

Distribution, fractionation and mobility assessment of heavy metals in a spiked soil using a multi-species soil system

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Abstract

The concentration, distribution and fractionation of metals in a metal spiked soils were studied using a Multi-Species-Soil-System (MS-3), a terrestrial microcosms that allows a combined assessment of the chemical fate and effects on terrestrial organisms. Samples of a natural soil were spiked with a mixture of the metals Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn and placed on the top of soil columns simulating the heavy metal concentration due to an agricultural organic amendment (manure, sewage sludge) equivalent to 2, 5 and 10 times the original background of soil metal concentration. After 21 days exposure, metal mobility was assessed using three complementary approaches: the metal concentrations at four depths in the soil profiles (I, II, III and IV), the metal fractionation in the top soil and the percent metal detected in leachates. The upper soil profile had the highest metal concentration for the majority of the metals. For Cd, Mn and Co highest concentrations were in the bottom profile. Maximum metal mobility in leachates was for Cd (24.67%) > Co (3.65%) > Mn (3.18%) > Zn (0.55%) > and Cu (0.012%). Nickel and Cr percentages were < 0.001%. Cobalt, in all leachates, control and treated, was > 0.1%. Lead mobility from soil to water was 0.004% and 0.008% for the lowest and highest treatments, respectively.

Additional key words: agricultural soil, microcosms, MS-3, sequential metal extraction.

Resumen

Valoración de la distribución, fraccionamiento y movilidad de metales pesados en un suelo fortificado utilizando un sistema multiespecie terrestre

En un suelo control fortificado con una mezcla de metales (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn), se ha estudiado la concentración, distribución y fraccionamiento de metales utilizando un microcosmos terrestre, Multi-Species-Soil-System (MS-3), que permite conocer el destino y los efectos de contaminantes sobre los organismos del suelo. Se fortificaron muestras de un suelo control con la mezcla de metales mencionada y se colocaron en la parte superior de las columnas de suelo, simulando el contenido de metales pesados que contiene una enmienda agraria, con residuos orgánicos (composts de residuos sólidos urbanos, lodos de depuradora), equivalente a 2, 5 y 10 veces la concentración basal de metales en el suelo control utilizado. Después de 21 días de exposición, se determinó la movilidad de los metales usando tres aproximaciones complementarias: la concentración de metales en los cuatro perfiles en que la columna de suelo fue dividida (I, II, III y IV), el fraccionamiento secuencial en el perfil superior del suelo y el porcentaje de metales que llega a los lixiviados. El perfil superior mostró, para la mayoría de los metales considerados, las concentraciones más altas, mientras que las concentraciones más elevadas de Cd, Mn y Co aparecieron en el perfil más profundo. En los lixiviados procedentes de las columnas tratadas con la mayor dosis de metales (10x) se detectaron Cd (24,67%) > Co (3,65%) > Mn (3,18%) > Zn (0,55%) > Cu (0,012%); sin embargo los valores Ni y Cr en estos mismos lixiviados fueron <0,001%. Valores >0,1% se encontraron para Co en cualquiera de los lixiviados, tratados o control. La movilidad del Pb fue 0,004% y 0,008% para las dosis más baja y más alta respectivamente.

Palabras clave adicionales: extracción secuencial de metales, microcosmos, MS-3, suelo agrícola.

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Introduction

Input of metals into soils has increased since the start of industrialization (Han *et al.*, 2000; Bialosi *et al.*, 2006). Anthropogenic inputs of trace elements in soils frequently comes from activities such as mining, smelting, waste, sewage sludge, transport, industrial activity or atmospheric deposition (Adriano, 2001; Kashem and Singh, 2001; Abollino *et al.*, 2002; Mantovi *et al.*, 2003). A new generation of organic soil amendments has appeared due to the use of waste and sewage sludge from municipal water treatment plants. Their use as fertilizers is attractive but it poses an environmental risk due to their metal content and other micropollutants, which may affect soil organisms, and be accumulated in plants or transferred to aquatic systems (Parkpian *et al.*, 2002; Bose *et al.*, 2008). Directive 86/278 (EEC, 1986) considers sewage sludge use on agricultural soil; mainly controlling metal accumulation in soil after its application as fertilizer. Metals are a major cause of concern, as they are potentially toxic and persistent in soil (Kabata and Pendias, 2001). Bioavailability and potential metal toxicity in soils depend on their persistence in bulk soil and the soil solution. Soil risk assessment should consider both, mobile and bioavailable fractions of pollutants (Clemente *et al.*, 2007). Metal distribution in soils is controlled by soil processes such as cation exchange, specific adsorption, precipitation and complexing (Rieuwerts *et al.*, 1998). A number of studies have tried to explain the mobility (*e.g.* Kuo *et al.*, 1985; Dragun, 1998; Howari, 2004; Hu *et al.*, 2006; Pelfrène *et al.*, 2007) or bioavailability (Rieuwerts *et al.*, 1998) of metals in soils. Soil organisms should be considered because they produce significant changes in metal mobilization patterns (Alonso *et al.*, 2006).

This soil microcosms constitutes a proper experimental tool for study of chemicals under conditions resembling the natural environment. An innovative soil microcosms design, the Multi-Species-Soil-System (MS-3) has been developed by the Ecotoxicology Laboratory (INIA) for mimicing agricultural soil conditions. It is planned for ecotoxicological studies covering different chemical groups and complex samples, including wastes. The main objective of this study was to describe the use of this tool for assessing the distribution, mobil-

ity and bioavailability of metals in soil after 21 days exposure to a metal mixture. This design allows simultaneous assessment of dose/responses relationships and a comparative assessment among metals.

Materials and methods

Soil

The soil used in this study was collected from a research facility located 40 km northeast of Madrid. It is a typical agricultural soil with a known history. Fertilisers have not been applied for at least the last 10 years. Unavoidable medium-range atmospheric deposition is considered the only source of anthropogenic metal input. A large soil sample was collected from the top 20 cm soil layer, sieved (≤ 4 mm) and homogenized before use. The soil is a loamy sand soil and is characterized as clay (8.4%), silt (0.002–0.02 mm, 5.1%), silt (0.02–0.05 mm, 8.7%) and sand (77.8%). Physical chemical properties were: pH 8.3; organic matter 1.7%, maximum water holding capacity (WHC); 20%, electric conductivity (EC), 5.1 meq 100 g⁻¹, cation-exchange capacity (CEC), 8.8 meq 100 g⁻¹ and carbonate content 6%.

The multi-species-soil-system (MS-3) design

The MS-3 design was described by Fernández *et al.* (2004) and Boleas *et al.* (2005). It is a microcosms system designed to assess the chemical fate and toxicity effects of substances and mixtures on soil organisms from three taxonomic groups (earthworms, plants and microorganisms). The MS-3 are artificial assemblages of soil macro-organisms lying on homogeneous columns of sieved natural soil. The columns of soil are adapted to study the objectives. In this study, PVC cylinders (20 cm internal diameter x 30 cm high) covered in a fine nylon mesh at the bottom to avoid soil loss were used. A polyethylene container on each system allowed leachate collection. Exposure was in a controlled temperature room ($21 \pm 1^\circ\text{C}$) with a light/dark cycle of 16:8 h (1150 lux \pm 13% coefficient of varia-

Abbreviations used: AAS (atomic absorption spectrometry), BCR (Community Bureau of Reference), CEC (cation-exchange capacity), CV (variation coefficient), dw (dry weight), EC (electric conductivity), FAAS (flame atomic absorption spectroscopy), GF-AAS (graphite furnace atomic absorption spectroscopy), PVC (polyvinyl chloride), WHC (water holding capacity).

tion). Ten adult laboratory reared earthworms (*Eisenia fetida*), were added to each MS-3. Certified seed of wheat (*Triticum aestivum* L.), rape (*Brassica napus* L.) and vetch (*Vicia sativa* L.) were supplied by the Spanish Office of Plant Varieties. A total of 30 seeds, 10 seeds of each species, were incorporated into each MS-3. Columns were watered (100 mL of spring-water MS-3⁻¹ day⁻¹) five days a week, simulating 1,000 mm rainfall year⁻¹. All MS-3s were in triplicate. The 12 columns were distributed as follows: 3 MS-3 systems without treatment as controls (Columns 1, 2 and 3), 3 MS-3 systems with 2 x control soil metal concentration (Columns 4, 5 and 6), 3 MS-3 systems with 5 x control soil metal concentration (Columns 7, 8 and 9) and 3 MS-3 systems with 10 x control soil metal concentration (Columns 10, 11 and 12).

Preparation of metal spiked soil

The MS-3s were filled with 8 kg of dried soil as follows. Six kilograms of sieved control soil were packed into the columns, after 24 h columns were irrigated to field capacity saturation (1,400 mL spring-water), followed by the addition of 10 adult earthworms in each MS-3. After that, 2 kg of control or spiked soil were placed on the top of the soil columns simulating an agricultural amendment.

To obtain the expected metal concentration the calculated weight of each metal was mixed with 200 g of unpolluted (control) soil; additional control soil was then added to final weight (2 kg MS-3⁻¹). Amounts of metal added to the sample 2 kg of soil were those that provided a relative increase of two (2 x), five (5 x) or ten (10 x) times background metal control concentrations considering the whole soil column (8 kg). Metal spiked soils were prepared with heavy metals added as chlorides (Cd, Co, Cr (III), Cu, Mn, Ni and Zn), or acetates (Pb) salts. Only Cd was added as an acidified aqueous solution (1 mg Cd L⁻¹). Finally, 30 seeds of the three plants were sown in each column, 10 seeds of each species. One hour later, all MS-3s were watered with 100 mL of spring-water.

During the 21 day exposure period from Monday to Friday all MS-3s were irrigated (100 mL column⁻¹ day⁻¹). After 24 h (t = 0) and 7 days (t = 7), leachates were collected. Because on day 14 leachates could not be collected, we decided to irrigate on day 16, to field capacity (1,800 mL of spring-water), the MS-3s to obtain leachate samples. After 21 days exposure, the MS-3s

were opened, the soil was removed from the PVC cylinders and divided into 4 equal profiles (Profiles I, II, III and IV; each 5 cm thick). The soil samples and leachates were collected and frozen at -20°C until metal analysis. Plants and earthworms were removed and placed at -20°C until analysis for metal concentration. Plant and earthworm results are not presented in this paper.

Heavy metal analysis

Heavy metals in soil profiles

The heavy metals in soil profiles I, II, III, and IV were analyzed at the end of the exposure time (21 days). Analyses were by Atomic Absorption Spectrometry (AAS) (Perkin-Elmer Analyst 800), using Flame Atomic Absorption Spectroscopy (FAAS) or Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), depending on metal concentration, equipped with Zeeman-effect background correction and an AS 800 autosampler. Soil metals were extracted using USEPA digestion method 3051 (USEPA, 1994). For most samples this method is not a total digestion technique. It is very strong acid digestion that will dissolve almost all elements that could become "environmentally available". A microwave laboratory unit (ETHOS SEL, Milestone) was used for soil samples digestion. Ultra pure water (Millipore Milli-Q system) and analytical grade reagent (65% HNO₃, Suprapur, Merck) were used. Standard metal solutions were prepared from commercial concentrated stock solutions (Merck, Germany). Chromium, spiked on soil as Cr (III) chloride, was determined as total Cr. All analyses were in duplicate.

Sequential extraction of heavy metals in soil

Metal fractionation was only analyzed in the top soil profile, Profile I, following the Community Bureau of Reference (BCR) protocol, this involved a four-stage sequential extraction of fractions (F₁: acid-soluble, exchangeable and carbonate bound, F₂: elements associated with Fe and Mn bound oxides, F₃: elements associated with organic matter and residual elements).

Following Quevaullier *et al.* (1993), the extractions were performed as follows. F₁: 15 mL of 0.11 M acetic acid was added to 1.5 g soil; the mixture was placed on an orbital shaker (16 h at 21°C) and centrifuged to sep-

arate the extract from the residue. F_2 : the residue of F_1 was extracted with 15 mL of 0.11 M hydroxylamine hydrochloride (99.999% trace metals basis, Aldrich) on an orbital shaker (16 h at 21°C) and centrifuged to separate extract from residue. F_3 : The residue from F_2 was extracted with 5 mL of 30% hydrogen peroxide (Trace Select Ultra, for trace analysis, Fluka) on an orbital shaker (1 h at 85°C, twice). F_4 : this fraction was calculated from the difference between the “environmentally available” metal concentration (digested with HNO_3 , USEPA 3051 Method) and the sum of the first three fractions. Between each successive extraction the suspension was centrifuged, the supernatant removed and the solid phase preserved for subsequent extraction, acidified with HNO_3 (pH = 2) and stored at 4°C until analysis. Leachates were acidified (pH = 1.5) before analysis and metal concentrations were determined by FAAS or GF-AAS, depending on the concentration range. Analyses were performed on leachates collected at 0, 7 and 16 days exposure.

Statistical analysis

Statistical comparisons between control and spiked soils were analyzed by one-way ANOVA and least significant differences (LSD) multiple range test at the $p < 0.05$ significance level (Statgraphics Plus 5.1).

Correlation coefficients, between bioavailable metal concentrations in Profile I and metal concentrations in leachates (16 day), were calculated using the least square method (Statgraphics Plus 5.1). Correlations were assumed to be statistically significant at $p < 0.05$.

Results

Heavy metals in soil profiles (I, II, III, and IV)

Concentrations of Co, Cr, Cu, Mn, Ni and Pb in the control soil were similar among the different profiles and were in accordance with the range of values in unpolluted soil. Cadmium and Zn concentrations (mean \pm SD) showed slight variation among profiles from 0.08 ± 0.02 to 0.12 ± 0.03 and 47.5 ± 3.8 to 62.6 ± 9.2 mg kg^{-1} dry weight for Cd and Zn respectively. In the metal spiked systems highest concentration were, in most cases, in the top soil profile. A vertical decrease (from top to bottom) among profiles was observed for Cu, Cr and Pb.

For all heavy metal concentrations were related to the dose but profile distributions differed among metals and treatments (Figure 1). At the lowest application rate (2x) the highest concentration was found in the top soil profile for all metals. In profile II an increase in concentration, particularly for Cd and Mn, was observed compared with control values. Metal concentration in profiles III and IV had similar values to the control soil. Mobility patterns in application rate 5x were similar to the trend observed in 2x. Metal concentrations in profiles II and III were similar to, or higher than, those in profile I for Cd and Mn respectively. At the highest (10x) application rate, the same trend was observed for Co and Ni.

Under these experimental conditions, where metal loads were related to background concentrations, Cd, Mn and Co had the highest mobility, followed by Ni and Zn. After 21 days Cd, Mn and Co concentrations, in the bottom profile were similar, or higher, than those in upper profiles. Copper, Cr and Pb remained almost entirely in the top soil profile.

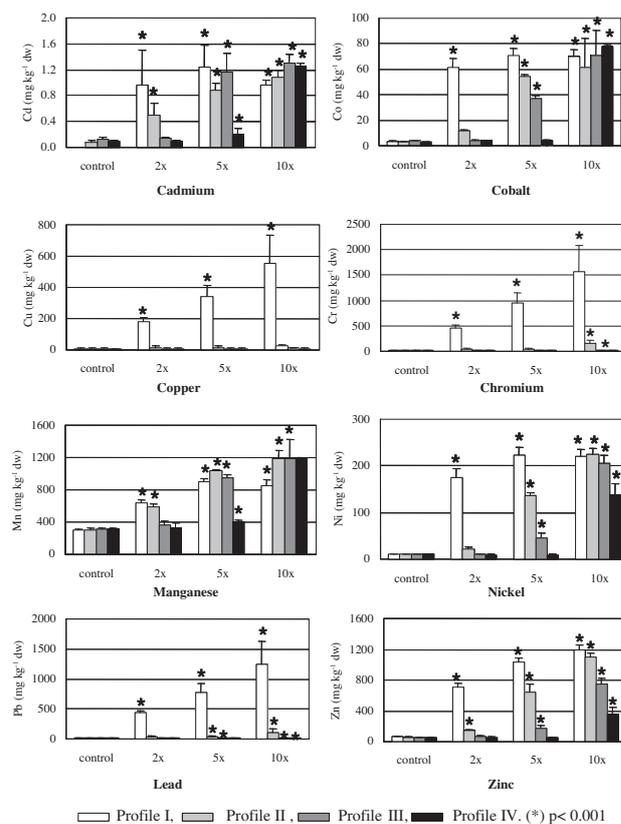


Figure 1. Distribution of metal concentrations (mean \pm SD, mg kg^{-1} dw) in soil samples. (*) $p < 0.001$.

Sequential extraction of soil heavy metals

The extractable soluble metals (F_1), as a percent of “environmentally available” metal concentration, are presented in Figure 2. The distribution of sequential extractions also expressed as percent of the metal in each fraction, corresponding to extractable soluble metals, iron-manganese oxides-bound, organic matter-bound and residual respectively can be seen in Figure 3. There were differences in distribution patterns among metals and treatments. As expected, metal application (in soluble forms) increased the percentage of Fraction 1. The results also indicate that within 21 days a large proportion of the metals studied interact with soil components. Metal additions were proportional to background levels; thus, these graphic representations offer an indication on the short time binding capacity of soil components. A low binding capacity would produce large treatment related effects on the distribution of the fractions, with an increase in Fraction 1, as can be observed for Cd. The mobility of this metal was so high that on day 21 the peak concentration, at the highest treatment level was already at Profile III (1.31 ± 0.14 mg kg⁻¹ dry weight, mean \pm SD (Figure 1). This was also observed in fraction distribution, with a reduction in Fractions 1 and 2 as a result of mobilization of Cd to deeper profiles. In contrast, a high binding capacity would produce no or minor changes in distribution of the fractions, as the added metal will suffer the same fate as the background metal. This was observed in the lowest and intermediate treatment for Cr, Cu, and Pb, where the largest amount of metal was in the residue. These results are consistent with the observed lack of metal movement from the top to the lower profiles. The situation differed slightly with Ni. Although the metal mostly appears in the residual, a dose response effect was observed for Fraction 2; highly significant and very

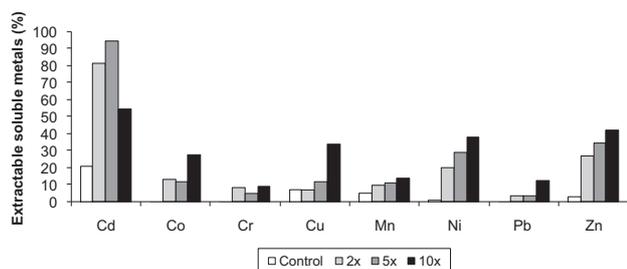


Figure 2. Community Bureau of Reference (BCR) procedure. Fraction 1: Percent extractable soluble metals (0.11 M acetic acid) in the top soil profile.

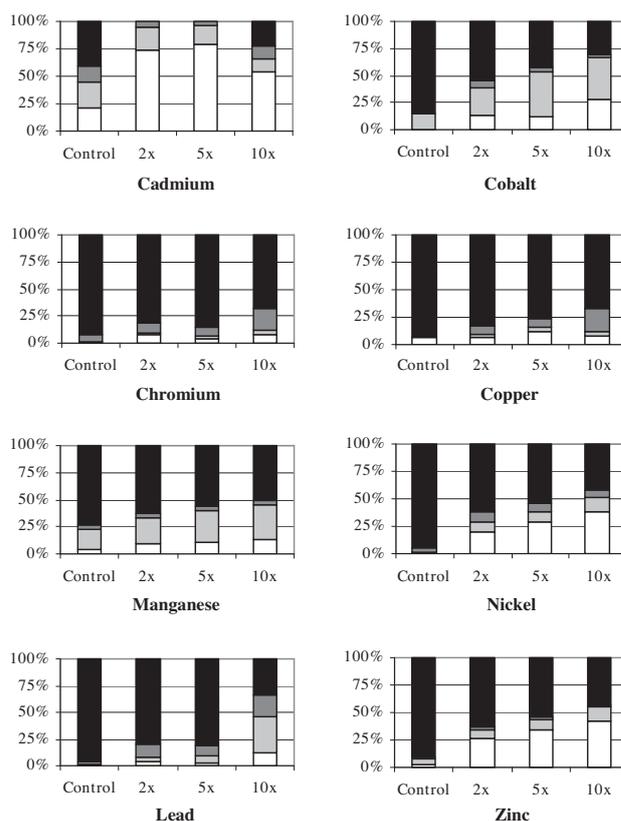


Figure 3. Sequential extraction of metals (%) in the top soil profiles. F_1 : Extractable soluble metals; F_2 : Fe-Mn oxides bound; F_3 : organic matter-bound; F_4 : Residual elements.

high movement of Ni were observed for the medium and high treatment rates respectively (Figure 1). Dose related increases in the summation of Fractions 1 and 2 were observed for Co, Mn, and Zn.

Heavy metals in leachates

Leachate volume and the amount of metal (μ g or mg) in the leachate of each MS-3 at 0, 7 and 16 days are shown in Table 1.

Leachate volume collected 24 h later at the beginning of exposure ($t = 0$) were similar for all MS-3s, with volumes (mean \pm SD) ranging from 111.3 ± 21.1 to 145 ± 27.2 mL. On day 0 two patterns could be observed for the metals. Concentration of Cd, Cr, Cu and Pb in leachates increased with application rate. This pattern was also observed on the other sampling days for Cd, Cr and Cu. However, Co, Mn, Ni and Zn showed similar values to the control for the lowest and intermediate

Table 1. Leachates analysis: volume and metal extraction by MS-3 system at different times and total extracted metal (%)

MS-3	Date	Leachate (mL)	Cd (μg)	Co (mg)	Cr (μg)	Cu (μg)	Mn (mg)	Ni (μg)	Pb (μg)	Zn (mg)
Control	0	114.7 \pm 10.3	0.01 \pm 0.00	0.11 \pm 0.05	ND	4.63 \pm 1.23	0.02 \pm 0.00	0.01 \pm 0.00	0.11 \pm 0.04	0.02 \pm 0.00
2x		111.3 \pm 21.1	0.07 \pm 0.04	0.06 \pm 0.02	0.02 \pm 0.01	5.34 \pm 2.30	0.04 \pm 0.03	0.02 \pm 0.01	0.25 \pm 0.10	0.05 \pm 0.03
5x		125.7 \pm 9.0	0.27 \pm 0.03	0.08 \pm 0.02	0.12 \pm 0.07	8.62 \pm 0.75	0.05 \pm 0.01	0.04 \pm 0.01	0.40 \pm 0.18	0.04 \pm 0.01
10x			111.0 \pm 33.6	0.32 \pm 0.15	0.49 \pm 0.11	12.69 \pm 0.99	21.86 \pm 6.30	0.23 \pm 0.10	1.62 \pm 0.79	0.28 \pm 0.37
Control	7	15.7 \pm 7.5	0.01 \pm 0.00	0.03 \pm 0.02	ND	1.11 \pm 0.61	0.01 \pm 0.01	ND	0.24 \pm 0.31	ND
2x		32.3 \pm 1.5	0.06 \pm 0.01	0.09 \pm 0.04	0.01 \pm 0.00	4.91 \pm 1.28	0.04 \pm 0.01	0.01 \pm 0.00	1.16 \pm 0.96	0.01 \pm 0.04
5x		23.3 \pm 6.4	4.30 \pm 1.23	0.02 \pm 0.00	0.01 \pm 0.00	5.27 \pm 1.55	0.25 \pm 0.20	0.02 \pm 0.00	0.42 \pm 0.46	0.01 \pm 0.00
10x		23.0 \pm 8.7	112.90 \pm 141.26	3.20 \pm 1.17	0.05 \pm 0.00	7.65 \pm 2.15	59.13 \pm 5.37	2.80 \pm 1.46	0.74 \pm 0.30	5.75 \pm 5.46
Control	16	143.0 \pm 35.7	0.19 \pm 0.04	0.02 \pm 0.01	ND	11.09 \pm 7.43	0.02 \pm 0.01	0.02 \pm 0.01	0.17 \pm 0.20	ND
2x		309.8 \pm 10.2	0.64 \pm 0.07	0.08 \pm 0.01	0.03 \pm 0.01	23.47 \pm 3.96	0.11 \pm 0.04	0.10 \pm 0.02	6.13 \pm 9.71	0.04 \pm 0.02
5x		388.0 \pm 2.6	72.46 \pm 37.48	0.22 \pm 0.06	0.05 \pm 0.01	59.08 \pm 12.91	0.83 \pm 0.89	0.24 \pm 0.07	4.65 \pm 1.35	0.08 \pm 0.04
10x		412.3 \pm 38.8	1945.53 \pm 160.96	12.52 \pm 4.46	0.07 \pm 0.001	85.06 \pm 0.88	757.68 \pm 76.22	12.97 \pm 3.70	73.57 \pm 25.54	18.16 \pm 13.53
Total										
Control		283.3 \pm 34.8	0.21 \pm 0.04	0.16 \pm 0.03	0.01 \pm 0.00	16.84 \pm 7.28	0.05 \pm 0.001	0.04 \pm 0.01	0.53 \pm 0.31	0.02 \pm 0.01
2x		453.5 \pm 29.2	0.77 \pm 0.10	0.23 \pm 0.04	0.06 \pm 0.01	33.72 \pm 1.37	0.18 \pm 0.01	0.13 \pm 0.02	7.53 \pm 895	0.10 \pm 0.04
5x		556.3 \pm 26.6	77.03 \pm 38.23	0.32 \pm 0.7	0.17 \pm 0.06	72.96 \pm 12.92	1.12 \pm 0.92	0.31 \pm 0.08	5.44 \pm 1.61	0.13 \pm 0.04
10x		561.0 \pm 55.0	2170 \pm 207	16 \pm 5.7	0.61 \pm 0.12	105.00 \pm 2.50	838.67 \pm 125.31	16 \pm 5.20	75.9 \pm 25.5	24.2 \pm 19.30
Extracted metal (%)										
Control			0.03	0.4	< 0.001	0.021	0.002	< 0.001	< 0.001	0.006
2x			0.03	0.19	< 0.001	0.014	0.003	< 0.001	0.008	0.008
5x			1.61	0.13	< 0.001	0.015	0.008	< 0.001	< 0.001	0.006
10x			24.67	3.65	< 0.001	0.012	3.18	0.002	0.004	0.550

ND: not detected

application rate. Considerable amounts of these metals in leachates only appeared at the highest application rate. The most significant increase in extracted metals in leachates was at the highest (10x) application rate. The greatest mobility, as a percent of applied metal in the leachate, was observed for Cd (24.67%), Co (3.65%), Mn (3.18%) and Zn (0.55%).

Correlation coefficients between metal concentration of sequential extracts of the top soil (Profile I), and

leachates at $t = 16$ were also evaluated. Table 2 shows the correlation coefficients and p -values; the relationship between sequential extraction fractions (F_1 , F_2 , F_3 , $F_1 + F_2$, $F_1 + F_2 + F_3$) in the top soil profile and the leachates (Table 2) was almost linear for Co, Cr, Cu and Pb. The nonlinear relationship observed for Cd, Mn, Ni and Zn indicates overloading of the soil capacity to retain the metals, since the metal concentrations in the leachates were higher than expected values.

Table 2. Correlation coefficients (r^2) between top soil profile (Profile I) and leachates metal concentrations. F_1 : exchangeable and carbonate bound, F_2 : bound Fe-Mn oxides; F_3 : bound organic mater; L: leachate

Metal	F_1 -L		F_2 -L		F_3 -L		(F_1+F_2) -L		$(F_1+F_2+F_3)$ -L	
	r^2	p -value	r^2	p -value	r^2	p -value	r^2	p -value	r^2	p -value
Cd	0.4469	0.2778	0.0129	0.9738	0.3279	0.3889	0.4701	0.2018	0.5411	0.1325
Co	0.9622	0.0000	0.4083	0.2753	-0.9287	0.0003	0.8165	0.0073	0.7826	0.0127
Cr	0.9539	0.0002	0.8102	0.0147	0.9812	0.0000	0.9457	0.0004	0.976	0.0000
Cu	0.9667	0.0021	0.9511	0.0001	0.9831	0.0000	0.9621	0.0000	0.9668	0.0000
Mn	0.6466	0.0598	0.4599	0.2131	-0.6619	0.0521	0.5228	0.1487	0.4463	0.2258
Ni	0.7071	0.0331	0.8581	0.0031	-0.7028	0.0347	0.7506	0.0198	0.7292	0.0258
Pb	0.9838	0.0000	0.9823	0.0000	0.9512	0.0001	0.9906	0.0000	0.9845	0.0000
Zn	0.8318	0.0054	0.9103	0.0007	-0.0472	0.9038	0.8537	0.0034	0.8499	0.0037

Discussion

The analytical results for the control soil indicated typical ranges for unpolluted soils (Alloway, 1990) with similar Co, Cr, Cu, Mn, Ni and Pb concentrations in the four profiles. Metal mobility in soil is a key element for assessing their health and environmental impacts. Heavy metal mobility in soil is influenced by various variables and environmental factors including the composition of the soil-aqueous phase (Lim *et al.*, 2002); the coexistence of several processes controlled by different mechanisms complicates the assessment process. A large number of studies describe some of these processes. In the short-term, the physical-chemical processes are regulated by partition coefficients (Carlson *et al.*, 2003); while in the long-term other processes such as clay-associated movement may become relevant (Sheppard, 2005). Further, metal-soil interactions are time related, and aging is relevant when estimating metal speciation in soil (Ma and Dong, 2004). Depending on soil conditions, preferential flow through macropores may become a critical route, and models based on partition coefficients may under-predict metal mobility. Plants and soil invertebrates also play significant roles in increasing metal mobility, as observed in ecotoxicological assays under realistic conditions (Alonso *et al.*, 2006).

The MS-3 appears a useful tool for assessing a combination of these processes, allowing an experimental estimation of metal mobility where the effect of the amount applied, the exposure pathway, soil characteristics and the weather can be studied and quantified. The results confirm that several mechanisms can be monitored in this soil microcosms. The capability of the estimation methods used in this study to monitor metal mobility was described by Li and Shuman (1997a,b) and Ma and Dong (2004).

The combination of 8 metals and 3 doses of each metal produced a large amount of information. In addition, the selected treatment regime, where the metal dose was related to soil background concentration of each metal, allowed estimates based on relative increases (2x, 5x and 10x) as well as on measured metal concentrations. Both, the metal and the treatment had significant effects on metal mobility and metal fractionation. The greatest mobility was observed for Cd, Co, Mn and Zn, as shown by soil profile and of leachate analysis. Mobility was observed for the majority of the metals, only Cu, Cr and Pb remained in the top soil profile at all application rates, so metal concentra-

tions did not increase in the lower profiles, being similar to the control soil level.

Leachate analysis showed some movement of Cd, Co or Mn, (Cu and Zn), but not of Cr, Ni or Pb. The study of sequential metal fractionation in the top soil profile, using a suitable procedure, gave additional information on mobilization mechanisms. Generally, good agreement was observed for the three processes. Kaasalainen and Yli-Halla (2003) used a similar sequential extraction method for studying metals in an agricultural soil profile near a smelter and observed differences in the fractionation pattern related to soil and depth. The results of this study indicate that for the same soil and profile, differences were observed depending on metal load. High mobility of Cd and a lack of mobility of Cr were observed. The mobility pattern, $Cd > Cu \geq Ni > Pb > Zn > Cr$, was clearly different for other metals such as Cu and Zn. Kaasalainen and Yli-Halla (2003) observed a high proportion of Cu and Ni in Fractions 1 and 2, which was not the case in this study. In contrast, Zn was mostly in Fraction 3, while in our study about 50% of Zn was in Fractions 1 and 2. Large soil-related differences in Zn were also observed by Lee and Kao (2004). Sorption of metals from the liquid phase onto the solid phase is considered to be the key chemical process affecting metal behaviour and bioavailability (Echevarría *et al.*, 1998). Anthropogenic metal inputs usually enter in exchangeable fractions (Lee and Kao, 2004) and suffer a rapid, competitive process (Echevarría *et al.*, 1998), followed by ageing where metal pore water concentrations may vary on a daily basis as demonstrated in incubation experiments (Pelfrène *et al.*, 2007). The process is highly relevant as it affects both metal fate and toxicity (Prokop *et al.*, 2003). The use of a soil microcosms, such as the MS-3, offers experimental possibilities for assessing the environmental fate of metals under more realistic situations; under the experimental conditions of this study, leachate measurements offered information on the mobility and pore water metal levels (Alonso *et al.*, 2006).

These results are in accordance with previous studies and offer quantitative assessments on the mobility and potential accumulation of metal additions in an agricultural soil. In conclusion, this work shows that a metal's mobility in soil profiles and their leaching to groundwater is governed by a complex combination of physical, chemical and biological mechanisms. Metal fractionation, in particular the binding to soil particles, plays a major role in this process, as it is regulated by the metal and the soil characteristics, as well as the amount of

metal reaching the soil, the contamination pathway, and the ageing processes. As large differences in natural metal background concentrations are observed in natural soils, comparisons of metal mobility patterns require setting equivalence setting.

The experimental approach used in this study, where the addition of each metal was proportional to the natural background, allowed comparisons in terms of the soil capacity for binding metal inputs in upper soil profiles. Three complementary approaches were used to follow metal mobility; the results demonstrated coherence and the added value of combining the results from each method, allowing mechanistic explanations of the observed effects. The greatest mobility, and therefore potential for groundwater contamination was observed for Cd, Co and Mn, followed by Cu and Zn. The lowest mobility (<0.001%) and therefore potential for accumulation in top soil, was observed for Cr, Ni and Pb.

The use of a soil microcosms allowed a realistic assessment of metal contamination. Factors and influences such as the role of soil macro-organisms or changes in temperature, humidity or microbial activity, considered in this experimental approach, provide more realistic estimations than those obtained from soil column leaching studies. Although data on the influence of organisms on mobility and distribution of metals in soil was not shown, in this work, this hypothesis was demonstrated in previous experiments in our laboratory (Alonso *et al.*, 2006). Metal accumulation in plants and soil invertebrates, that were introduced into the MS-3s in the study, will be the reported in a further publication.

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