PHYTOREMEDIATION OF POLLUTED SOILS: RECENT PROGRESS AND
DEVELOPMENTS

**Assessment of trace elements phytoavailability in compost amended soils using
different methodologies**

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Abstract

Purpose: This study evaluates the effects of two soil amendments and the growth of two plant species on labile trace elements (TE) fractions in two different contaminated soils.

Materials and methods: We studied the effects of two organic amendments (biosolid compost and alperujo compost) and two plant species (*Medicago polymorpha* and *Poa annua*) on pH, total organic carbon (TOC) and TE availability, by three extraction methods (CaCl₂ aqueous solution, Soil Pore Water-SPW and Diffusive Gradient in Thin Film-DGT), in two contaminated soils with contrasting pH values (Aznalcázar, 6.32 and Vicario, 3.23) in a 118-days pot experiment. The effects of the composts on labile TE fractions were compared with elements concentrations in plants.

Results and discussion: No relevant effects of amendments and plants were found on the physical and chemical characteristics of the Aznalcázar soil. However, the addition of amendments was essential for plant species growing in the acid Vicario soil. In this soil, amendments and plant growth increased pH and TOC and reduced substantially TE bioavailability. Although absolute values of bioavailable TE contents obtained by the three methods were very different and followed the trend CaCl₂ extraction>SPW>DGT, these values follow a similar behavior in the two studied soils and for the two species.

Conclusions: the results demonstrate that the application of organic amendments are suitable for remediating acid TE-contaminated soils, for the establishment of a vegetation cover on previously bare soils for reducing wind and water erosion and for reducing labile TE fractions to prevent leaching of pollutants into subsoil or groundwater layers. Moreover the results obtained in this study pointed out that under microcosm conditions the three methods tested (CaCl₂ extraction, SPW and DGT) to predict TE bioavailability were highly correlated.

Keywords CaCl₂ extraction • DGT • Soil pore water • Soil remediation

1 Introduction

In the last century the human activity has been responsible for the contamination of a vast number of sites worldwide (He et al. 2005). Trace elements (TE) have been deposited in soils through agricultural and industrial activities and have subsequently led to considerable detrimental effects on the environment and human health (Adriano 2001).

Trace elements remain in soils for very long periods of time (Brookes 1995). The extent of TE contamination is typically characterized by the total concentration of TE. However, this is a poor indicator of potential metal toxicity, since it does not give information on the ability of the elements to be absorbed by plants or the transfer of toxic elements through the food chain (Morel 1997). Bioavailable TE concentrations reflect the greatest potential risks for the ecosystem (Adriano et al. 2004). The bioavailable contaminant fraction in soil, typically, refers to the most labile fraction (sum of soluble and weakly absorbed fractions) (Adriano 2001). Numerous procedures have been developed to estimate labile TE fractions in soils. Extraction with weak neutral salt solutions ($0.01 \text{ mol L}^{-1} \text{ CaCl}_2$; $0.1 \text{ mol L}^{-1} \text{ NaNO}_3$) is commonly used for the assessment of the exchangeable fraction of TE in soils. In addition, the extraction of interstitial soil pore water (SPW) is considered a valid alternative for the assessment of TE availability (Beesley et al. 2010; Clemente et al. 2008; Ciadamidaro et al. 2013) as it isolates the aqueous phase to which plant roots and microorganisms are exposed (Nolan et al. 2003). Moreover, SPW extraction does not require highly invasive or destructive procedures and allows repeated sampling (Clemente et al. 2008). However, several authors have shown that extractable or soluble TE fractions often show poor correlation with concentrations in organisms like plants (Zhang et al. 2001; Degryse et al. 2009; Tandy et al. 2011). A holistic method, the diffusive gradient in thin films (DGT), was

proposed by Zhang and Davison (1995) as a tool for quantifying exchange between the solid phase and the soil solution. This technique, which is a diffusion driven passive sampling device developed and used for quantitative determination of labile metal fractions, has been reported to measure satisfactorily metal bioavailability (Zhang et al. 2001; Degryse et al. 2009; Zhang and Davison 2006). The DGT is a more mechanistic surrogate of diffusion controlled plant uptake since it acts as a sink, like plant roots, and accounts for diffusion of elements to the root surface, driven by depletion and subsequent resupply from the solid phase (Degryse et al. 2009).

The objective of this study was to evaluate the short-term effects of two soil amendments and the growth of two plant species, *Medicago polymorpha* L. and *Poa annua* L., on the labile TE fractions in two different contaminated soils. To assess which of the amendments and plant species were better improved soil quality, available TE concentrations were determined by using three different procedures (0.01 mol L⁻¹ CaCl₂ extraction, SPW and DGT). We also compared these results with the response of plants to the changed TE bioavailability upon amendment addition. This study hypothesized that the addition of the amendments and the growth of plant species would influence available TE fractions by changing soil pH and organic C contents. It was also hypothesized that the different methods to predict TE availability to plant could be comparable and correlated with plant uptake.

2 Materials and methods

2.1 Soil and compost characterization

The experiment was carried out using two TE contaminated soils with different pH values, Aznalcázar (AZ) and Vicario (V) (Table 1). Both soils were collected in an area affected by a mine sludge spill, (Aznalcóllar mine accident; Grimalt et al. 1999). Soils

were collected from the upper 25 cm of soil. Soil AZ was a sandy loam with neutral pH and relatively high TOC concentrations; whereas soil V was a very acid loam with relatively low TOC concentrations.

Two different composts were used as amendments: Biosolid compost (BC) and “alperujo” compost (AC), since they were locally available at low cost and were already successfully applied in the affected area (Burgos et al. 2010). Biosolid compost was collected from the composting plant “EMASESA” (Seville, Spain) and was produced from sewage and pruning from parks and gardens in the city of Seville. The “alperujo compost” was prepared by the cooperative "Coto Bajo" Guadalcazar (Córdoba, Spain) by mixing legume residues and manure from organic farming. The alperujo compost was more alkaline but contained lower total N concentrations than the biosolids compost (Table 1).

Compost samples were analysed following standard procedures for soil improvers and growing media of the European Committee for Standardization (CEN). Moisture was determined and results were expressed on a dry matter basis. Fresh compost samples were used for the determination of electrical conductivity and pH in a 1:5 (V:V) aqueous extract. The organic matter content (OM) was determined by dry combustion at 450 °C and total N content was determined by distillation after Kjeldahl digestion. Water-soluble organic carbon was determined using a TOC analyser (Simadzu TOC-VCSCH) after water extraction. Nutrients and TE contents were determined after *aqua regia* digestion in a microwave oven by ICP–OES. Compost samples from the WEPAL programs were also analyzed for quality control of analytical procedures. The results obtained for these samples agreed $\pm 5\%$ with the certified results.

2.2 Experimental design and samplings

The experiment was carried out in a greenhouse using pots (3 L of volume each) that were filled with the experimental soils. A factorial design was used consisting of the two soil types, two plant species, and three compost treatments (control with no compost (control, CO), biosolid compost, BC, and alpurujo compost, AC) with three replications for a total of 36 pots. The pots were distributed following a randomized block design. The amendments were added in the upper layer of the pots and mixed with the top 10-15 cm of soil. The application rate was 26 g kg⁻¹, corresponding to a field dose of 50,000 kg ha⁻¹. No fertilizers were added to the pots during the whole experiment.

Two plant species were selected for the experiment: ME, *Medicago polymorpha* (*Leguminosae*) and PO, *Poa annua* (*Poaceae*). Both species are common in the Mediterranean area and also occur in the region where the soils were obtained (Madejón et al. 2006).

At the beginning of the experiment, ‘Rhizon’ samplers (MOM 10 cm, Eijkelkamp Agrisearch Equipment, The Netherlands) were inserted laterally in three pots per soil, species and treatment (total of 36 pots).

Four days after the addition of amendments, bulk soil samples (initial sampling) were collected from the top 10 cm of each pot using an auger. Immediately after this sampling the seeds were sown. Plant germination was relatively consistent for all the plants and treatments, excepted in control pots with the acid soil (V) without amendments where no successful seed germination occurred. Plants were grown in the pots from November 2011 to March 2012, and the pots were regularly irrigated with tap water from the bottom (about twice a week) to meet the water demand of the plants (water holding capacity 32.4 % for AZ soil and 29.6 % for V soil). The greenhouse temperature was maintained between 19 °C and 29 °C. Minimum temperatures occurred

between 6 a.m. and 7 a.m. (19-19.5 °C) and maximum temperatures between 5 p.m. and 6 p.m. (28.6-29 °C).

Plant biomass was estimated by two harvests: 68 and 118 days after the sowing. In both samplings biomass was weighted. Plant shoots were washed for 15 s with a 0.1 mol L⁻¹ HCl (30 %, Suprapur) solution then for 10 s with deionised water. Washed samples were oven dried at 70 °C. Dried plant material was ground and passed through a 500 µm mesh stainless-steel sieve (MF 10 basic, IKA Labortechnik) prior to analysis. After 118 days of plant growing SPW was sampled using removable needles attached by a Luer-Lock connection to the sampler and vacuum tubes. Finally soil samples at 0–10 cm depth were also taken.

Soil samples were air-dried and passed through a 2 mm mesh sieve, homogenized and oven-dried at 40 °C for at least 48 h for chemical analyses. Dry samples were ground to < 60 µm for pseudo-total TE analysis.

2.3 Plant analyses

Trace elements (As, Cd, Cu, Mn, Pb and Zn) concentrations in plant tissues were determined by ICP-OES (Varian ICP 720-ES) after wet oxidation with concentrated HNO₃ (65 %, trace analysis grade) under pressure in a microwave digester. The samples were then diluted to 50 ml with deionized water and then filtered (pore diameter < 45 µm). The recovery efficiencies during digestion were determined using a certified reference material (INCT-TL-1, Tea leaves). The limits of quantification, (LOQ) of the method used were: As 0.1 mg kg⁻¹, Cd 0.03 mg kg⁻¹, Cu 0.03 mg kg⁻¹, Pb 0.3 mg kg⁻¹, and Zn 0.03 mg kg⁻¹. Recoveries for TE concentrations in the reference plant samples ranged between 90 and 110 %. Total TE uptake by plant species (shoots) was calculated in both samplings having dry biomass values and TE concentrations.

2.4 Soil chemical analyses

Soil pH was measured in 1 mol L⁻¹ KCl extracts (1:2.5, *m/V*) after shaking for 1 h (Hesse 1971) using a pH meter (CRISON micro pH 2002). Total organic carbon (TOC) in soil was analyzed by wet oxidation using dichromate (K₂Cr₂O₇) and titration with diammonium iron (II) bis(sulfate) (Walkley and Black 1934). Pseudo-total element concentrations in the soils (< 60 µm) were determined by ICP-OES (Varian ICP 720-ES) following *aqua regia* digestion in a microwave oven (ISO 1995). The quality of the analysis was assessed using the reference soil sample BCR-143R (sewage sludge amended soil EUR 15284 EN). Obtained recoveries ranged from 96.7% (Mn) to 103% (Zn).

2.5 Trace elements availability analysis

The 0.01 mol L⁻¹ CaCl₂-extractable TE concentrations in soils were determined in 0.01 mol L⁻¹ CaCl₂ (1:10, *m/V*) extracts after shaking for 3 h (Houba et al. 2000), then filtered through Whatman No. 2 (pores diameter < 25 µm) filters. Analysis of TE concentrations in soil extracts was performed using ICP-OES.

Soil pore water (SPW) samples were analyzed for pH, and for elements using ICP-OES. The limits of quantification (LOQ) were as follows: Cd 0.1 µg L⁻¹, Cu 0.1 µg L⁻¹, Mn 1.0 µg L⁻¹, Pb 3.0 µg L⁻¹, and Zn 0.2 µg L⁻¹.

Diffusive fluxes of metals in soil samples were determined according to Zhang et al. (2005) using DGT fitted with Chelex-100-resin-impregnated gels. Diffusive gradient in thin films devices were prepared according to the procedure described by Zhang et al. (2005). Air-dried soil samples (from final soil sampling) were moistened by adding sufficient deionized water to adjust the soil moisture to 80 % of water-holding capacity. The samples were placed in an incubator (Cooled Incubators IPP with Peltier-

technology, IPP 500, Memmert) at 20 °C for 24 h for equilibration. Approximately 5 g of moist soil sample (three replicates per sample) were placed on the top of the DGT devices, stored in closed plastic boxes containing a water-saturated atmosphere, and kept in an incubator at 20 °C for 4 h. After incubation, DGT devices were washed with deionized water to remove residual soil. Subsequently, the metals adsorbed to the chelex resin gels were eluted in 10 mL of 1 mol L⁻¹ HNO₃. The eluate was analysed for TE by ICPMS (Perkin Elmer 9000 DRCe). The time-averaged interfacial concentrations (C_{DGT}) were calculated according to Zhang et al. (2005). The concentration of all TE were referred to the total amount of soil contained in the pot. For that reason the results of TE availability are presented as amount (µg of each TE in the pot at the end of the experiment).

2.6 Statistical analysis

All statistical analyses were carried out with the program SPSS 20.0 for Windows. Comparisons of treatment means were performed using post-hoc tests following a one-factorial ANOVA. The Tukey's test was performed in case of homogeneity of variance between the three subgroups, or Student's t test only if there were only two subgroups. Correlation analysis was performed to determine the relationship between the different parameters methods assayed. The significance level reported ($p < 0.01$ and $p < 0.05$) is based on Pearson's coefficients.

3 Results and discussion

3.1 Effect of compost on plant performance and elements uptake

In soil AZ the addition of amendments had no significant influence on plant yield (except in *P. annua* first harvest) (Table 2). In the acid soil V, addition of amendments

was essential for the growth of plant, especially for *M. polymorpha* and the treatment with BC, for which significantly higher biomass values were obtained, compared to AC treatment (Table 2). No plant growth was observed on V control soils. The amendment addition was the key factor on the growth of plants in this soil. In the amended V soils the plant biomass was similar to that obtained in soil AZ (except in *M. polymorpha* first harvest). The improvement of biomass production found in V soil seems apparently related to the increase of soil pH and a corresponding reduction of labile TE fractions (Pérez de Mora et al. 2011).

In general, in AZ soil for both species, Cd, Cu, Mn and Zn concentrations in plant were in the background range for plants on neutral soils (Table 3) (Chaney 1989). However, the addition of amendments had no relevant effect on TE concentrations in plant tissues; a significant decrease in TE in plant tissues due to BC was only observed in the first harvest for *M. polymorpha* for Cd and in the second harvest for both species in case of Zn (Table 3).

In the acid soil V, TE concentrations in shoots tended to be higher than normal levels in plants (even with the addition of amendments) (Table 3), especially in case of *P. annua*. Manganese concentrations were much higher in plant tissues of *P. annua* (~ 2000 mg kg⁻¹) than those observed in *M. polymorpha* and those found by Madejón et al. (2006) for the same species in the same soil under field conditions. The enhanced Mn concentrations in plant shoots were reflecting an increase of the labile Mn fractions (Table 3).

Figures 1, 2, 3 and 4 (a, e) showed the amount of TE uptake for both plant species in both soils with different treatments. As plant biomass was similar for both species in each soil and treatment, *P. annua* uptook more amount of TE (except for Cu) than *M. polymorpha* due to the higher concentration of these elements in the Gramineae family

(Table 3). These data confirm the findings of Kuboi et al. (1986), in which Leguminosae family is classified for Cd as a low accumulator and Graminae family as a moderate accumulator.

3.2 Effects of amendments and plant species on soil pH and organic matter

Soils were analysed for pH and TOC before plant growth (initial sampling), and after plant growth (final sampling) (Table 4). Initially in both soils the addition of amendments increased the values of pH, and significant differences compared to the control in both soils were found (Table 4). However the effects of amendments were more noticeable in the acid soil (V). It is known that the application of organic amendments to acid soil increases pH values and reduces the concentration of soluble and exchangeable Al. Different mechanisms have been proposed to explain the alkalizing effect of the organic matter: 1) consumption of protons during the decarboxylation of organic acid taking place in the decomposition of amendments added to the soil; 2) Proton consumption by functional groups associated to the organic material; 3) specific adsorption of organic molecules on surfaces minerals by ligand exchange, releasing OH (Mokolobate and Haynes 2002; Naramabuye and Haynes 2006).

The pH values of the rooted soil in amended V soil, obtained in the final sampling (Table 4), were similar to those obtained in the first sampling. In this soil it was not possible to evaluate the direct influence of the plants (without amendment addition) because, in this soil, plants were not able to grow. In AZ soil, the pH of control treatments increased for both plant treatments (Table 4), probably due to root activities causing alkalization (Dunbabin et al. 1988).

Total organic carbon content in both soils was also improved by amendment addition (Table 3). Due to the high initial organic matter content, the addition of amendments did not induce a considerable change in the TOC content of soil AZ. In case of the acid soil (V) the amendment effects were more noticeable. The soil V was characterised by a low initial content of organic matter due to the removal of the sludge from the first 10 cm of soil after the mine spill accident (Aguilar et al. 2004). For this reason, the application of organic amendments increased TOC in V soil threefold compared with the unamended control. Whereas the values of TOC did not increased in time and remained similar at the final sampling, the presence of roots had no significant effect on the total content of C (TOC) in amended soils (at least in short term experiments) (Table 4).

3.3 Effects of amendments and plant species on trace elements availability

Figures 1, 2, 3 and 4 showed the quantity (μg) of available TE (Cd, Cu, Mn and Zn) in the pots obtained by the three methods assayed and by the plants. Concentrations of As and Pb were below the detection limit when CaCl_2 extraction and SPW were used.

Absolute values obtained by the different methods were different and followed the extraction trend $\text{CaCl}_2 > \text{SPW} > \text{DGT}$. However the tendency respect to the effect of the amendments in both soils was very similar.

In soil AZ labile TE concentrations measured with the three different methods were low for all treatments and the application of amendments did not induce important changes compared to the control. Both target species had a remarkable influence on the availability of trace elements in this soil.

In AZ soil results obtained by extraction with CaCl_2 showed little differences between treatments and only in the case of Cu significant differences were found (Figure 2), with higher values in soils under *P. annua* amended with both composts. Results obtained by

the direct measure in the SPW showed some differences between treatments; however these differences depended on the species and on the element without a clear trend. In the case of the results obtained with DGT procedure significant differences between treatments were not observed for any of the studied elements or plant species. Nevertheless values of TE availability were very low compared to the pseudo-total TE content in this neutral soil. In general, the amount of available TE in acid soil V was much higher than those found in neutral soil AZ. In V soil the application of amendments clearly influenced the availability of trace elements. Both composts reduced the CaCl_2 -extractability of Cd, Cu and Zn. Moreover, concentrations of TE in SPW decreased in soils treated with both composts. These reductions are likely related to the increase of soil pH (Pierzynsky and Schwab 1993; Mench et al. 1994). On the other hand, CaCl_2 -extractable Mn increased in both soils in response to both amendments; probably due to the high initial Mn concentrations coming from both composts (Table 1). In accordance with these results, several authors (De la Fuente et al. 2008; Burgos et al. 2010) found increases in the extractability of Mn in contaminated soils treated with alperujo compost (AC) or derivatives. This kind of compost from olive wastes is known to be an important source of phenolic compounds. Several reports (Lehman et al. 1990; Huang 1990) have demonstrated that oxidative polymerisation of phenols can be accelerated non-enzymatically by several soil mineral fractions that can act as catalysers. Soluble monomeric phenols are oxidised, yielding water-insoluble polymers, while soil Mn will be reduced to oxidation states that originate more soluble chemical species.

In V soil, comparison between amended and non-amended treatments using DGT procedure cannot be shown, since soil without composts had pH values around 3, and DGT recoveries for cations in solutions are only in the range of 30-55 % (Zhang and

Davison 1995). Chelex-100 is less efficient at binding metals at low pH as metal ions are in competition with high concentration of hydrogen ions present in solution (Gimpel et al. 2001).

Based on SPW and CaCl_2 extracts, we could show that the application of both amendments reduced trace elements availability, likely by inducing various sorption processes upon pH increase. In general, TE sorption/dissolution processes are influenced by many factors: pH, redox potential, type of soil constituents, cation exchange capacity, etc. (Kumpiene et al. 2008). It is well known that pH is generally the main factor governing concentrations of soluble or labile TE fractions (Clemente et al. 2006). The incorporation of organic matter through the two different amendments produced a raise in soil pH and thus triggered the establishment of vegetation into the pots, whereas in non-amended soils no plant was growing.

3.4 Soil-plant correlations

Correlations between the amount of TE extractable by CaCl_2 , present in SWP, and determined by DGT and those found in plant tissues were calculated (Tables 5 and 6) to assess the predictability of the different methods and shoot concentrations.

Despite the differences in the absolute values, in TE amounts obtained by the three methods, a similar trend was observed that was supported by the high correlations between values of available Cd, Cu and Zn ($p < 0.01$) (Table 5). In the case of Mn no correlation coefficients were found and only between SPW and DGT values correlations were significant ($p < 0.05$) (Table 5).

Correlations between total amount of each TE uptake by the plants and the amount of TE available estimated by the three methods showed different results depending on the species evaluated (Table 6). In the case of *M. polymorpha* these correlations were

positive and significant only for Mn, and no correlations (or even negative values) were observed for the rest of the elements. A possible explanation would be that *M. polymorpha* is an excluder plant able to avoid TE absorption or retaining them in the root system. For Mn, the correlations were higher ($p < 0.01$) for SPW and less significant for CaCl_2 extraction and DGT ($p < 0.05$). In a previous study by Mundus et al. (2012) a lower predictability of plant Mn concentrations by DGT-available was reported, in particular for aerated soils. However, the higher correlations observed in our study (in both species) can be explained by the much greater range of available Mn concentrations in both soils.

For *P. annua*, correlation coefficients between the different values of available Cd, Mn and Zn obtained by the three methods and total plant uptake were higher than for *M. polymorpha* with high significance ($p < 0.01$) in most cases (except Mn with SPW; Table 6). For Cu these correlations were not significant. This finding is in contrast to previous observations, where high correlations between DGT-available Cu in soil and plant Cu concentrations (Zhang et al. 2001; Tandy et al. 2011) and between Cu extracted by CaCl_2 and Cu in plants (Burgos et al. 2008) were shown. In contrast to Mn, Cd and Zn, plant accumulation of Cu in our experiment was to a large extent independent from the amount of Cu extractable in soil. Since the available fraction concentrations of the other studied elements occurred in a much larger range, we assume that under conditions of high availability of Mn, Cd and Zn, the uptake of Cu could be decreased by competition with other elements. Trace elements correlation coefficients between plants and soil were more dependent on the plant species than the methodology used, and the better correlation obtained for *P. annua* demonstrated that it could be considered as indicator plant. For *P. annua* and for Cd and Zn high correlations coefficients were also observed using the three methods. Puschenreiter et al. (2013) have obtained similar results using

other two indicator species for Zn, whereas for Cd lower correlation coefficients were reported than in Table 6. For both elements, the highest correlations were found for SPW, due to the fact that the TE fraction extracted by this method represents the primary exposure to plant root system in soil (soil solution). The high reliability of DGT-labile TE concentrations in soil has previously been explained by the fact that DGT mimics the soil chemical processes that are also dominating the availability for plants, i.e. diffusion and labile metal release (Zhang et al. 2001).

4 Conclusions

The results of this experiment demonstrate that the application of organic amendments such as biosolid and alperujo compost, are suitable for the improvement and restoration of TE contaminated soils. The effectiveness of the restoration was especially noticeable in the acid TE contaminated soil, in which the addition of the composts allowed the establishment of a vegetation cover. This vegetal cover contributes to the prevention of wind and water erosion decreasing the mobilization of TE in these polluted soils to the adjacent areas.

The results obtained in this study pointed out that under our microcosm conditions the three methods tested (CaCl_2 extraction, SPW and DGT) to predict TE phytoavailability were highly correlated. Among the three methods tested, pore water is probably the most realistic and cost effective method. However, the extraction of the soil pore water is not always possible under Mediterranean field conditions because the low moisture content in the vadose zone only permits this extraction after rainfall episodes. Despite the accuracy of the diffusive gradient in thin film (DGT), this method could underestimate concentrations and new gels adapted to very acid soils have to be

developed. Furthermore the extraction with neutral salts, may overestimate the availability, however is a simple method adapted to acid soils.

These results also show that the correlation between TE concentrations in plants and different TE concentrations in soils extracted by the three methods is more dependent on the plant species than the method of extraction. The best coefficients that correlated the three methods were found for elements like Cd and Zn and especially when *P. annua*, an indicator plant, was the test plant.

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Table 1. Main characteristics of studied soils and amendments. Standard errors in bracket (n=10).

Parameter	Soils		Amendments	
	Vicario (V)	Aznalcázar (AZ)	Alperujo Compost (AC)	Biosolid Compost (CB)
pH	3.48 (0.04)	6.53 (0.08)	8.10 (0.21)	7.09 (0.28)
TOC	7.60 (1.09)	26.5 (2.00)	301 (1.63)	246 (0.4)
Texture	Loam	Sandy-Loam	-	-
Sand	393	500	-	-
Silt	346	280	-	-
Clay	393	220	-	-
N	0.16 (0.14)	0.54 (0.17)	156 (0.25)	227 (0.45)
Tot-As (0.1-40) ^a	250 (51)	112 (53)	2.45 (0.22)	13.5 (0.60)
Tot-Cd (0.01-2) ^a	1.30 (0.23)	3.82 (1.23)	0.25 (0.00)	1.94 (0.09)
Tot-Cu (2-250) ^a	180 (18.0)	166 (42.9)	94.2 (1.1)	188 (11)
Tot-Mn (20-10,000) ^a	520 (11)	135 (12)	360 (5)	573 (36)
Tot-Pb (2-30) ^a	600 (140)	236 (13.5)	9.77 (0.06)	61.4 (3.7)
Tot-Zn (1-900) ^a	370 (42.2)	507 (34.9)	185 (11)	601 (32)

TOC = total organic carbon; Pseudo-total values of trace elements (mg kg⁻¹).

^a Normal levels in soils according to Bowen (1979) (mg kg⁻¹)

Table 2. Biomass (g) obtained in both samplings. Standard error in brackets (n=3). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost

Soil	Species	Treatment	Fresh Biomass Harvest 1	Dry biomass Harvest 1	Fresh Biomass Harvest 2	Dry biomass Harvest 2
AZ	<i>M.polymorpha</i>	C	30.6 ab (2.1)	9.18 ab (0.6)	10.0 a (0.3)	3.00 a (0.1)
		AC	38.3 b (2.2)	11.5 b (0.7)	11.8 a (0.4)	3.54 a (0.1)
		BC	27.9 a (2.3)	8.37 a (0.7)	11.3 a (0.8)	3.39 a (0.2)
	<i>P. annua</i>	C	15.1 a (0.9)	4.65 a (0.3)	7.30 a (0.2)	2.19 a (0.1)
		AC	22.9 b (1.5)	6.87 b (0.5)	10.2 a (1.0)	3.06 a (0.3)
		BC	34.0 c (2.6)	10.2 c (0.8)	8.80 a (1.9)	2.64 a (0.6)
V	<i>M.polymorpha</i>	C	-	-	-	-
		AC	12.3 b (1.9)	2.46 b (0.4)	4.41 a (0.5)	0.88 a (0.1)
		BC	1.90 a (0.4)	0.38 a (0.1)	7.62 b (0.9)	1.52 b (0.2)
	<i>P. annua</i>	C	-	-	-	-
		AC	37.9 b (3.6)	11.4 b (1.1)	9.30 a (1.1)	2.79 a (0.3)
		BC	26.3 a (3.1)	7.89 a (0.9)	10.7 a (1.4)	3.21 a (0.4)

Difference between values are reported with different letter in each column and for each soil (p<0.01).

Table 3. Trace elements concentrations (mg kg⁻¹) in the aerial part of the plants for the two harvests studied. Standard error in brackets (n=3). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost

Soil	Species	Harvest	Treatment	Cd	Cu	Mn	Zn
AZ	<i>M. polymorpha</i>	1	C	0.09 (0.003) b	13.9 (1.63)	24.3 (3.51)	55.5 (1.06)
			AC	0.05 (0.01) ab	12.6 (1.23)	20.5 (0.57)	47.4 (2.22)
			BC	0.07 (0.01) a	13.0 (0.55)	17.5 (1.37)	84.0 (28.8)
		2	C	0.09 (0.01)	22.6 (3.15)	14.1 (0.74)	69.5 (1.24) b
			AC	0.07 (0.01)	21.6 (4.89)	15.9 (2.52)	70.0 (4.12) b
			BC	0.09 (0.01)	16.8 (1.62)	17.0 (1.56)	54.5 (2.89) a
	<i>P. annua</i>	1	C	0.36 (0.01) b	23.7 (6.38)	57.0 (4.68)	124 (24.2)
			AC	0.28 (0.02) a	19.1 (5.57)	44.9 (1.74)	100 (11.9)
			BC	0.57 (0.02) c	18.9 (3.39)	49.0 (0.65)	111 (2.37)
		2	C	0.41 (0.02) a	16.6 (3.14)	50.5 (2.57)	139 (3.00) b
			AC	0.41 (0.004) a	16.5 (1.47)	50.8 (3.09)	112 (3.47) a
			BC	0.52 (0.02) b	18.3 (4.77)	50.5 (1.86)	103 (2.50) a
V	<i>M. polymorpha</i>	1	AC	0.09 (0.01) a	19.9 (4.85)	164 (6.31) a	80.9 (6.73) a
			BC	0.16 (0.01) b	21.0 (0.20)	243 (13.9) b	101 (2.44) b
		2	AC	0.07 (0.01) a	42.9 (19.7)	184 (19.5) a	70.2 (1.05) a
			BC	0.23 (0.01) b	21.9 (2.12)	270 (11.4) b	101 (3.67) b
	<i>P. annua</i>	1	AC	1.09 (0.15)	11.1 (0.95)	1750 (297)	301 (40.1)
			BC	1.13 (0.01)	10.7 (0.48)	2287 (42.9)	319 (2.37)
		2	AC	1.21 (0.32)	17.9 (3.16)	816 (113)	215 (26.7)
			BC	1.23 (0.34)	13.2 (1.57)	1238 (222)	249 (45.8)
Normal values in plants ^a				0.01-1	3-20	15-150	15-150

Significant difference between values are reported with different letter in each column and for each soil (p<0.01).

^a Normal levels in plants (Chaney, 1989)

Table 4. pH, TOC (g kg⁻¹) of bulk soil (initial sampling) and of rhizosphere soils of both species (Standard error is given in brackets (n=3). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost.

Soil	Treat	pH	TOC	Species	Treat	pH	TOC
Bulk				Rhizosphere			
AZ	C	6.32 a (0.15)	26.5 a (0.63)	<i>M. Polymorpha</i>	C	7.34 b (0.02)	29.9 a (4.04)
	AC	7.00 b (0.85)	33.4 b (1.16)		AC	7.20 ab (0.11)	32.9 a (8.83)
	BC	7.39 c (0.41)	29.4 ab (1.87)		BC	7.04 a (0.03)	30.8 a (0.71)
				<i>P. annua</i>	C	7.19 a (0.03)	32.6 a (4.48)
					AC	7.20 a (0.01)	32.4 a (3.69)
					BC	7.15 a (0.04)	29.2 a (1.27)
V	C	3.23 a (0.02)	7.60 a (0.72)	<i>M. Polymorpha</i>	C	3.19 a (0.04)	11.5 a (0.59)
	AC	5.98 b (0.11)	23.0 b (0.88)		AC	6.40 b (0.02)	21.6 ab (2.25)
	BC	6.07 b (0.04)	22.7 b (1.67)		BC	6.07 b (0.27)	26.6b (3.94)
				<i>P. annua</i>	C	2.98 a (0.03)	6.00 a (0.63)
					AC	6.22 b (0.08)	23.5 b (3.46)
					BC	6.36 b (0.20)	19.4 b (0.79)

Significant differences between treatments are indicated by different letters in each column and for each soil separately (p<0.01).

Table 5. Correlations between the three studied methods (CaCl₂ for n=36, n=24 for SPW, n=24 for GDT)

Element	Method	SPW	DGT
Cd	CaCl ₂	0.733**	0.675**
	SPW	-	0.997**
Cu	CaCl ₂	0.479**	0.847**
	SPW	-	0.972**
Mn	CaCl ₂	n.s	n.s
	SPW	-	0.435*
Zn	CaCl ₂	0.823**	0.767**
	SPW	-	0.774**

**p<0.01; * p<0.05. n.s : no significant

Table 6. Correlations of trace elements in plants and the method of extraction (n=15)

Plant	Element	CaCl ₂	SPW	DGT
<i>M. polymorpha</i>	Cd	n.s.	n.s.	n.s.
	Cu	-0.608*	-0.751*	n.s.
	Mn	0.754*	0.769**	0.525*
	Zn	-0.822**	-0.779**	-0.868**
<i>P. annua</i>	Cd	0.582*	0.827**	0.715**
	Cu	n.s.	n.s.	n.s.
	Mn	0.863**	n.s.	0.820**
	Zn	0.855**	0.879**	0.747**

**p<0.01; * p<0.05. n.s : no significant

Figure 1

Figure 2

Figure 3

Figure 4

Figure Caption

Figure 1. Cd concentration in plant shoots, Cd concentration in soils extracted with 0.01M CaCl_2 , soil pore water (SPW), and DGT for the two studied soils, with and without plants and amendments. For each soil and plant columns with different letter differ significantly ($p < 0.05$). Error bars show standard error of the mean ($n=3$). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost; Soil type codes: AZ = Aznalcázar (neutral contaminated soil); V = Vicario (acid contaminated soil); Plant species code: ME: *Medicago polymorpha*; PO: *Poa annua*.

Figure 2. Cu concentration in plant shoots, Cu concentration in soils extracted with 0.01M CaCl_2 , soil pore water (SPW), and DGT for the two studied soils, with and without plants and amendments. Error bars show standard error of the mean ($n=3$). For each soil and plant columns with different letter differ significantly ($p < 0.05$). Error bars show standard error of the mean ($n=3$). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost; Soil type codes: AZ = Aznalcázar (neutral contaminated soil); V = Vicario (acid contaminated soil); Plant species code: ME: *Medicago polymorpha*; PO: *Poa annua*.

Figure 3. Mn concentration in plant shoots, Mn concentration in soils extracted with 0.01M CaCl_2 , soil pore water (SPW), and DGT for the two studied soils, with and without plants and amendments. Error bars show standard error of the mean ($n=3$). For each soil and plant columns with different letter differ significantly ($p < 0.05$). Error bars show standard error of the mean ($n=3$). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost; Soil type codes: AZ = Aznalcázar (neutral contaminated soil); V = Vicario (acid contaminated soil); Plant species code: ME: *Medicago polymorpha*; PO: *Poa annua*.

Figure 4. Zn concentration in plant shoots, Zn concentration in soils extracted with 0.01M CaCl_2 , soil pore water (SPW), and DGT for the two studied soils, with and without plants and amendments. Error bars show standard error of the mean ($n=3$). For each soil and plant columns with different letter differ significantly ($p < 0.05$). Error bars show standard error of the mean ($n=3$). Amendment treatment codes: C = non-amended; AC = alperujo compost; BC = biosolid compost; Soil type codes: AZ = Aznalcázar (neutral contaminated soil); V = Vicario (acid contaminated soil); Plant species code: ME: *Medicago polymorpha*; PO: *Poa annua*.