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Soil Properties Affecting metal Extractability Patterns in Periurban Calcareous Agricultural Soils in the Mediterranean Area

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ABSTRACT: The potential risk of metal accumulation in periurban agricultural areas is a matter for concern. The climate characteristics and carbonate content of calcareous agricultural Mediterranean soils typical in these areas favour metal accumulation at the surface level; however there is also a risk of potential metal mobility. Our study focuses on the soil properties affecting metal mobility in these soils. Metal extractability patterns were assessed in soils after they were spiked with a mixture of Cd, Cu, Pb, and Zn and incubated up to 12 months, using one-step extraction methods ($NaNO_3$, a mixture of low molecular weight organic acids –LMWOA and diethylene triamine pentaacetic acid –DTPA–). The concentration of extractable Cd was 50% lower in the soil with the highest carbonate content. LMWOA-extractable Cu was highest in soils with the lowest organic matter (OM) content at day 1 of the incubation experiment and in soils with the lowest carbonate and recalcitrant OM contents at 12 months. Fine mineral fractions determined the lowest DTPA-extractable Cu. The highest Pb retention was in soils with the highest carbonate and clay contents. However, we were unable to establish any soil component affecting Pb extractability patterns. The Zn extractability pattern was related to particle-size distribution, which was highest in soils with a low proportion of fine mineral fractions. To summarise, carbonate, particle-size distribution and OM are relevant to potential metal mobility in these soils. The high DTPA-extractable metal values are evidence of a potential risk of metal mobility in the soils in the study.

Key words: Soil pollution, Carbonate, Metal mobility, One-step extractions, Incubation experiment

INTRODUCTION

In recent decades, increasing industrialization and urbanization has led to a rise in the accumulation of trace metals in periurban agricultural areas (Roca-Perez *et al.*, 2010). The risk of metal contamination of groundwater and its accumulation in food crops is directly related to the potential mobility of metals in soils. The mobility, and availability metals in soils depends on the combined action of various factors, including the nature of the metal and its concentration in the soil, environmental factors, and soil components (Vega *et al.*, 2010). These factors determine the soil solution-solid phase equilibrium of metals through the mechanisms of sorption-desorption and dissolution-precipitation. In the Mediterranean climate, low rainfall and high evapotranspiration limit metal mobility in soils, and metal retention is increased in calcareous Mediterranean soils owing to their carbonate content. It has been suggested that these characteristics imply that these soils are not vulnerable to chemical degradation (Conde *et al.*, 2007). However, their high metal retention capacity enhances metal accumulation in the first few centimetres of the soil, where plants can increase their concentrations of soluble metal, thereby leading to significant leaching and particularly to the uptake of metals (Sayyad *et al.*, 2010). Martínez & Motto (2000) observed that metal solubility is more susceptible to decreases in pH in calcareous soils than in non-calcareous soils, making them more vulnerable. Although carbonates play a decisive role in sorption-desorption processes, some studies have highlighted the fact that other soil constituents could play a key role in metal distribution and availability in calcareous soils (Buekers *et al.*, 2007; Jalali & Khanlari, 2008; Micó *et al.*, 2006; Sánchez-Camazano *et al.*, 1998; Sipos *et al.*, 2008). However, the information in the literature is far from homogeneous. In previous studies we observed a high metal retention capacity (> 90 %) in

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calcareous agricultural soils in the Mediterranean area and we reported that metal mobility is conditioned by the mineralogical characteristics (Lafuente *et al.*, 2008). The complexity of the interaction between metals and the constituents of calcareous soils, combined with the vulnerability of these soils which allows metals to move from one environmental echelon to another, highlights the importance of the study of the soil properties affecting the mobility –and thus the availability– of metals in these soils. It is generally accepted that to assess the mobility and availability of metal in soils it is not sufficient merely to analyse the total metal content in soils, nor is this a useful tool for determining potential risks. One-step extraction methods are widely used in studies of the potential mobility and availability of metals: chelating agents to assess metal phytoaccessibility and ecotoxicity, neutral salts for toxicity-related measurement of metal availability (Conder *et al.*, 2001; Gupta & Aten, 1993), and recently low molecular weight organic acids to simulate the rhizosphere ambient (Feng *et al.*, 2005). These procedures are useful as they may provide comparative information on the potential mobility of metals, and help to evaluate the relative availability of various metals in multi-element contaminated soils.

In the present work, we placed particular emphasis on the soil constituents and properties affecting metal availability in calcareous soils, since the role of soil constituents in metal desorption processes is not well defined for these soils. We therefore selected various periurban calcareous agricultural soils representative of the Mediterranean area with a gradient in their carbonate content, and whose remaining soil components differed. In order to obtain information on metal availability in soils with these characteristics, samples were spiked with a mixture of metals and incubated for up to 12 months. Metals were extracted with one-step extraction methods ($NaNO_3$, LMWOA and DTPA) at different time intervals.

MATERIALS & METHODS

The study area is located in Alcalá de Henares (Spain) at an altitude of 588 m. It is part of a periurban axis that combines agricultural activity and the main residential and industrial uses in the Madrid region. This scenario is typical of European metropolitan areas, and contains agricultural soils which are affected and likely to become more so in the future unless sustainability criteria are applied (Steinitz *et al.*, 2011). The soil samples come from different plots in the “El Encín” Agricultural Research Station, located on Henares River on quaternary sediments (IGME, 1990). These alluvial sediments have led to an ancient calcaric Fluvisol (Moreno Merino, 1998) which presents Anthric characteristics today (FAO, 2006) mainly as a result of agricultural use. The average annual temperature is slightly over 13 ºC; average annual rainfall is 401 mm/yr; and potential evaporation is about 760 mm/yr. The site is typical of a Mediterranean pluviseasonal-oceanic bioclimate on an upper meso-Mediterranean low dry bioclimatic belt (Rivas-Martínez, 1987). We selected six agricultural soil samples with different carbonate contents and amounts of organic matter. Samples included a mixture of 30-40 kg of the soil surface horizons (0-30 cm), which were not been differentiated due to agricultural activity. Soil samples were air-dried and passed through a 2 mm sieve.

For the experimental design, we selected unpolluted agricultural soil samples that are at potential risk of contamination due to their location. Based on the premise that the addition of a mixture of heavy metals in the soil samples would mimic the situation which would occur in a multi-element contaminated soil, the unpolluted soil samples were spiked with a multi-element metal salt solution. Cd, Cu, Pb, and Zn were selected owing to their different speciation, mobility and extractability in soils. Ten kg of each soil sample were subjected to an incubation experiment. Each soil sample was placed in an individual plastic container (40 cm wide x 59 cm long x 21 cm high) and spiked with nitrate salts from heavy metals in an aqueous solution at the rate of 3 mg/kg of Cd, 140 mg/kg of Cu, 300 mg/kg of Pb, and 300 mg/kg of Zn, corresponding to the limit values proposed by current European legislation (Directive 86/278/EEC, 1986). The soil samples and the metallic solution were mixed and left to equilibrate for a period of 12 months at room temperature without cover or drainage. During this equilibration period, the soils were air-dried, mixed and rewetted with distilled water in cycles of about 2 weeks, in order to favour metal redistribution into the soil matrix and to minimize the disruption of the metal speciation patterns (Zheng & Zhang, 2011). When a soluble metal salt is added to soil, it enters the soil pore water and initially increases the concentration of soluble metal ions, as a function of contact time; metal ions are redistributed from weakly-binding sites to more strongly-binding sites (McLaughlin, 2001). These processes play a key role in determining the extractability and availability of metals in soils and the soil toxicity status (Zapusek & Lestan, 2009). Three duplicate subsamples were randomly removed from each spiked soil sample at different time intervals (1 day, 1, 3, 6 and 12 months) for one-step extraction methods of metals ($NaNO_3$, LMWOA and DTPA-methods). Metal chemical extractions were also performed in unpolluted soils for use as a reference. In order to evaluate the soil sorption capacity, a sorption test was conducted prior to the incubation experiment. All chemicals were obtained from Merck (Germany). All glassware used was pre-washed with an aqueous solution of HNO_3 1:1000 for 24 h and rinsed with distilled water. According to ISRIC-methods (2002), the following parameters were determined: soil pH in a 1:2.5 soil to water ratio, equivalent $CaCO_3$ (ECC) by the acid neutralization method, total organic C by Walkley and Black wet oxidation procedure, particle-size distribution by the Robinson's pipette method, cation exchange capacity (CEC) by the ammonium acetate method and crystalline and amorphous Fe and Mn oxide contents by dithionite-citrate extraction, followed by acid oxalate extraction. We used the two-step acid hydrolysis procedure with H_2SO_4 to determine recalcitrant pool of organic matter (Rovira & Vallejo, 2000). Total N was determined by elemental analysis (LECO CNS 2000I analyzer). Total Ca, Mg, Cd, Cu, Pb, and Zn contents of the soil samples were determined after wet digestion with a mixture of nitro-perchloric-hydrofluoric acids under high-pressure conditions (SISS, 1985). Fe, Mn, Ca, Mg, Cd, Cu, Pb, and Zn concentration in the corresponding extracts was quantified by atomic absorption spectroscopy –AAS– (Analytikjena NovAA 300). All samples were extracted and analyzed in duplicate.

Mineral composition of soil samples (fine earth) was examined by X-ray diffraction (XRD) using an EQ 0434520 31 02 (X'Pert MPD) diffractometer with Cu Kα radiation operated at 45 kV and 40 mA. All XRD patterns were recorded with a dwell time of 1s and 0.04º2θ step. Soils were examined on randomly-orientated powders. Abundance of soil minerals was semi-quantitatively (Bish, 1994).

$NaNO_3$-extractable metals were determined by shaking 16 g of soil with 40 ml of 0.1 M $NaNO_3$ for 2 h (Gupta & Aten, 1993). LMWOA-extractable metals were determined by shaking 5 g of soil with 50 ml of a combined solution of low molecular weight organic acids (acetic, lactic, citric, malic and formic acids with a molar ratio of 4:2:1:1:1) for 16 h (Feng *et al.*, 2005). DTPA-extractable metals were determined by shaking 20 g of soil with 40 ml of 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.01 M triethanolamine (TEA) for 2 h (Lindsay & Norwell, 1978). For the sorption capacity test, the metallic solution and each soil sample (1:2 w:v) were mechanically shaken for 24 h in centrifuge tubes. The supernatant of each extraction was subsequently centrifuged at 3500 rpm for 15 min and then filtered. Cd, Cu, Pb and Zn concentration in the supernatant was quantified by AAS. Distribution coefficients (K_d) of metals were calculated from the sorption data obtained (Lafuente *et al.*, 2008) using the equation: $K_d = (M_{sorbed})/(M_{solution})$; where M_{sorbed} is the amount of sorbed metal per unit weight of soil (mg/kg), and $M_{solution}$ is the amount of metal in solution per unit volume of liquid (mg/L).

RESULTS & DISCUSSION

The main results of the soil physicochemical analyses are shown in Table 1. All unpolluted soils showed pH values above 8. The ECC content ranged from moderate (M1, M2, and M3 soils) to low (L1 and L2 soils) and very low (VL1 soil). The total organic C (TOC) ranged from very low to high content and recalcitrant pool (RP) was about 67 %. The textural classes of soils ranged from sandy-loam to sandy-clay loam. Fine sand was the most frequent size fraction. Crystalline Fe oxide content was low. Crystalline Mn and amorphous Fe-Mn oxide content was very low (data not shown). The total Cu, Pb, and Zn content were similar to those obtained by other authors for agricultural soils in the Mediterranean area (Jiménez Ballesta *et al.*, 2010; Micó *et al.*, 2006; Peris *et al.*, 2007) and in no case exceeded the levels established by the European Union (Directive 86/278/EEC, 1986). The total Cd content was lower than the quantification limit. In the metal-spiked soil samples, pH values decreased by 0.2-0.6 units as a result of the metallic solution added (pH 3.5). We also noted that there was a 2.8-fold decrease of RP content in L2 soil, 1.7 in VL1 soil, and 1.3 in L1 soil. These soils corresponded to the soil samples with a lower ECC content, which suggests that soils with low ECC levels show lower OM stabilization (Grünewald *et al.*, 2006). The decrease in RP may be related to the toxicity produced by the mixture of metals added. In a previous experiment we observed an increase in the fungal:bacterial ratio in some metal-spiked soils (de Santiago *et al.*, 2011), leading to a disruption of key soil processes such as the fungal decomposition of recalcitrant OM (de Boer *et al.*, 2005). Examination of the fine earth fractions by X-ray diffraction (Table 2) indicated high quartz and low kaolinite contents. Phyllosilicates 2:1 were the main mineral in this fraction with peaks at 0.99 and 0.33 nm and in varying proportions. The presence of plagioclase feldspars was confirmed by the reflections at 0.32 and 0.31 nm. Calcite (0.303, 0.209 and 0.187 nm) was the predominant mineral in the carbonate group. Dolomite appeared only in M1 and M2 soil samples (0.288 nm and 0.180 nm).

The metal sorption capacity was above 98 % for Cd and 99 % for Cu, Pb, and Zn in all six soils studied (data not shown). Distribution coefficients (K_d) are shown in Fig. 1. High K_d values indicate that the metal was retained in the solid phase through adsorption, precipitation and complexing reactions, whereas low

Table 1. Selected physicochemical parameters of the unpolluted soil samples

Soil sample	pH	ECC	OM	CS	FS	Silt	Clay	cry-Fe	CEC	Total Ca	Total Mg	Total Cu	Total Pb	Total Zn
		g/kg							$cmol_c$/kg	g/kg		mg/kg		
M1	8.4	190	10.2	111	603	126	161	8.1	7.1	94.2	10.5	8.0	14.0	54.7
M2	8.1	148	20.3	114	569	124	193	8.5	8.7	93.5	10.7	12.1	55.8	62.7
M3	8.1	125	30.6	23	592	215	170	11.7	10.6	41.3	10.5	10.0	23.8	63.3
L1	8.2	106	31.6	159	590	78	172	13.4	6.9	40.1	7.0	12.0	25.5	52.9
L2	8.1	100	13.4	112	462	167	259	10.3	13.3	33.8	9.4	8.5	21.5	55.1
VL1	8.1	32	21.0	45	459	168	328	11.4	19.1	10.0	6.2	10.1	24.0	62.1

ECC = equivalent $CaCO_3$; OM =organic matter; CS = coarse sand; FS = fine sand; cry-Fe = crystalline Fe oxides; CEC = cation exchange capacity.

Table 2. Composition and relative abundance of fine earth in the unpolluted soil samples

Soil Sample	Phyllosilicates 2:1	Kaolinite	Feldspars	Quartz	Calcite	Dolomite
M1	++	tr	++	++++	+++	++
M2	+	tr	+	++++	+++	+
M3	++	+	++	++++	++	tr
L1	++	tr	++	++++	++	tr
L2	+	tr	++	++++	++	nd
VL1	++	tr	++	++++	tr	nd

Number of +′s is proportional to abundance: (++++) most abundant, (+) least abundant, (tr) trace, and (nd) not detected.

K_d values show that high concentrations of metals remain in solution (Anderson & Christensen, 1988). Pb was retained in the highest quantities: as shown, the calculated K_d ranged from 5 to $7\cdot10^3$. Cu and Zn presented intermediate values of K_d while Cd had the lowest K_d value. These results concur with the lowest percentage of extractable Pb and the highest percentage of extractable Cd obtained with all the extraction methods tested. The metal concentration values obtained in single chemical extractions in the unpolluted and metal-spiked soils incubated for 1 day and 12 months are shown in Table 3. Other values were not included for reasons of space. The metal concentration values in the metal-spiked soils as a percentage of total metal are shown in Fig. 2. In general, the extraction efficiency at the end of the incubation experiment for all tested soils and metals applied as a multi-element metal salt solution followed the extraction sequence: DTPA (36-80 %) > LMWOA (1-11 %) > $NaNO_3$ (0-3 %), as specified by Feng *et al.* (2005). In some cases, the metal concentrations extracted were too low to allow a consistent interpretation of the data ($NaNO_3$-extractable Cu, Pb and Zn and LMWOA-Pb). Moreover, the $NaNO_3$ method was incapable of discriminating among different soil types for metal extractability. However, the influence of soil properties on metal extractability could be observed with LMWOA and more clearly with the DTPA method from the start of the experiment, and more markedly so at the end. This behaviour is reflected in the trend plots shown in Fig. 2. $NaNO_3$-extractable metal percentages showed a general downward trend throughout the incubation experiment, mainly during the first month of contact time. The percentage values of LMWOA-extractable Cd and Cu decreased throughout the incubation experiment, while LMWOA-extractable Zn showed a slight upward trend. The temporal trends with the DTPA-method were more irregular. These differing temporal trends thus revealed the strong affectation of soil constituents on metal extractability with these extraction methods, as discussed below for each metal studied.

As expected, the values for $NaNO_3$-extractable Cd concentration presented a downward trend during the first month of the incubation experiment (Fig. 2) and similar percentage values of Cd –about 14 % at day 1 and about 3 % at 12 months– were extracted from different soils. At 12 months, the relative concentration of extractable Cd in M1 soil (with the highest ECC content) was half that found in L1 and VL1 soils (with a lower ECC content). This pattern was more evident in the case of DTPA than with the LMWOA method.

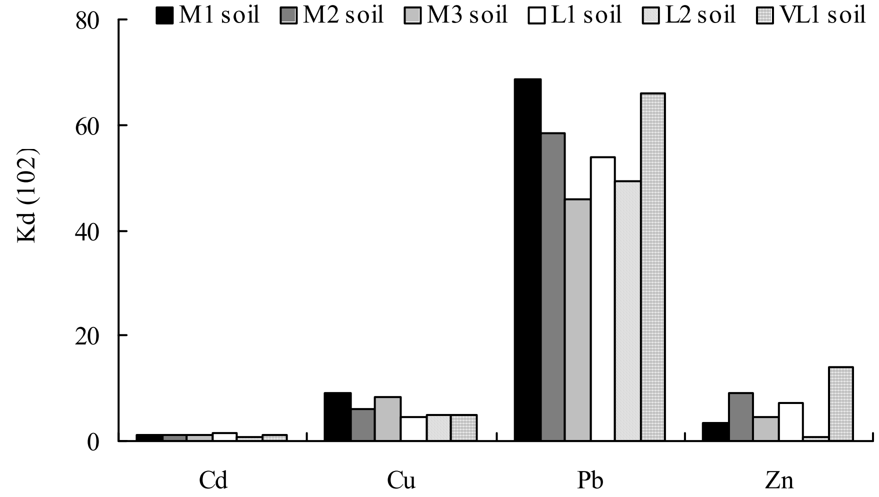

Fig. 1. Distribution coefficient (K_d) calculated in the metal-spiked soil samples at 24 h of contact time

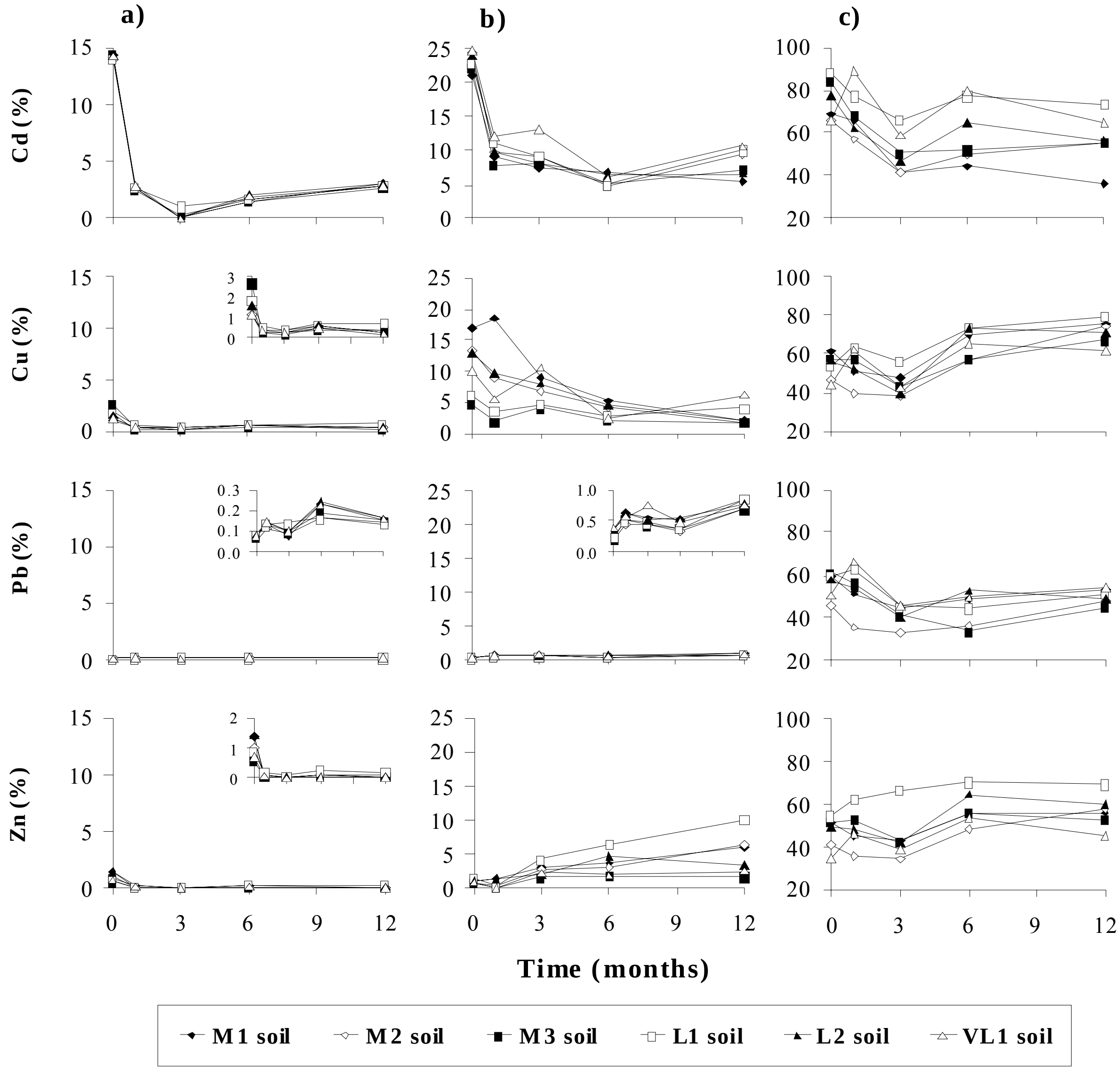

Fig. 2. Relative metal extractability (%) with $NaNO_3$ (a), LMWOA (b) and DTPA (c) methods in the metal-spiked soil samples as a function of the incubation time

Table 3. Extractable Cd, Cu, Pb, and Zn concentrations (mg/kg) in the unpolluted and in the metal-spiked soil samples at day 1 and at 12 months of contact time

Metal	Extraction method	Contact time	Soil sample					
			M1	M2	M3	L1	L2	VL1
Cd	$NaNO_3$	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	0.43	0.42	0.43	0.42	0.43	0.43
		12 months	0.09	0.08	0.08	0.09	0.09	0.08
	LMWOA	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	0.63	0.73	0.66	0.69	0.71	0.74
		12 months	0.17	0.28	0.22	0.30	0.20	0.32
	DTPA	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	2.06	1.97	2.53	2.65	2.32	1.97
		12 months	1.07	1.67	1.65	2.20	1.70	1.93
Cu	$NaNO_3$	Unpolluted	0.59	0.42	0.43	0.58	0.58	0.55
		1 day	1.65	1.79	4.04	2.75	2.34	1.74
		12 months	0.43	0.63	0.53	1.06	0.43	0.29
	LMWOA	Unpolluted	0.19	0.18	0.18	0.91	0.32	0.21
		1 day	25.27	20.20	6.87	9.33	19.30	15.16
		12 months	3.06	2.35	2.68	6.27	3.22	9.30
	DTPA	Unpolluted	1.32	1.47	1.70	0.93	0.85	1.49
		1 day	90.91	71.08	86.31	81.64	83.50	65.97
		12 months	111.70	112.70	100.90	121.00	104.90	92.64
Pb	$NaNO_3$	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	0.24	0.22	0.25	0.27	0.28	0.26
		12 months	0.49	0.48	0.48	0.45	0.52	0.52
	LMWOA	Unpolluted	0.98	0.56	0.68	0.54	1.08	0.93
		1 day	1.09	0.62	0.72	0.74	1.15	1.25
		12 months	2.65	2.44	2.19	2.74	2.45	2.42
	DTPA	Unpolluted	2.24	7.12	5.38	3.63	3.52	6.27
		1 day	186.76	161.64	197.09	193.09	184.96	162.88
		12 months	167.20	171.20	145.50	166.20	157.70	174.80
Zn	$NaNO_3$	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	4.91	3.54	2.04	3.02	4.98	2.65
		12 months	0.24	0.21	0.10	0.64	0.20	0.07
	LMWOA	Unpolluted	LQL	LQL	LQL	LQL	LQL	LQL
		1 day	3.19	2.74	2.64	4.99	3.79	3.01
		12 months	20.76	22.97	5.98	35.38	11.94	8.77
	DTPA	Unpolluted	1.37	1.84	2.22	2.36	1.88	2.53
		1 day	181.34	148.85	188.73	193.12	175.16	125.39
		12 months	198.90	210.00	193.20	244.60	213.20	164.20

LQL = lower quantification limit.

This highlighted the fact that the ECC content plays a key role in Cd sorption-desorption processes in calcareous soils at the Cd concentration used in this study (Buekers *et al.*, 2007; Sánchez-Camazano *et al.*, 1998). However, although this behaviour is clearly shown in the soils with very different ECC contents, no clear pattern could be established between the carbonate gradient in the soils tested and the percentage of extractable Cd. Hence other soil properties and/or constituents such as CEC and OM (Krishnamurti & Naidu, 2003) may affect Cd extractability as the carbonate gradient decreases. In previous studies on these types of soil, we reported that Cd retention was affected by mineralogy (Lafuente *et al.*, 2008). However, in the present research, we observed that although the M1 soil had the highest content of calcite and dolomite (trace in L1 soil and not detected in VL1 soil), the mineralogical study did not offer any additional information on the extractability pattern. From these results it can be concluded that although the ECC content appears to play a key role, this constituent alone proved insufficient for us to establish a pattern of Cd extractability when it is added as a multi-element metal salt solution.

The study of soil properties affecting Cu extractability was more complex. In the case of the $NaNO_3$-extraction method, it should be noted that similar Cu concentrations were extracted in unpolluted soils and in metal-spiked soils at 12 months (Table 3 and Fig. 2). This may suggest that Cu redistributes with contact time, reducing its concentration in exchangeable positions (Sayen & Guillon, 2009). However, the low extraction percentage was not sufficient to reveal a Cu extractability pattern with this method. LMWOA-extractable Cu decreased throughout the incubation experiment, showing a different pattern of extractability at the beginning and at the end. At day 1, the soils with the highest OM contents (M3 and L1 soils) showed the lowest % of extractable Cu (about 5 %), while soils with the lowest OM content (M1 soil) showed the highest extractability values (about 17 %). However, after 12 months, soils with a low ECC content (VL1 and L1 soils), regardless of their OM content, showed the highest Cu extractability, which coincides with the pattern observed in unpolluted soils. This observation could indicate *a priori* that the ECC content affects Cu extractability with LMWOA. Nevertheless, the alteration of the organic fractions noted in metal-spiked soils at the end of the incubation experiment in soils with less ECC content could explain this behaviour. This fact suggests that Cu-OM complexes may be established at the start and then degrade over the course of the incubation experiment (Martínez *et al.*, 2003) and facilitating its subsequent extraction with LMWOA. This suggests that OM and carbonate may compete for Cu retention in these calcareous soils (Besnard *et al.*, 2001). In view of these results, we cannot determine whether higher Cu-extractability with LMWOA is affected by either a lower ECC content or by further degradation of recalcitrant OM in soils with a lower ECC content, or possibly both. In the case of DTPA, soils with the highest silt and clay contents (VL1, M3 and L2 soils) presented the lowest Cu extractability after 12 months. There was no pattern related to OM content. However, Jalali & Khanlari (2008) highlighted the influence of the silt fraction on the amount of Cu bound to the organic fraction in calcareous soils. In these soils, clay-humic complexes dominate organometallic complexes, and the former are more stable. If this type of complex had been established, the DTPA method, due to its greater extracting power, might have been able to release the Cu retained in these complexes. Studies of soil particle-size fractionation have shown that Cu was mainly concentrated in the clay-sized fraction < 2 μm, and in the coarse particulate organic matter > 50 μm (Besnard *et al.*, 2001). This was attributed to the high reactivity of the mineral constituents and organo-mineral associations present in the < 2 μm fraction, and would explain the relationships found between fine mineral fractions and OM, and Cu extractability with DTPA. As noted by Jalali & Khanlari (2008), further research is required in this direction. Although Cu extractability was affected by ECC, silt and OM, the OM appears to be the most influential factor. This reveals that in the soils studied with a high Cu sorption capacity the sorption-desorption processes could be affected by the OM, despite their low OM content (Buekers *et al.*, 2007; Micó *et al.*, 2006), and highlights the vulnerability of these soils, since an increase in the mineralization of OM (very common in the Mediterranean climate) could affect the storage capacity, mobility and availability of metals in soils, leading to a significant risk of environmental contamination (Hernandez-Soriano & Jimenez-Lopez, 2012; Martínez *et al.*, 2003). It is worth noting that we extracted about 60-80 % of Cu with DTPA. This high extraction percentage reveals the risk of the potential mobility and availability of Cu, even in soils such as these, where the Cu sorption capacity is high.

The chemistry of Pb in soils is mainly affected by specific adsorption, precipitation of stable compounds, and formation of relatively stable complexes with OM. As shown by the calculated K_d values, the highest retention of Pb occurred in M1 and VL1 soils, with the highest ECC and clay contents respectively, thus showing the influence of these constituents on Pb retention after 24 h of contact time (Fig. 1). However, at 12 months, DTPA-extractable Pb concentrations were very similar for all soils, making it impossible to discern which soil properties affect Pb extractability with this method (Fig. 2). Jalali & Khanlari (2008) observed that with longer contact times than the first 3 h-1 day, Pb was distributed from exchangeable forms to more strongly-binding sites (reducible and oxidizable fractions). The sorption-desorption processes in these fractions are strongly regulated by soil pH. However, the pH range of the soils in the study is so narrow that we are unable to distinguish which of the constituent parts of these fractions affect Pb extractability with DTPA. Therefore, the DTPA method was impractical for fulfilling the aims of this paper. Nevertheless, it is worth noting the high percentage of DTPA-extractable Pb obtained in the metal-spiked soils (about 50 %).

Zn concentrations extracted with the LMWOA and DTPA methods showed similar extractability patterns, with an upward trend over the course of the incubation experiment (Fig. 2). All soils showed similar LMWOA-extractable Zn concentration values at the beginning of the study and became differentiated during the incubation experiment. After 12 months, the different Zn extractability pattern corresponded to the soil silt content, and the highest extractable Zn values were obtained in soils with the lowest proportions of silt, following the soil sequence: L1>M2>M1>L2>VL1>M3. In this regard, Jalali & Khanlari (2008) noted that a lower proportion of Zn in the exchangeable fraction of calcareous soils was observed in soil samples with the highest silt content. In the case of the DTPA method, the highest extraction percentage corresponded to soils with the highest coarse sand content, following the soil sequence: L1>L2=M2=M1>M3>VL1. In the unpolluted soils (Table 3), more Zn was extracted with DTPA in the soil with the lowest silt content (L1 soil). Although the distribution of forms of Zn is dose-dependent (Xiang *et al.*, 1995), in our case both unpolluted soils and metal-spiked soils appeared to show the same extractability pattern. For the Zn-K_d value, the highest sorption was obtained in VL1 soil, which had the highest CEC values and clay content. The latter is dominated by 2:1 silicates (Table 2), which have a greater fixing capacity for this metal. Nevertheless, due to the low percentage of $NaNO_3$-extractable Zn, the main mechanism for binding to these constituents appears not to be adsorption, but immobilization in the octahedral layers of phylosilicates (Jacquat *et al.*, 2009; Sipos *et al.*, 2008). In view of these results, we conclude that particle-size distribution can be the most important factor affecting both the sorption and extractability of Zn with all the selected extraction procedures in these calcareous agricultural soils, under experimental conditions. As in the case of the other metals, Zn extractability with DTPA was high (45-70 %), which may indicate a risk of phytotoxicity, despite the high sorption capacity of these soils.

CONCLUSION

The information obtained shows that the carbonate content alone cannot explain the extractability patterns of Cd, Cu, Pb, and Zn in the soils studied, under the experimental conditions used. Thus although carbonate was observed to play a key role in regulating Cd extractability, carbonate content was insufficient to establish a Cd-extractability pattern. Cu-extractability was affected by the content of carbonate, silt and organic matter, with the latter as the most influential constituent. Although carbonate and clay content was seen to govern Pb retention, it could not be established which soil components affect Pb-extractability patterns. Particle-size distribution was found to be a significant factor affecting both sorption and extraction processes of Zn. It has become apparent that the combination of several soil properties and constituents, such as particle-size distribution and organic matter, plays a key role in metal extractability patterns in calcareous soils. These results point to the conclusion that, despite the low organic matter content, long-term transformations of organic matter and organo-mineral associations could alter metal storage capacity and, consequently, metal availability. From the high Cd, Cu, Pb, and Zn concentrations extracted with the DTPA method we can infer a potential risk of metal mobility and availability in the periurban calcareous agricultural soils of the Mediterranean area studied, with a high metal retention capacity (> 98 %). Since our results provide evidence that these soils could not withstand the environmental impact from metal accumulation in periurban areas, we believe that they should be considered vulnerable in the long term. Nevertheless, further research work is necessary.

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