Effects of Zinc Complexes on the Distribution of Zinc in Calcareous Soil and Zinc Uptake by Maize

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The movement and availability of Zn from six organic Zn sources in a Typic Xerorthent (calcareous) soil were compared by incubation, column assay, and in a greenhouse study with maize (*Zea mays* L.). Zinc soil behavior was studied by sequential, diethylenetriaminepentaacetate, and Mehlich-3 extractions. In the incubation experiment, the differences in Zn concentration observed in the water soluble plus exchangeable fraction strongly correlated with Zn uptake by plants in the greenhouse experiment. Zinc applied to the surface of soil columns scarcely moved into deeper layers except for Zn-ethylenediaminetetraacetate (EDTA) that showed the greatest distribution of labile Zn throughout the soil and the highest proportion of leaching of the applied Zn. In the upper part of the column, changes in the chemical forms of all treatments occurred and an increase in organically complexed and amorphous Fe oxide-bound fractions was detected. However, the water soluble plus exchangeable fraction was not detected. The same results were obtained at the end of the greenhouse experiment. Significant increases were found in plant dry matter yield and Zn concentration as compared with the control treatment without Zn addition. Increasing Zn rate in the soil increased dry matter yield in all cases but Zn concentration in the plant increased only with Zn-EDTA and Zn-ethylenediaminedi-o-hydroxyphenyl-acetate (EDDHA) fertilizers. Higher Zn concentration in plants (50.9 mg kg^{-1}) occurred when 20 mg Zn kg^{-1} was added to the soil as Zn-EDTA. The relative effectiveness of the different Zn carriers in increasing Zn uptake was in the order: Zn-EDTA > Zn-EDDHA > Zn-heptagluconate $\geq$ Zn-phenolate $\approx$ Zn-polyflavonoid $\approx$ Zn-lignosulfonate.

INTRODUCTION

Zinc can be a limiting factor for plant growth in alkaline soils (*1*). Crop response to Zn fertilization varies with the source of Zn fertilizer (*2*) and soil physicochemical properties (*3*). Among the plants, maize (*Zea mays* L.) is very susceptible to Zn deficiency (*4*), particularly in calcareous soils where high pH and high CaCO$_3$ content may affect Zn availability (*5*). The Zn sources mostly used to correct Zn deficiencies are inorganic salts, synthetic chelates, natural organic complexes, and inorganic complexes (*6*). Several authors reported that Zn chelates are more effective than inorganic Zn forms (*7−9*). Anderson (*10*) reported that the relative effectiveness of different Zn carriers on crop growth was in the order: Zn-DTPA (Zn-diethylenetriaminepentaacetate) > Zn-EDTA (Zn-ethylenediaminetetraacetate) > Zn-EDDHA (Zn-ethylenediaminedi-o-hydroxyphenyl-acetate) > ZnSO$_4$ > Zn-Rayflex (polyflavonoid). Rico et al. (*11*) found that the application of Zn-EDTA and Zn-lignosulfonate led to larger increases in maize dry matter yield.

The movement of Zn in the soil profile markedly influences Zn concentrations in plants and organic ligands, especially chelating agents, which contribute to Zn mobility in soils (*12−14*). Mobility and extractability have been studied by means of column experiments (*15*). Alvarez et al. (*16*) found differences between Aquic Haploxeralf (acidic) and Calcic Haploxeralf (neutral) soils when Zn-EDDHA, Zn-EDTA, Zn-heptagluconate, Zn-polyflavonoid, Zn-lignosulfonate, and Zn-phenolate were applied in a column experiment. In the acidic soil, the application of the six organic Zn complexes produced little migration and very little leaching of Zn in soil columns. The same occurred in the neutral soil except with Zn-EDTA, which produced a loss of Zn by leaching.

Sequential extraction procedures were also useful to assess indirectly the potential mobility and bioavailability of metals in soils (*17*). Zinc can be found in the following forms in soils: (i) as free and complexed ions in soil solution, (ii) as nonspecifically and specifically adsorbed cations, (iii) as ions occluded mainly in soil carbonates and hydrous oxides, (iv) in biological residues and living organisms, and (v) in the lattice structure of primary and secondary minerals (*18*). Metal fractions in soils can be operationally classified as water soluble, exchangeable, carbonate bound, Mn oxides bound, amorphous and crystalline Fe oxides bound, and residual (*19*, *20*). Zinc in soils can be separated into fractions relevant to the physicochemical forms

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Table 1. Selected Properties of Soil Used in the Incubation, Column, and Greenhouse Experiments[a]

property		ref
clay (g kg^{-1})	280	25
texture (USDA)	clay loam	
predominant clay	montmorillonite	26
permeability	moderate	27
pH (1:2.5, w:v)	8.3	
oxidizable OM (g kg^{-1})	9.1	28
extractable P (mg kg^{-1})	34.9	29
total N (g kg^{-1})	0.90	30
CEC (cmol$^+$ kg^{-1})	31.3	31
base saturation (%)	100	
total CaCO$_3$ (g kg^{-1})	206	32
free CaCO$_3$ (g kg^{-1})	24.2	33
total Zn (mg kg^{-1})[a]	87.3	
poorly crystalline (active) oxides (g kg^{-1} Fe$_2$O$_3$)	8.0	34

[a] Soil digestion in a microwave oven with an HCl–HNO$_3$–HF mixture (1:14, w/v).

while the distribution of Zn depends on the extracting reagents and the sequence of extraction (*21*). This topic was reviewed comprehensively by Ure (*22*). According to Li and Shuman (*23*), the most labile Zn fractions have the greatest leaching potential and may be a threat to groundwater quality. Simple extraction methods including DTPA-CaCl$_2$-TEA and Mehlich-3 are normally used to predict Zn availability to plants (*4*, *18*).

The objective of this study was to investigate the influence of six organic ligands on the behavior of Zn found in Zn fertilizers, when they were added to a calcareous soil under laboratory and greenhouse conditions. The distribution in the soil and leachability were determined by incubation and column experiments. Plant uptake efficiency and availability of residual Zn were also determined by growing maize in a greenhouse experiment. Finally, the results of different extraction procedures were compared with the yield of maize and Zn concentration in plants.

MATERIALS AND METHODS

The surface 27 cm layer (Ap horizon) of a calcareous soil was collected at Loeches (Madrid, Spain, latitude 40°23′ N, longitude 3°24′ W). The soil was classified as a Typic Xerorthent (*24*) (**Table 1**) and had no B horizon. The soil sample was air-dried and crushed, and the fraction of less than 2 mm was used in the experiments.

Six liquid fertilizers with different organic Zn complexes were selected as follows: Zn-EDDHA (70 g Zn L^{-1}), Zn-EDTA (88 g Zn L^{-1}), Zn-heptagluconate (60 g Zn L^{-1}), Zn-polyflavonoid (50 g Zn L^{-1}), Zn-lignosulfonate (75 g Zn L^{-1}), and Zn-phenolate (70 g Zn L^{-1}). All of them were commercial trademarks, and their characteristics were described by Liñán (*35*).

The soil was treated with aqueous suspensions of the six fertilizers to obtain samples of each one with a concentration of added Zn of 0 (control), 10, and 20 mg Zn kg^{-1} soil. Weighed samples were incubated in appropriate containers for direct analysis: 5 g in 100 mL centrifuge glass tubes for chemical fractionation and 10 g in 125 mL conical glass flasks for DTPA extractable Zn and maintained at 22 ± 1 °C with soil moisture at field capacity (26.9% w/w) for 15, 30, and 60 days. Soil moisture content was maintained by weighing every 3 days and adding water as required. Three replicates of each sample were analyzed.

Homogeneous columns of each fertilizer treatment were prepared by uniformly packing 1.2 kg of soil in 40 cm long, transparent Plexiglas tubes (inside diameter, 7.2 cm) with two filter papers (Whatman no. 4) and gravel at the bottom. The bulk density of the packed soil was 1.1 g cm^{-3}. The packed soil was saturated by adding water and by permitting the excess to drain overnight. The soil occupied about 27 cm of the column. Variable amounts of fertilizer were added to each

Table 2. Procedure for the Sequential Extraction of Zn from Soil[a]

step	form/association	extracting solution	ref
1	water soluble plus exchangeable (WSEX)	1 M Mg(NO$_3$)$_2$ (pH 7.0)	20
2	organically complexed (OC)	0.7 M NaOCl (pH 8.5)	20
3	carbonate bound (CAR)	1 M NaOAc (adjusted to pH 5.0 with HOAc)	19
4	Mn oxides bound (MnO)	0.1 M NH$_2$OH·HCl (pH 2.0)	37
5	amorphous Fe oxides bound (AFeO)	0.2 M (NH$_4$)$_2$C$_2$O$_4$ + 0.2 M H$_2$C$_2$O$_4$ (pH 3.0)	20
6	crystalline Fe oxides bound (CFeO)	solution as for previous step plus 0.1 M ascorbic acid	20
7	residual (RES)	acid mixture[a]	

[a] Two grams of the soil residue of step 6 air-dried and ground, digested in a microwave oven with HCl (2 mL) + HNO$_3$ (5 mL) + HF (5 mL).

column (except the control) containing 24 mg of Zn in all cases and mixed with the soil in the zone of 1.5 cm from the top. Two groups of columns were maintained at room temperature (18−23 °C), and 30 mL days^{-1} of water were added to them in both the 30 and the 60 day experiments. Three replicates were prepared of each fertilizer treatment plus the control soil with measurements at 30 and 60 days. Leachates were collected in eight fractions of 150 mL (0.235 pore volume) in the 60 day experiment, and the Zn concentration of each portion was measured. After leaching, the two halves of the columns were separated along the longitudinal axis and the soil was handled as described by Alvarez et al. (*16*). The columns were collected at depths of 0−7 (312 g), 7−17 (444 g), and 17−27 cm (444 g). Each zone was analyzed by chemical fractionation and DTPA and Mehlich-3 extractable Zn methods.

In the greenhouse experiment, three seeds of maize were sown in polyethylene pots containing 8 kg of air-dried soil, uniformly fertilized with 75 mg N kg^{-1} (as urea), 75 mg P kg^{-1} (as superphosphate), and 75 mg K kg^{-1} (as K$_2$SO$_4$) and spiked with 0 (control), 10, and 20 mg Zn kg^{-1} (as organic Zn fertilizer). Additional applications of 37.5 mg N kg^{-1} were added to the pots at 7 and 30 days after sowing the seeds. The maize genotype (A-33, ASGROW Seed Co., Madrid, Spain) double hybrid was a short growth season variety extensively used as fodder. The pots were placed in a greenhouse with average day and night temperatures of 42 and 16 °C, respectively, and were irrigated slightly above field capacity moisture. The experimental design was a randomized complete block with three replications. After 45 days, the plants were cut at soil level, washed in deionized water, and then dried in an oven at 65 °C until a constant weight was obtained. Zinc concentrations in the plants were determined after treating 1 g of the dried samples with 14 mL of an acid mixture (HCl + HNO$_3$ + HF) and digesting them in Teflon tubes in a microwave oven. Soil Zn concentrations were determined after harvesting the maize crop by sequential fractionation and DTPA and Mehlich-3 extraction procedures.

The chemical fractionation of Zn was performed by sequentially treating 5 g subsamples of soil (except in step 7) with 50 mL (*36*) of the corresponding extracting solution (**Table 2**). The total concentration of Zn in soil was calculated as the sum of Zn extracted from the seven fractions. The form of Zn available for plants was extracted with DTPA (*38*) and Mehlich-3 (*39*). In all cases, Zn concentrations were determined by atomic absorption spectrophotometry.

Analysis of variance was performed on the data, and the mean values were separated by the LSD method ($P \leq 0.05$), using the Statgraphics Plus software, version 5.1 (Manugistic Inc., Rockville, MD).

RESULTS AND DISCUSSION

Incubation Effects on the Chemical Extractions of Zinc from Soil. Concentrations of Zn extracted with DTPA from the incubated control soil and from the incubated samples with soil plus fertilizers are shown in **Figure 1**. The concentration of Zn extracted from the control soil was hardly affected by
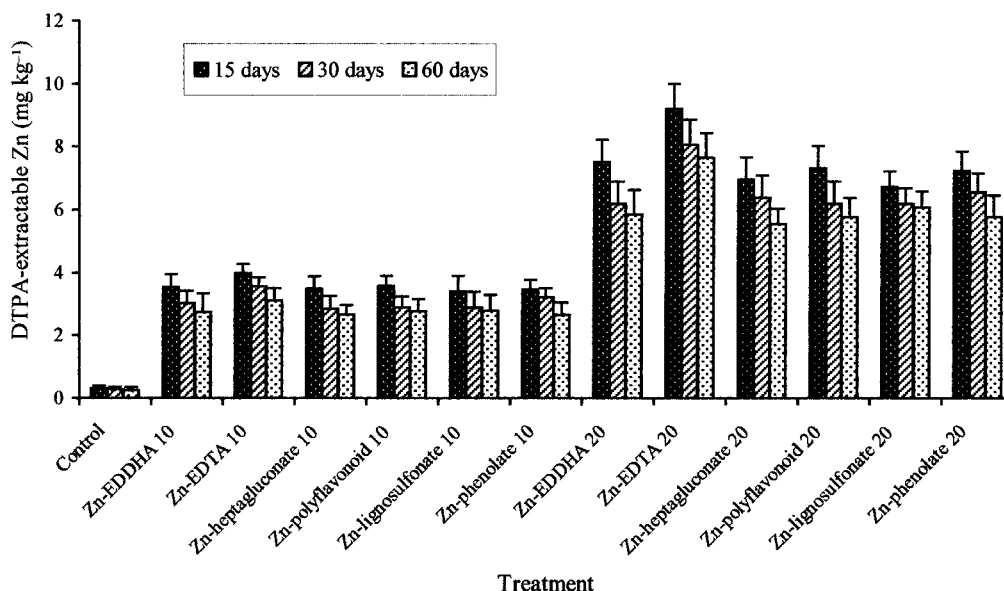
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Figure 1. DTPA extractable Zn in soil with different Zn rates (10 and 20 mg Zn kg^{-1}) of fertilizers and incubation period. Error bars are based on one standard deviation.

Table 3. Average Values of DTPA Extractable Zn and WSEX and OC Fractions (mg kg^{-1})^a with Different Rates (mg kg^{-1}) of Zn Fertilizers as Influenced by Incubation Period (Days) and Zn Source

	method of extraction					
	DTPA		WSEX		OC	
	Zn (mg kg^{-1}) applied to soil					
source of variation	10	20	10	20	10	20
	Zn mg kg^{-1}					
incubation period (days)						
15	3.57^c	7.49^c	0.31^b	0.77^b	0.37^b	0.73^c
30	3.07^b	6.59^b	0.15^a	0.46^a	0.22^a	0.54^b
60	2.78^a	6.10^a	0.08^a	0.36^a	0.24^a	0.31^a
fertilizer						
Zn-EDDHA	3.10^a	6.51^b	0.29^b	0.72^b	0.39^c	0.39^a
Zn-EDTA	3.54^b	8.30^c	0.67^c	2.33^c	0.24^a	0.46^a
Zn-heptagluconate	3.00^a	6.29^a	0.02^a	0.03^a	0.32^b	0.75^c
Zn-polyflavonoid	3.07^a	6.42^{ab}	0.03^a	0.04^a	0.25^a	0.56^b
Zn-lignosulfonate	3.02^a	6.32^{ab}	0.05^a	0.05^a	0.24^a	0.61^a
Zn-phenolate	3.10^a	6.51^b	0.01^a	0.01^a	0.25^a	0.38^a

^{a} In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability.

the incubation and was in all cases less than 0.5 mg kg^{-1}, which is considered deficient in alkaline soils for most crops. Levels lower than 0.8 mg kg^{-1} DTPA extractable Zn are insufficient for maize production (*38*) and require application of Zn fertilizers. Potentially available Zn decreased during the incubation period in soil treated with both Zn rates ($P < 0.0001$; **Table 3**). After incubation of ZnSO$_4$ with alkaline soils, Ma and Uren (*40*) reported that drying and rewetting at a relatively high temperature might be an important factor in the process of decreasing the availability of Zn to plants. Significant differences were found between fertilizer treatments with each Zn rate ($P < 0.0001$). The highest concentration of DTPA extractable Zn occurred with the Zn-EDTA treatments. Statistical analysis showed that for both Zn rates significant differences existed between Zn-EDTA and the other five fertilizers. According to Norvell (*41*), little chelated Zn is substituted by Ca in calcareous soils and Zn-EDTA remains effective for plants in these soils. A significant interaction existed between the incubation period and the fertilizer treatment ($P < 0.0001$). At the end of the incubation, the percentage of available Zn extracted was between

20.8 and 24.8 for the 10 mg Zn kg^{-1} rate and between 24.8 and 33.5% for a rate of 20 mg Zn kg^{-1}. These values are large enough to consider a favorable influence of the ligands on the availability of Zn although not all of them behave the same.

Chemical fractionation of the originally collected soil showed the following distribution of Zn in the different fractions: WSEX, not detected; OC, 0.3%; CAR, 0.1%; MnO, 0.1%; AFeO, 4.8%; CFeO, 1.7%; and RES, 93.0%. As observed, Zn concentrated mostly in the RES fraction reflecting the tendency of Zn to transform into forms unavailable to plants. Among the nonresidual fractions, the Fe oxide fraction contained a larger amount of Zn than the other fractions, probably due to the high stability of the Fe−Zn oxides (*42*). In the control soil, Zn distribution among the fractions remained in the order as in the original soil (except that OC was larger than CAR), RES, AFeO, CFeO, CAR, OC, MnO, and WSEX, and hardly varied during the whole incubation period. Also, Zn in the WSEX fraction was not detected (**Table 4**). The addition of Zn complexes led to different increases in all fractions, but as time passed, Zn concentrations diminished for WSEX, OC, MnO, and CFeO ($P < 0.0001$, except the WSEX fraction $P < 0.0002$) and increased for CAR, AFeO, and RES fractions ($P < 0.0001$, except the AFeO fraction with a rate of 10 mg Zn kg^{-1}, $P < 0.05$). The order between Zn fractions was the same as for the control soil for all fertilizers except Zn-EDDHA and Zn-EDTA, which changed the order of the three last fractions, where both had the smallest MnO fraction.

Among the fertilizer treatments, significant differences were found in WSEX and OC fractions with both Zn rates ($P < 0.0001$; see **Table 3**). The highest Zn concentration in the most labile fraction (WSEX) occurred with the Zn-EDTA treatments, and Zn concentrations with both rates of Zn-EDDHA fertilizer were approximately three times lower, although Zn-EDTA and Zn-EDDHA complexes in aqueous solutions have similar pK values (p$K_{Zn-EDTA} = -17.4$ and p$K_{Zn-EDDHA} = -17.8$ with ionic strength of 0.01 M; *42*). Aboulroos (*43*) reported that the Zn-EDDHA molecule was unstable in a calcareous soil and that adsorption of the Zn-EDDHA molecule by soil was the main process of removing Zn from solution. The influence of time on Zn associated with this fraction (WSEX) can be observed in **Figure 2** for Zn-EDTA and Zn-EDDHA fertilizers. During

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Table 4. Zinc Fractions (mg kg^{-1})^a in Soil at the End of the Incubation Time (60 Days) as Influenced by Fertilizer Treatments

treatment	Zn applied (mg kg^{-1})	Zn mg kg^{-1}						
		WSEX	OC	CAR	MnO	AFeO	CFeO	RES
control	0	nd^a	0.08^a	0.37^a	0.05^a	4.51^a	0.90^a	81.04^a
Zn-EDDHA	10	0.11^a	0.32^e	0.59^{cd}	0.05^a	8.89^b	1.49^b	87.02^{bc}
	20	0.48^c	0.35^e	0.71^e	0.05^a	14.26^d	1.53^b	91.61^{de}
Zn-EDTA	10	0.35^b	0.26^d	0.54^{bcd}	0.05^a	10.00^{bc}	1.59^b	85.24^{bc}
	20	1.65^d	0.34^e	0.81^{ef}	0.08^{bc}	11.41^c	1.61^b	91.76^{de}
Zn-heptagluconate	10	nd	0.25^{cd}	0.60^d	0.06^{ab}	9.51^b	1.55^b	86.19^{bc}
	20	nd	0.51^f	0.73^{ef}	0.09^c	9.98^{bc}	1.59^b	95.67^e
Zn-polyflavonoid	10	nd	0.25^{cd}	0.45^{ab}	0.06^{ab}	10.31^{bc}	1.47^b	86.51^{bc}
	20	nd	0.31^e	0.76^{ef}	0.10^c	16.15^e	1.52^b	87.49^{bcd}
Zn-lignosulfonate	10	nd	0.21^{bc}	0.49^{bc}	0.05^a	10.22^{bc}	1.41^b	84.60^{ab}
	20	nd	0.26^d	0.74^{ef}	0.08^{bc}	16.11^e	1.55^b	88.00^{bcd}
Zn-phenolate	10	nd	0.19^b	0.71^e	0.09^c	9.50^b	1.46^b	85.19^{ab}
	20	nd	0.21^{bc}	0.82^f	0.10^c	14.24^d	1.59^b	89.54^{cd}

^{a} In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability; nd, not detected.
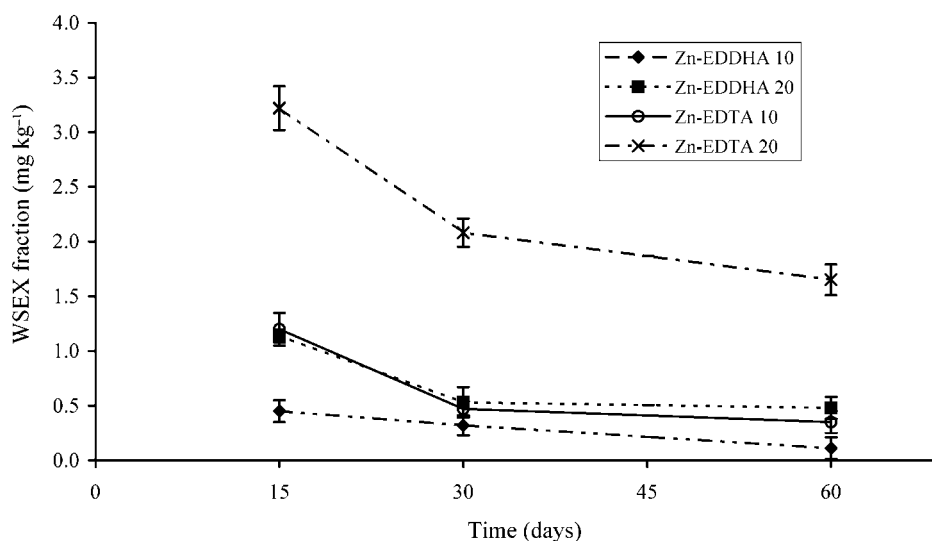

Figure 2. Water soluble plus exchangeable fraction (WSEX) concentrations vs incubation period with different Zn rates (10 and 20 mg Zn kg^{-1}) of Zn-EDDHA and Zn-EDTA fertilizers. Error bars are based on one standard deviation.

the first 30 days of the experiment, a decrease was noticeable followed by a period of little changes. Differences between fertilizers should be pointed out, especially for a rate of 20 mg Zn kg^{-1}. Significant differences were found in this fraction between these two fertilizers and the other four (see **Tables 3** and **4**). For the other four Zn fertilizers after 15 days, the concentration of Zn in the WSEX fraction was less than 0.1 mg kg^{-1} in all cases and was not detected by the end of the experiment. In the OC fraction, which also decreases with time, the behavior of the fertilizers is different from that observed in the DTPA and WSEX extractions. The highest concentrations were for Zn-EDDHA at a rate of 10 mg Zn kg^{-1} and Zn-heptagluconate at a rate of 20 mg Zn kg^{-1}. EDTA and EDDHA are able to complex Zn more strongly than the others and therefore decrease the sorption of Zn by soil constituents (e.g., the high montmorillonitic clay content). On the other hand, as Ca is abundant in this soil, it competes with Zn, displacing it from the organic Zn chelates and more easily from the natural Zn chelates because these complexes are less stable (*8*). With respect to the other fractions, most of the Zn applied was found in relatively high amounts in the RES and AFeO fractions. The results of the incubation experiment suggest that in general the Zn-EDTA fertilizer behaves as the best micronutrient source, followed by the Zn-EDDHA fertilizer, on the type of soil studied.

Zinc Mobility in the Soil Columns. Despite the fact that the soil columns were well drained and their permeability was moderate, the amount of Zn leached was very small, not only for control columns but also for those treated with Zn fertilizer (less than 1% of the Zn applied) except in the case of Zn-EDTA. The results of total Zn leached (mg Zn) from the control columns and fertilizer treatments were as follows: control, 0.09; Zn-EDDHA, 0.22; Zn-heptagluconate, 0.15; Zn-polyflavonoid, 0.15; Zn-lignosulfonate, 0.20; and Zn-phenolate, 0.22. With the Zn-EDTA treatment, the concentration of Zn (mg L^{-1}) in the eight leachates averaged 0.07, 0.73, 3.33, 4.27, 5.40, 2.07, 1.67, and 0.93. The concentration increased with time and reached a maximum in the fifth fraction (approximately 40 days, 1.18 pore volume) and then decreased. Total Zn recovered in the leachates was 2.68 mg (11.2% of Zn applied). These results can be explained by the stability of the complexes, which is in agreement with that obtained in the fractionation of soil (WSEX values) in the incubation experiment. This behavior has also been observed with the same fertilizers in a Calcic Haploxeralf (neutral) soil. The addition of Zn-EDTA produced a loss of 36% Zn by leaching, and Zn leached very little with the other five treatments (*16*).

It should be pointed out that the soil column methods used are a simplification of the natural processes. According to Li and Shuman (*44*), the Zn movement with soluble organics in

Table 5. Concentrations of Total Zn and DTPA and Mehlich-3 Extractable Zn (mg kg^{-1})^a in Soil Columns with Different Zn Fertilizer Treatments

		Zn mg kg^{-1}					
		days after application					
		30 days			60 days		
treatment	depth (cm)	total Zn	DTPA	Mehlich-3	total Zn	DTPA	Mehlich-3
control	0–7	84.89^a	0.50^a	2.23^a	84.52^a	0.52^a	2.14^{ab}
	7–17	83.00^a	0.39^a	2.15^a	83.11^a	0.36^a	1.95^a
	17–27	83.25^a	0.39^a	2.11^a	83.03^a	0.38^a	1.80^a
Zn-EDDHA	0–7	147.39^d	19.58^d	26.17^f	144.17^e	18.13^d	24.21^e
	7–17	89.11^{ab}	2.59^{ab}	5.80^{abc}	90.15^{ab}	2.05^a	5.32^b
	17–27	86.62^a	1.63^a	3.47^{ab}	87.77^{ab}	0.98^a	2.97^{ab}
Zn-EDTA	0–7	110.92^c	6.05^c	10.58^{de}	102.56^{cd}	4.78^{bc}	9.27^c
	7–17	103.95^c	6.93^c	13.52^e	106.57^d	6.65^c	12.70^d
	17–27	94.91^b	4.45^{bc}	8.84^{cd}	95.28^{bc}	5.76^c	9.23^c
Zn-heptagluconate	0–7	153.89^d	30.23^f	44.06^h	151.23^e	26.30^{ef}	42.50^g
	7–17	85.04^a	0.44^a	2.20^a	85.02^{ab}	0.46^a	2.15^{ab}
	17–27	85.20^a	0.49^a	2.17^a	86.66^{ab}	0.53^a	1.96^{ab}
Zn-polyflavonoid	0–7	152.82^d	29.95^f	48.75^i	151.70^e	28.75^f	42.55^g
	7–17	87.45^{ab}	0.69^a	2.39^a	88.03^{ab}	0.63^a	2.86^{ab}
	17–27	84.89^a	0.43^a	2.24^a	84.89^{ab}	0.69^a	2.41^{ab}
Zn-lignosulfonate	0–7	149.19^d	26.88^e	39.55^g	147.40^e	25.76^e	38.54^f
	7–17	86.00^a	0.64^a	3.06^a	87.61^{ab}	0.87^a	3.26^{ab}
	17–27	85.96^a	0.39^a	2.38^a	86.96^{ab}	0.40^a	1.83^a
Zn-phenolate	0–7	148.05^d	24.20^e	36.25^f	146.71^e	23.75^e	37.09^f
	7–17	90.07^{ab}	3.01^{ab}	7.11^{bcd}	91.15^{ab}	2.86^{ab}	3.07^{ab}
	17–27	84.62^a	0.39^a	2.13^a	83.12^a	0.43^a	1.96^{ab}

^{a} In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability.

the field will be much slower than in the soil columns due to the heterogeneity of soils. The results of leaching with organic Zn complexes in soil columns may represent a long period of time under natural field conditions.

The concentration values of total Zn and DTPA and Mehlich-3 extractable Zn at depths of 0–7, 7–17, and 17–27 cm after 30 and 60 days of leaching are presented in **Table 5**. For total Zn concentrations, the differences observed between the control columns and the columns with fertilizers were very large in the upper part of the soil profile in all cases and in general diminished with the depth. With time, small decreases in Zn concentrations were observed in the 0–7 cm depth and small increases can be observed at depths of 7–17 and 17–27 cm. For the different fertilizers, the order of Zn concentrations in the 0–7 cm depth was the opposite of that in the 7–17 and 17–27 cm depths; this implied higher mobility for the Zn-EDTA fertilizer.

In all cases, Zn extracted with Mehlich-3 was higher as compared to DTPA. The order of these values was the same, in general, as for total Zn in the three sampling depths showing a relationship between mobility and availability. Significant differences for the three variables (total Zn and DTPA and Mehlich-3 extractable Zn) were found among the fertilizer treatments (P < 0.001) and among sampling depths (P < 0.0001). In addition, there were significant main effects caused by time for DTPA (P < 0.001) and Mehlich-3 extractable Zn (P < 0.0001), which decreased with time after application. For DTPA and Mehlich-3 extractable Zn, significant interactions (P < 0.01 and P < 0.0001, respectively) were obtained among the type of fertilizer, sampling depth, and time.

The results of the Zn fractionation procedure in the three soil layers at the end of the column experiment showed that in all of the cases, the WSEX fraction was negligible after adding water. For Zn materials that hardly moved into the 7–17 and 17–27 cm soil layers, the distribution in the different fractions was similar in both layers and it was also similar to the control columns, which did not appreciably vary from the original soil. In these layers, Zn concentrations increased slightly in the OC

Table 6. Zinc Fractions (mg kg^{-1})^a at 0–7 cm in Soil Columns Amended with Zn Fertilizers and Incubated for 60 Days

| | Zn mg kg^{-1} | | | | | |
treatment	OC	CAR	MnO	AFeO	CFeO	RES
control	0.38^a	0.15^a	0.08^a	4.53^a	1.99^b	77.39^a
Zn-EDDHA	26.38^c	0.74^c	0.79^b	19.41^e	2.35^c	94.50^b
Zn-EDTA	9.18^b	0.50^b	0.68^b	12.63^b	1.50^a	78.07^a
Zn-heptagluconate	32.72^d	1.05^d	1.07^c	15.43^c	2.37^c	98.56^b
Zn-polyflavonoid	33.84^d	1.00^d	1.07^c	14.43^{bc}	2.23^{bc}	99.03^b
Zn-lignosulfonate	30.11^{cd}	1.28^f	1.37^d	15.73^{cd}	2.12^{bc}	96.79^b
Zn-phenolate	29.06^{cd}	1.03^d	1.02^c	18.23^{de}	2.05^{bc}	95.32^b

^{a} Water soluble plus exchangeable fraction was not detected. In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability.

fraction with the Zn-EDDHA treatment and more so with the Zn-EDTA treatment. The distribution of Zn in the 0–7 cm soil layer (**Table 6**) differs from the control columns in this experiment and also in the soil incubated with fertilizers, because a significant part of the added Zn accumulated in the OC fraction. This relates to the results of extractable Zn (see **Table 5**) and can be caused by irrigation that has been associated with significant increases in plant available Zn (DTPA and Mehlich-3 extractions). According to Teutsch et al. (*45*), Zn in soils is affected by rainfall in an indirect way through the phases with which it is associated. Differences in behavior among the fertilizer treatments were also noted in the 0–7 cm soil depending on the mobility and leachability of the fertilizers. Zinc concentrations in the OC fraction were approximately three times lower with Zn-EDTA fertilizer than with the other five Zn fertilizers. The AFeO fraction was slightly lower as compared to the other fertilizers, and the RES fraction did not increase as compared to the control columns.

Relative Effectiveness of Zinc Sources for Maize. The effect of fertilizer treatments on maize dry matter and Zn concentrations in corn seedling plants can be observed in **Table 7**. With regard to dry matter, significant yield increases as

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Table 7. Dry Matter Yield and Zn Concentration in Corn Seedlings as Affected by Different Fertilizer Treatments[a]

treatment	Zn applied (mg kg^{-1})	dry matter yield (g per pot)	Zn concn (mg kg^{-1})
control	0	9.32[a]	8.69[a]
Zn-EDDHA	10	59.07[ef]	13.54[ab]
	20	60.94[f]	24.24[c]
Zn-EDTA	10	53.42[ef]	25.20[c]
	20	60.72[f]	50.91[d]
Zn-heptagluconate	10	31.86[bc]	12.92[ab]
	20	57.17[ef]	16.61[b]
Zn-polyflavonoid	10	26.69[b]	11.76[ab]
	20	45.29[cde]	12.66[ab]
Zn-lignosulfonate	10	25.77[b]	11.26[ab]
	20	47.53[def]	11.69[ab]
Zn-phenolate	10	31.52[bc]	12.56[ab]
	20	34.89[bcd]	15.61[ab]

[a] In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability.

compared with the control (untreated Zn) were found in all of the cases due to the application of Zn fertilizers ($P < 0.0001$). In the fertilized pots, the yield was between 2.8 and 6.5 times larger as compared to the control. With each fertilizer, the yield increased with the increasing rate of Zn applied. However, the application of Zn-EDDHA and Zn-EDTA fertilizers at a rate of 10 mg Zn kg^{-1} led to a similar yield as the application of 20 mg Zn kg^{-1} of Zn-heptagluconate fertilizer and the yield reached was less in the pots fertilized with the other three Zn sources at a rate of 20 mg Zn kg^{-1}.

Differences in the Zn concentration in plant samples were observed between fertilizer treatments ($P < 0.0001$). In comparison with the control treatment, Zn concentration in plant was 2.8 times larger for 20 mg Zn kg^{-1} of Zn-EDDHA fertilizer and 2.9 and 5.9 times larger for 10 and 20 mg Zn kg^{-1} of Zn-EDTA fertilizer. Besides, the influence of the rate is significant only with Zn-EDDHA and Zn-EDTA fertilizers. The rest of the fertilizer treatments produced similar concentrations with no influence of the Zn rate. According to Jones (*4*), a typical sufficiency range for a maize crop is 25−100 mg Zn kg^{-1} in dry matter of mature leaves from new growth. Although the Zn rates applied in this study were very high, only the Zn-EDTA (10 and 20 mg Zn kg^{-1} rates) and Zn-EDDHA (20 mg Zn kg^{-1} rate) treatments produced a sufficient concentration in the plant tissue. On the other hand, Zn-EDTA at a rate of 20 mg Zn kg^{-1}

was the only treatment that led to a concentration of 50 mg Zn kg^{-1} in dry matter, which is given by some authors as the convenient minimum for this plant to be used as feeding fodder for different animals (*46*).

To compare fertilizer efficiency, Zn uptake was calculated per pot. A higher Zn rate increased Zn uptake, as was reported by Maftoun and Karimian (*47*). This is due to a higher dry matter yield in all cases, but for Zn-EDTA and Zn-EDDHA, an increase in Zn concentration in plants was also observed. Zn-heptagluconate, with a rate of 20 mg Zn kg^{-1}, produced more Zn uptake by the plants (0.95 mg Zn per pot) than Zn-polyflavonoid, Zn-lignosulfonate, and Zn-phenolate. Zn-polyflavonoid, Zn-lignosulfonate, and Zn-phenolate showed little differences between the Zn uptakes with the two rates of applications. Zinc uptake with Zn-EDTA was the largest with a rate of 20 mg Zn kg^{-1} reaching 3.09 mg Zn per pot, followed by Zn-EDDHA that reached 1.47 mg Zn per pot with the same rate. López-Valdivia et al. (*48*) reported that when the same six fertilizers were applied to an Aquic Haploxeralf (acidic) soil in a greenhouse maize growth experiment, the highest percentages of Zn taken up by the plants occurred when a rate of 20 mg Zn kg^{-1} was applied as Zn-EDTA fertilizer and a rate of 10 mg Zn kg^{-1} as Zn-lignosulfonate fertilizer, and the Zn percentage taken up by the plants with Zn-EDDHA was bigger than with the other three fertilizers. Prasad and Sinha (*49*) as well as Maftoun and Karimian (*47*) suggested that diffusion is the main mechanism contributing to Zn nutrition of crops in alkaline and calcareous soils, and the application of very stable sources enhances the diffusion flow and the uptake of Zn by maize roots.

The results of the extractions performed on soil collected after the harvest of maize are shown in **Table 8**. All Zn complexes were effective in increasing soil DTPA and Mehlich-3 extractable Zn, which was largest at a rate of 20 mg Zn kg^{-1} for all sources. In all fertilizer treatments, extracted Zn concentrations reached much larger values than those reported as critical for the two methods in alkaline soils: 1.0 mg kg^{-1} by the DTPA extraction (*50*) and 1.8 mg kg^{-1} by the Mehlich-3 extraction (*51*). The amount of Zn that remained available in the soil also depends on the source. With the results of the column and greenhouse experiments, a simple linear regression analysis defining the relationship between the two extractions of Zn available in soil was computed. The equation of the fitted model is Mehlich-3 = 1.71 + 1.44 DTPA ($P < 0.0001$). The R-squared

Table 8. Zinc Fractions, DTPA, and Mehlich-3 Extractable Zn (mg kg^{-1})[a] in Soil after Maize Harvest

treatment	Zn applied (mg kg^{-1})	Zn mg kg^{-1}							
		Zn fraction						DTPA	Mehlich-3
		OC	CAR	MnO	AFeO	CFeO	RES		
control	0	0.30[a]	0.06[a]	0.02[a]	4.13[a]	1.64[a]	79.55[a]	0.29[a]	0.79[a]
Zn-EDDHA	10	1.45[cd]	0.22[bcd]	0.04[bc]	8.84[cd]	2.24[cd]	79.64[a]	1.97[bc]	5.07[c]
	20	2.63[f]	0.32[d]	0.04[bc]	10.96[ef]	2.45[d]	84.61[bc]	3.93[e]	8.36[e]
Zn-EDTA	10	1.59[d]	0.17[bc]	0.02[a]	8.15[c]	2.23[cd]	79.71[a]	2.29[c]	4.43[bc]
	20	3.97[g]	0.52[e]	0.04[bc]	12.67[fg]	2.36[d]	79.74[a]	4.34[ef]	9.87[f]
Zn-heptagluconate	10	1.41[cd]	0.18[dc]	0.03[ab]	8.42[c]	1.94[abc]	80.36[a]	1.84[b]	4.16[bc]
	20	3.89[g]	0.33[d]	0.03[ab]	13.11[g]	2.30[d]	81.39[ab]	4.55[f]	10.27[f]
Zn-polyflavonoid	10	1.19[bc]	0.13[ab]	0.02[a]	6.21[b]	1.65[a]	82.35[abc]	2.14[bc]	3.93[b]
	20	2.19[e]	0.40[e]	0.05[c]	10.47[de]	1.72[ab]	84.94[bc]	2.86[d]	6.80[d]
Zn-lignosulfonate	10	0.83[b]	0.11[ab]	0.02[a]	9.43[cde]	1.67[a]	79.80[a]	1.81[b]	3.88[b]
	20	2.65[f]	0.23[cd]	0.04[bc]	10.51[de]	1.87[ab]	84.31[bc]	3.20[d]	7.86[e]
Zn-phenolate	10	1.23[cd]	0.11[ab]	0.03[ab]	8.79[cd]	1.78[ab]	80.59[a]	1.79[b]	3.55[b]
	20	2.32[ef]	0.30[de]	0.03[ab]	9.62[cde]	1.99[bc]	85.11[c]	3.25[d]	8.23[e]

[a] Water soluble plus exchangeable fraction was not detected. In the same column, means followed by the same letter are not significantly different at the 0.05 level of probability.

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statistic indicates that the fitted model explains 99.1% of the variability in Mehlich-3 extractable Zn. Novillo et al. (*52*) reported, in soils treated with organic Zn sources, that the Mehlich-3 extraction can be an acceptable soil analysis alternative to the DTPA-CaCl$_2$-TEA extraction. Abreu et al. (*53*) found that high correlation coefficients are obtained when the concentrations of Zn in the extracted soils were high. Both extraction procedures correlated with plant yield (DTPA $P <$ 0.01, $R^2 = 60.6\%$, and Mehlich-3 $P < 0.01$, $R^2 = 58.2\%$) and with lower significance level with Zn concentration in plants (DTPA $P < 0.05$, $R^2 = 34.8\%$, and Mehlich-3 $P < 0.1$, $R^2 = 29.4\%$).

For sequential Zn fractionation in the control soil, differences with regard to the original soil were not observed. In the pots with fertilized soil, the MnO fraction did not increase as compared to the control soil at any Zn rate and the CFeO and RES fractions increased slightly with little influence of the rate. The order of the OC and CFeO fractions changed with the rate of Zn applied. With a rate of 10 mg Zn kg^{-1}, the order was the same as with the original soil: RES, AFeO, CFeO, OC, CAR, and MnO fractions. With a rate of 20 mg Zn kg^{-1}, the OC fraction was larger than the CFeO fraction for all fertilizer treatments. A correlation can again be noted between the Zn concentration in the OC fraction and the Zn extractable with DTPA and Mehlich-3. The differences noted by means of the sequential extraction would be due to the physicochemical changes in the soils caused by the maize cropping. Ahumada et al. (*54*) reported that crops can affect the soil Zn distribution; Zn is distributed in all fractions, but the prevailing form is that associated with iron oxides and organic matter, in the case of the soil cultivated with lettuce, while Zn associated with iron oxides prevails in the soil cultivated with celery.

The addition of Zn complexes did not produce an increase in Zn in the most labile fraction (WSEX) after the maize harvest. Besides, this fraction is depleted by crop uptake, and the physicochemical characteristics of this soil (e.g., the high montmorillonitic clay content, alkaline pH, and the presence of CaCO$_3$) can result in less lability of Zn complexes. Tiller et al. (*55*) found that with soil clay containing a high proportion of montmorillonite, specific Zn sorption was still high after 2 weeks. In addition, according to Jeffery and Uren (*56*), Zn availability decreases to very low levels at high soil pH. The information supplied by sequential Zn fractioning corresponded with that provided by the maize plant. Significant correlations between dry matter yield ($P < 0.01$, $R^2 = 89.3\%$), Zn concentration in plant tissue ($P < 0.1$, $R^2 = 80.5\%$), and soil Zn fractions were obtained. These results indicated the contribution of the different forms or associations of Zn in soil to the uptake by plants.

Finally, the incubation and column experiments provided complementary information to the greenhouse experiment on fertilizer mobility. The better short-term response of maize to Zn-EDTA might be attributed to the greater mobility and adequate distribution of Zn in soil from that fertilizer source. The irrigation regimen in soil columns influenced the distribution of Zn in the different fractions as the OC fraction value was enhanced by the copious irrigation. The order of the available quantities extracted in the two laboratory experiments cannot be compared due to the migration and leaching that occurred in the columns. The incubation experiment showed that when Zn-EDTA was applied, Zn remained more labile (WSEX fraction and available Zn) than with Zn-EDDHA and, in both cases, more labile than with the other four fertilizers. These results are verified by Zn uptake by the maize plants. Thus,

Zn-EDTA fertilizer proved to be the most efficient source of Zn for maize seedling in this soil (under greenhouse conditions) than the others five Zn sources.

LITERATURE CITED

(1) Takkar, P. N.; Walker, C. D. The distribution and correction of zinc deficiency. In *Zinc in Soils and Plants*; Robson, A. D., Ed.; Developments in Plant and Soil Sciences 55; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993; pp 151−160.

(2) Murphy, L. S.; Walsh, L. M. Corrección de deficiencias de micronutrientes o fertilizantes. In *Micronutrientes en Agricultura*; Mortvedt, J. J., et al., Eds.; AGT Editor: México, D. F., México, 1983; pp 379−424.

(3) Marschner, H. Zinc uptake from soils. In *Zinc in Soils and Plants*; Robson, A. D., Ed.; Developments in Plant and Soil Sciences 55; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993; pp 59−77.

(4) Jones, J. B., Jr. In *Laboratory Guide for Conducting Soil Tests and Plant Analysis*; CRC Press: Boca Raton, FL, 2001.

(5) Karimian, N.; Yasrebi, J. Prediction of residual effects of zinc sulphate on growth and zinc uptake of corn plants using three zinc soil tests. *Commun. Soil Sci. Plant Anal*. **1995**, *26*, 277−287.

(6) Martens, D. C.; Westerman, D. T. Fertilizer applications for correcting micronutrient deficiencies. In *Micronutrient in Agriculture*, 2nd ed.; Mortvedt, J. J., et al., Eds.; SSSA: Madison, WI, 1991; pp 549−592.

(7) Prasad, B.; Sinha, M. K.; Randhawa, N. S. Effect of mobile chelating agents on the diffusion of zinc in soils. *Soil Sci.* **1976**, *122*, 260−266.

(8) Mortvedt, J. J.; Gilkes, R. J. Zinc fertilizers. In *Zinc in Soils and Plants*; Robson, A. D., Ed.; Developments in Plant and Soil Sciences 55; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993; pp 33−34.

(9) Shuman, L. M. Micronutrient fertilizers. *J. Crop Prod.* **1998**, *1*, 165−195.

(10) Anderson, W. B. Zinc in soils and plant nutrition. *Adv. Agron.* **1972**, *24*, 147−186.

(11) Rico, M. I.; Alvarez, J. M.; Mingot, J. I. Efficiency of zinc ethylenediamine-tetraacetate and zinc lignosulfonate soluble and coated fertilizer for maize in calcareous soil. *J. Agric. Food Chem.* **1996**, *44*, 3219−3223.

(12) Hodgson, J. F. Contribution of metal-organic complexing agents to the transport of metals to roots. *Soil Sci. Soc. Am. Proc.* **1969**, *33*, 68−75.

(13) McBride, M. B. Reactions controlling heavy metals solubility in soils. *Adv. Soil Sci.* **1989**, *10*, 1−56.

(14) Modaihsh, A. S. Zinc diffusion and extractability as affected by zinc carrier and soil chemical properties. *Fert. Res.* **1990**, *25*, 85−91.

(15) Chatzoudis, G. K.; Rigas, F. Macroreticular hydrogel effects on dissolution rate of controlled-release fertilizers. *J. Agric. Food Chem.* **1998**, *46*, 2830−2833.

(16) Alvarez, J. M.; Novillo, J.; Obrador, A.; López-Valdivia, L. M. Mobility and leachability of zinc in two soils treated with six organic zinc complexes. *J. Agric. Food Chem.* **2001**, *49*, 3833−3840.

(17) Ma, L. Q.; Rao, G. N. Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. *J. Environ. Qual.* **1997**, *26*, 259−264.

(18) Reed, S. T.; Martens, D. C. Copper and zinc. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Spark, D. L., Ed.; SSSA Book Series 5; SSSA and ASA: Madison, WI, 1996; p 706.

(19) Tessier, A.; Campbell, P. G. C.; Bisson, M. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* **1979**, *51*, 844−851.

(20) Shuman, L. M. Fractionation method for soil micronutrients. *Soil Sci.* **1985**, *140*, 11−22.

Zinc in Calcareous Soil and Zinc Uptake by Maize

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(21) Kiekens, L. Zinc. In *Heavy Metals in Soils*, 2nd ed.; Alloway, B. J., Ed.; Blackie Academic & Professional: London, U.K., 1995; pp 284−305.

(22) Ure, A. M. Single extraction schemes for soil analysis and related applications. *Sci. Total Environ.* **1996**, *178*, 3−10.

(23) Li, Z.; Shuman, L. M. Mobility of Zn, Cd and Pb in soils as affected by poultry litter extract−II. Redistribution among soil fractions. *Environ. Pollut.* **1997**, *95*, 227−234.

(24) Soil Survey Staff. In *Keys to Soil Taxonomy*, 8th ed.; USDA-NRCS: Washington, DC, 1998.

(25) Day, P. R. Particle fractionation and particle-size analysis. In *Methods of Soil Analysis, Part 1: Agronomy 9*; Black, C. A., et al., Eds.; ASA: Madison, WI, 1965; pp 545−567.

(26) Wiklicky, L.; Nemeth, K. Düngungsoptimierung mittels EUF-Bodenuntersuchung bei der Zückerrube (Optimization of sugar-beet fertilization with the aid of EUF soil testing). *Zuckerindustrie* **1981**, *106*, 982−988.

(27) Monturiol, F.; Alcalá, L. In *Mapa de Asociaciones de Suelos de la Comunidad de Madrid*; Inst. Edafología y Biología Vegetal, CSIC: Madrid, Spain, 1990.

(28) Jackson, M. L. In *Soil Chemical Analysis*; Prentice Hall Inc.: Englewood Cliffs, NJ, 1958.

(29) Olsen, S. R.; Cole, C. V.; Watanabe, F. S.; Dean, L. A. Estimation of available phosphorous in soils by extraction with sodium bicarbonate; Circ. 939; USDA: Washington, DC, 1954.

(30) Bremner, J. M. Nitrogen-total. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Sparks, D. L., Ed.; SSSA Book Series 5; SSSA and ASA: Madison, WI, 1996; pp 1085−1121.

(31) Bower, C. A.; Reitemeier, R. F.; Fireman, M. Exchangeable cation analysis of saline and alkali soils. *Soil Sci.* **1952**, *73*, 251−261.

(32) Allison, L. E.; Moodie, C. D. Carbonate. In *Methods of Soil Analysis: Part 2: Agronomy 9*, 2nd ed.; Black, C. A., et al., Eds.; ASA: Madison, WI, 1965; pp 1379−1400.

(33) Nijensohn, L.; Pizarro, O. C. Un procedimiento para la determinación del calcáreo activo en suelos orgánicos (A procedure for the determination of the active carbonate in organic soils); Boletín Técnico 2; Inst. Prov. Agropecuario: Madrid, Spain, 1960.

(34) McKeague, J. A.; Day, J. H. Dithionite and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Can. J. Soil Sci.* **1966**, *46*, 13−22.

(35) Liñán, C. Correctores de carencias simples (Corrector of simple deficiencies). In *Vademecum de Productos Fitosanitarios Nutricionales*; Liñán, C., Ed.; Agrotécnicas: Madrid, Spain, 2000; pp 552−554.

(36) Mandal, B.; Chatterjee, J.; Hazz, G. C.; Mandal, L. N. Effect of preflooding on transformation of applied zinc and its uptake by rice in lateritic soils. *Soil Sci.* **1992**, *153*, 250−257.

(37) Chao, T. T. Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. *Soil Sci. Soc. Am. Proc.* **1972**, *36*, 764−768.

(38) Lindsay, W. L.; Norvell, W. A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421−428.

(39) Mehlich, A. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409−1416.

(40) Ma, Y. B.; Uren, N. C. The fate and transformations of zinc added to soils. *Aust. J. Soil Res.* **1997**, *35*, 727−738.

(41) Norvell, W. A. Reactions of metal chelates in soils and nutrients solutions. In *Micronutrient in Agriculture*, 2nd ed.; Mortvedt, J. J., et al., Eds.; SSSA: Madison, WI, 1991; pp 187−227.

(42) Lindsay, W. L. Chelate equilibria. In *Chemical Equilibria in Soils*; John Wiley & Sons: New York, 1979; pp 238−265.

(43) Aboulroos, S. A. Reaction of EDTA, DTPA, and EDDHA complexes of zinc, copper, and manganese with a calcareous soil. *Z. Pflanzenernaehr. Bodenk.* **1981**, *144*, 164−173.

(44) Li, Z.; Shuman, L. M. Mobility of Zn, Cd and Pb in soils as affected by poultry litter extract−I. Leaching in soil columns. *Environ. Pollut.* **1997**, *95*, 219−226.

(45) Teutsch, N.; Erel, Y.; Halicz, L.; Chadwick, O. A. The influence of rainfall on metal concentration and behavior in the soil. *Geochim. Cosmochim. Acta* **1999**, *63*, 3499−3511.

(46) McDonald, P.; Edwards, R. A.; Greenhalgh, J. F. D. In *Nutrición Animal*, 3rd ed.; Acribia: Zaragoza, Spain, 1981; p 497.

(47) Maftoun, M.; Karimian, N. Relative efficiency of two zinc sources for maize (*Zea mays* L.) in two calcareous soils from an arid area of Iran. *Agronomie* **1989**, *9*, 771−775.

(48) López-Valdicia, L. M.; Fernández, M. D.; Obrador, A.; Alvarez, J. M. Zinc transformations in acidic soil and zinc efficiency on maize by adding six organic zinc complexes. *J. Agric. Food Chem.* **2002**, *50*, 1455−1460.

(49) Prasad, B.; Sinha, M. K. The relative efficiency of zinc carriers on growth and zinc nutrition of corn. *Plant Soil* **1981**, *62*, 45−52.

(50) Brennan, R. F.; Armour, J. D.; Reuter, D. J. Diagnosis of zinc deficiency. In *Zinc in Soils and Plants*; Robson, A. D., Ed.; Developments in Plant and Soil Sciences 55; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993; pp 167−181.

(51) Tran, T. S.; Simard, R. R. Mehlich III-extractable elements. In *Soil Sampling and Methods of Analysis*, 1st ed.; Carter, M. R., Ed.; Canadian Society of Soil Science; Lewis Publishers: Boca Ratón, FL, 1993; pp 43−49.

(52) Novillo, J.; Obrador, A.; Alvarez, J. M.; López-Valdivia, L. M.; Rico, M. I. A comparison of DTPA-TEA and Mehlich-3 extractants as predictors of zinc availability in three different soils. *VII International Symposium on Analytical Methodology in the Environmental Field*; Association Environmental Sciences and Techniques: Valladolid, Spain, 2002.

(53) Abreu, C. A.; Abreu, M. F.; Andrade, J. C.; van Raij, B. Restrictions in the use of correlation coefficients in comparing methods for the determination of the micronutrients in soils. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 1961−1972.

(54) Ahumada, I.; Mendoza, J.; Navarrete, E.; Ascar, L. Sequential extraction of heavy metals in soils irrigated with wastewater. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1507−1519.

(55) Tiller, K. G.; Gerth, J.; Brümmer, G. The sorption of Cd, Zn, and Ni by soil clay fractions: procedures for partition of bound forms and their interpretation. *Geoderma* **1984**, *34*, 1−16.

(56) Jeffery, J. J.; Uren, N. E. Copper and zinc species in the soil solution and effects of soil pH. *Aust. J. Soil Res.* **1983**, *21*, 479−488.