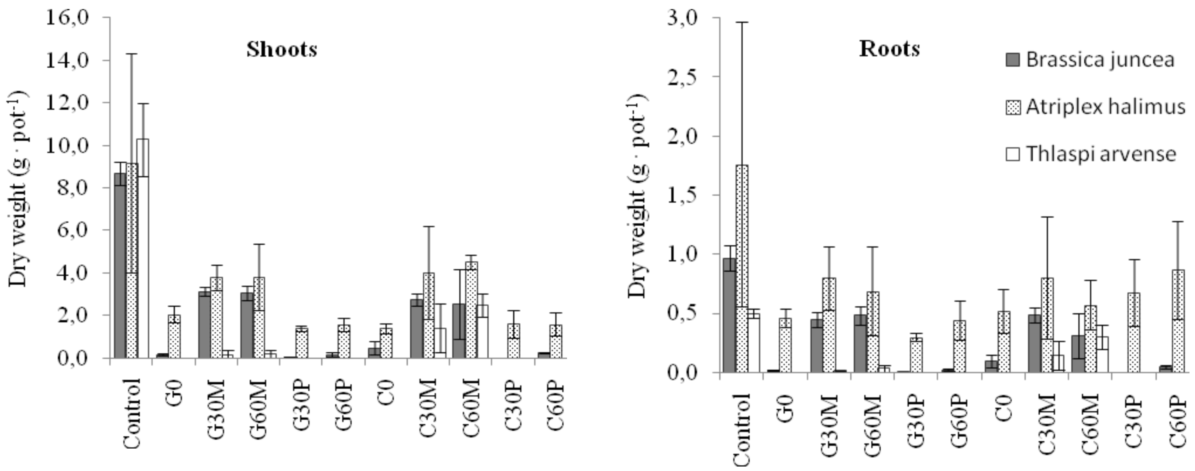

Figure 1. Shoot and root dry weight after harvest of each crop in different soils and amendments (Error bars represent standard deviation)

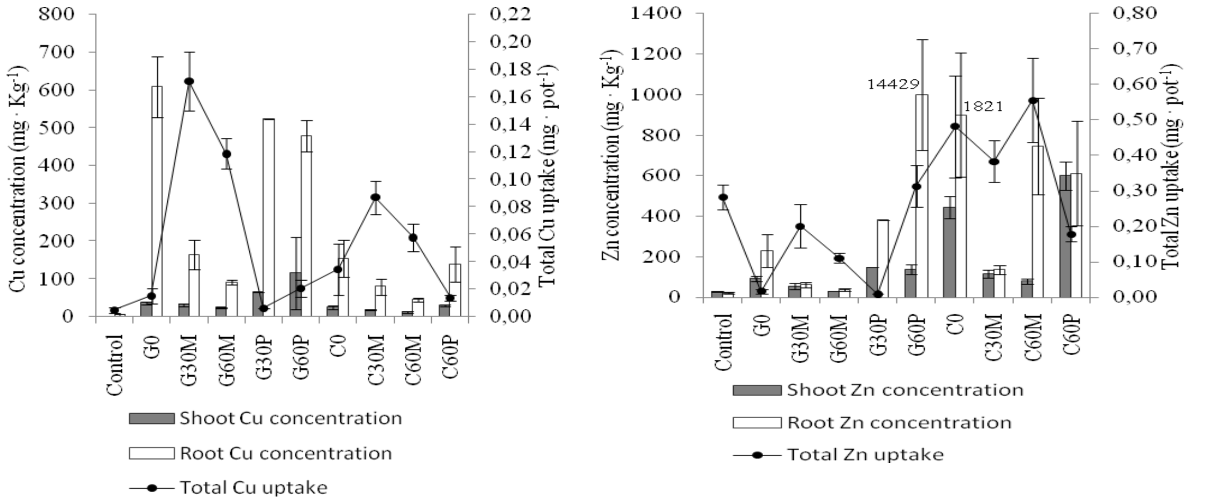

Figure 2. Metal concentration in shoot and root and total metal uptake by *B. juncea* in different soils and amendments (Error bars represent standard deviation. Bars of G60P and G0 of Zn concentration were reduced in order to maintain the graphic scale. Their total values are shown on the top of the bar and error bars were proportionally reduced)

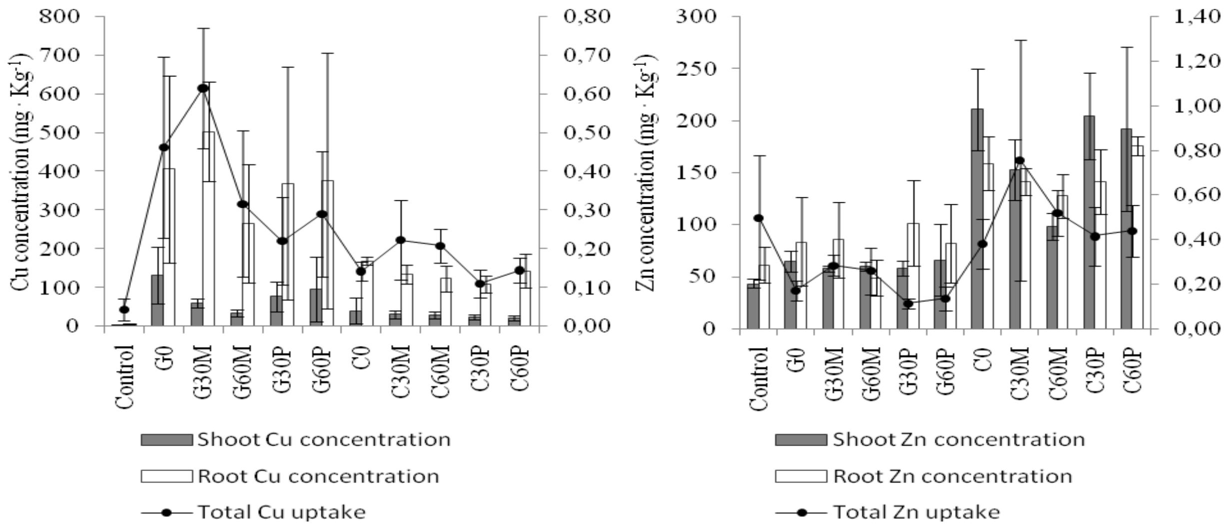

Figure 3. Metal concentration in shoot and root and total metal uptake by *A. halimus* in different soils and amendments (Error bars represent standard deviation)

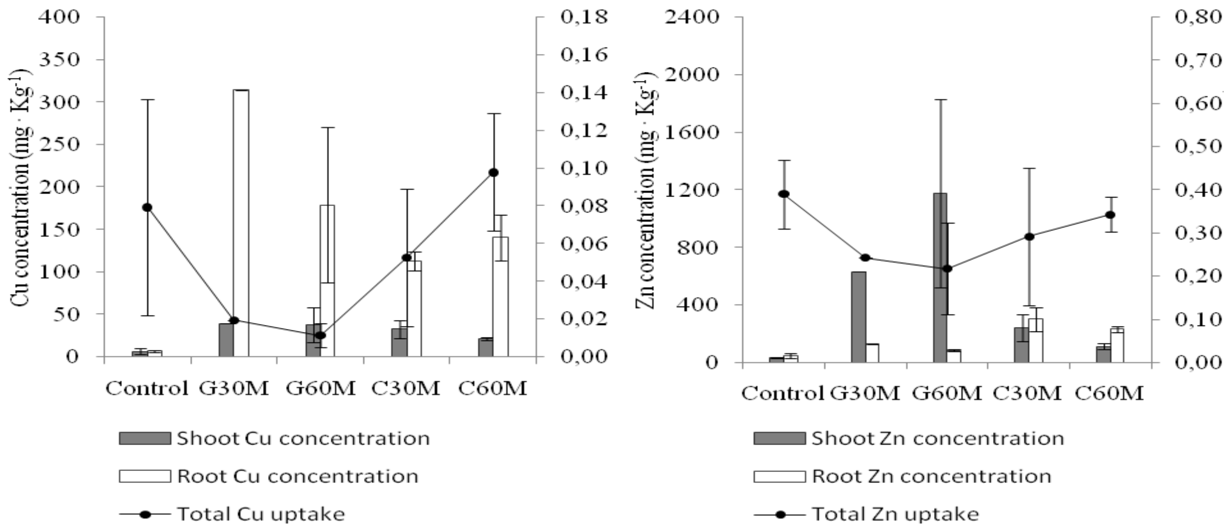

Figure 4. Metal concentration in shoot and root and total metal uptake by *T. arvense* in different soils and amendments (Error bars represent standard deviation)

A sequential extractions procedure (Tessier et al., 1979) was carried out in order to observe the different chemical fractions of Zn and Cu (

Figure **5**). Residual fraction was not measured because its little influence on metal bioavailability. Results show that a high amount of Zn was bounded to Fe-Mn oxides because it is strongly associated with oxides whereas a considerable amount of Cu, which has a high affinity for carboxylic and phenolic groups, was associated to organic matter. Also the water soluble + exchangeable fraction of Zn was high, specially in Cuadron treatments.

Althought *A. halimus* presented a lower Zn concentration it was the species that achieved the greatest metal uptake (Cu and Zn) because its greater growth, with the highest accumulation in aerial organs and therefore suitable for phytoextraction techniques. Lutts et al. (2004) observed that *A. halimus* accumulates Zn and Cd in aerial organs without showing any significant decrease in biomass production during 3 weeks in nutrient solution. On the contrary, *T. arvense* had the lowest metal uptake.

The most labile fraction of Cu and Zn (water soluble + exchangeable) decreased while carbonate fraction increased when manure amendments were added to soils. Xian and In Shokohifard (1989) found that the proportion of Zn in the exchangeable fraction increased and that Zn in the carbonate fraction decreased when soil pH was lowered. This loss in soluble + exchangeable fraction was balanced with an increasing organic-bounded Cu or Zn bounded to Fe-Mn oxides. These changes were due to the increasing pH of manure amendments that affected the variable charge of organic compounds and Fe-Mn oxides. The two soils followed the same pattern but Cuadron treatments presented more Cu and Zn bioavailability whereas in Garganta treatments the sum of Cu recovered across the four fractions was considerable higher.

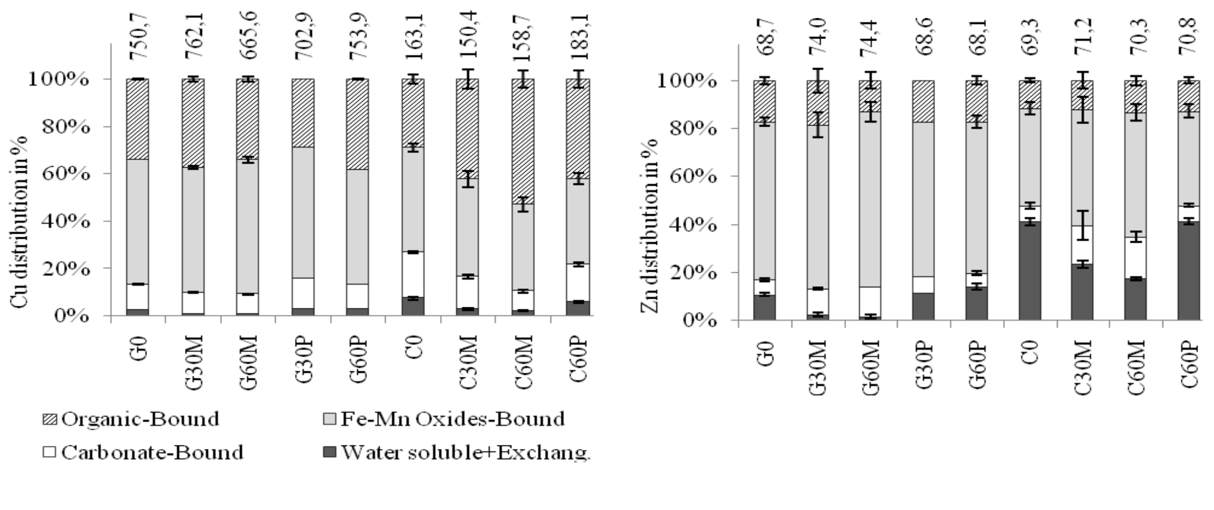

Figure 5. Distribution of Zn and Cu in various non-residual chemical fractions in different treatments after the harvest of *B. juncea* by a sequential extraction procedure (Tessier et al., 1979). For each treatment, the sum recovered (mg kg^{-1}) across the four fractions appears above the bar (Error bars represent standard deviation)

Table 2. Pearson's correlation coefficients (r) between metal uptake and concentration in plant organs (shoots and roots) of *B. juncea* and metal concentration in the different fractions by the Tessier et al. (1979) procedure

Correlation coefficient (r)		I.Water soluble+ exch.	II.Carbonate	III.Fe-Mn oxides	IV.Organic	Sum of I+II
Zn	Shoot	0.90**	-0.29	-0.36*	-0.27	0.77**
	Root	0.06	-0.29	0.07	0.11	-0.04
	Total uptake	0.40*	0.33	-0.42*	-0.38*	0.48**
Cu	Shoot	0.62**	0.48**	0.41*	0.49**	0.54**
	Root	0.89**	0.80**	0.63**	0.66**	0.86**
	Total uptake	-0.40*	0.24	0.42*	0.30	0.10

** Significant at probability level $P < 0.01$; and * Significant at probability level $P < 0.05$

Table 2 shows correlation coefficients between metal concentration in plants and total uptake by *B. juncea* and concentration of the different non-residual fractions in soils. The strong correlation between water soluble + exchangeable fraction and Zn shoot (r = 0,90) and Cu root (r = 0.89) concentration stands out and this fraction is also good correlated with Cu shoot concentration. Therefore, metal concentration in plant tissues increased in response of a higher soluble and exchangeable metal content in soil. There was also a good correlation between carbonate fraction and Cu in roots and less with this metal in shoots, whereas Zn concentration did not present such relation. If the sum of these two first and most available fractions is considered these results are consistent with Xian (1989) who found that the sum of exchangeable and the carbonate-bound forms were strongly correlated with Zn uptake by *Brassica oleracea*.

4. CONCLUSION

The addition of manure compost to metal contaminated soils resulted in a higher biomass production and a lower Cu and Zn concentration in plant tissues. This was probably due to a low metal bioavailability caused by an increasing pH and the contribution of organic matter that not only reduced metal phytotoxicity but was also a source of nutrients for plants. Moreover, a larger amount of metal was removed from soils when adding this amendment given the higher plant growth. Addition of this type of organic amendment could be effective for stabilization of metals in contaminated soils. In contrast, pine bark amendments did not achieve such results.

It was observed that *A. halimus* developed a considerable growth in spite of metal stress conditions and achieved the greatest Cu and Zn uptake making it suitable for phytoextraction purposes. The sequential extractions procedure of Tessier et al. (1979) was designed to separate Zn and Cu in different fractions showing that the most labile fractions decreased with the application of manure amendment increasing the proportion of metals associated to more unavailable chemical forms. This decrease was reflected in a lower metal concentration in plant tissues of *B. juncea*.

5. ACKNOWLEDGEMENT

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