

Influence of Soil Type on the Mobility and Bioavailability of Chelated Zinc

JOSE M. ALVAREZ*

Departamento de Química y Análisis Agrícola, E.T.S.I. Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, s/n, 28040 Madrid, Spain

The objective of this study was to compare the distribution, mobility, and relative effectiveness of Zn from Zn-amino acids (Zn-AA) and Zn-DTPA-HEDTA-EDTA (Zn-CH) (DTPA, diethylenetriaminepentaacetate; HEDTA, *N*-2-hydroxyethyl-ethylenedinitriacetate; and EDTA, ethylenedinitriacetate) sources by applying different Zn levels to weakly acidic and neutral soils in laboratory (incubation and soil column studies) and greenhouse conditions. The experiments were carried out for 60 days in incubation and column experiments and for 45 days in a greenhouse experiment. The zinc soil behavior was evaluated by DTPA-TEA and Mehlich-3 extractions and sequential speciation. The incubation experiment showed that the highest concentrations of available Zn in weakly acidic soil occurred with Zn-AA treatments, whereas in the neutral soil Zn-CH treatments produced the highest quantities of available Zn. The column experiment showed that in neutral soil, with slow to moderate permeability in the A_p and B_t horizons, only Zn-CH significantly increased the mobility of Zn through the column with respect to the control and the Zn-AA source: 31% of the Zn applied as synthetic chelate was leached from the column. The greenhouse experiment showed that, at different rates of Zn application, the Zn carriers increased Zn uptake by maize (*Zea mays* L.). The use of applied Zn by maize, or Zn utilization, was greatest when the Zn treatments were Zn-CH (3.3%) at 20 mg kg⁻¹ and Zn-CH (4.9%) at 10 mg kg⁻¹, in weakly acidic and neutral soils, respectively.

KEYWORDS: Maize response; bioavailability; chelates; mobility; fractions; zinc

INTRODUCTION

Zinc deficiency is one of the most widespread nutritional disorders observed for sustained crop production and quality food, especially when the soil pH is high (1, 2). Zinc applied to soil may be involved in several physical, chemical, and biological reactions that control its concentration in the soil solution (3, 4). Zinc deficiency frequently occurs in maize, which is very sensitive to low Zn availability (5–7). The crop response to Zn fertilization varies with the Zn fertilizer source (8, 9): In general, synthetic chelates are more effective micronutrient sources, but they are also usually the most costly in terms of micronutrient per unit. The stability of the metal–chelate bond affects the amount of chelated metal made available to plants. An effective chelate is one in which the rate of substitution of the chelated metal for other cations in the soil is quite low (10). According to Anderson (11), the effectiveness of Zn-EDTA (ethylenedinitriacetate) and Zn-DTPA (diethylenetriaminepentaacetate) sources on growth was superior to a ZnSO₄ source. Lopez-Valdivia et al. (12) reported that the relative effectiveness of the different Zn carriers in increasing the Zn concentration in plants, in acidic soil, could be expressed as: Zn-EDTA ≥ Zn-lignosulfonate > Zn-EDDHA > Zn-

phenolate ≈ Zn-polyflavonoid ≈ Zn-glucoheptonate. According to Dwyer and Miller (13), long-chain natural organic compounds, such as Zn-amino acids, are intermediate in terms of chelating strength. Thus, amino acids can effectively supply Zn when applied to a soil, although at a slightly higher application rate than required for chelates (14).

The measurement of the total Zn concentration in soil provides inadequate information to enable assessment of the bioavailability of this micronutrient. The distribution of metals in the different phases and forms in which they occur in soil can be determined using single and sequential extraction procedures (15). A definition of the various chemical pools of Zn exists in noncalcareous soils in relation to its bioavailability: water soluble, exchangeable, complexed with organic matter, inorganically precipitated/occluded with iron or manganese oxide, and entrapped in primary and secondary minerals (16, 17). Research has tended to focus on characterizing these pools of soil Zn and their availability for plant uptake on acid soils (18–21). Sequential speciation, through the use of an extractant of increasing strength, has been used widely to determine the distribution of metals in soils. The distribution of Zn also depends on the extracting reagents and the sequence of extraction (22, 23). DTPA- and Mehlich-3-extractable Zn are indexes for estimating bioavailability in numerous soils around the world. A high relationship is observed between the

* To whom correspondence should be addressed. E-mail: josemanuel.alvarez@upm.es.

Table 1. Selected Properties of the Soils Used in the Experiments

parameter	soil, horizon, and depth (cm)			
	soil 1		soil 2	
	A _p (0–23)	B _t (23–93)	A _p (0–25)	B _t (25–70)
sand (g kg ⁻¹)	540	390	390	360
silt (g kg ⁻¹)	220	190	320	200
clay (g kg ⁻¹)	240	420	290	440
pH _w (1:2.5)	6.28	6.10	7.02	7.13
OM (g kg ⁻¹) ^a	11.0	4.00	5.90	4.00
total N (g kg ⁻¹)	11.0	8.3	8.5	6.7
available P (mg kg ⁻¹)	12.92	ND ^c	18.51	ND ^c
CEC (cmol ⁺ /kg)	10.4	24.3	21.7	24.0
base saturation (%) ^b	56	61	71	76
total free iron oxides (Fe ₂ O ₃) (g kg ⁻¹)	7.87	15.16	11.58	17.73

^aOrganic matter = organic carbon × 1.724. ^bBase saturation (%) = (exchangeable bases/CEC) × 100. ^cND, not determined.

Zn available to plants and the most labile fractions of Zn in the soil (24). These are the most labile forms of metal in the soil environment and have a greater leaching potential than other forms (7, 25).

The increasing concern to assess the bioavailability of the metal fraction and the mobility of trace metals in the environment is reflected clearly by the considerable increase in the frequency of analyses based on operationally defined extraction procedures witnessed over the last 15 years. However, less attention was given to trying to understand various pools of Zn in soils associated with added fertilizers containing Zn chelates. When a concentrated fertilizer solution of Zn is applied to soil, the chemical composition of the soil solution is altered drastically and modifies the chemical availability of the micronutrient. After incubating soil, the availability of Zn to plants diminishes, presumably due to its transformation into less available forms (26, 27). The migration and leaching of different nutrients are usually monitored by means of packed soil columns (28). Soil pH and other soil properties influence the mobility of Zn sources (29, 30). The mobility of Zn at depth depends on soil texture. The movement of Zn, the metal included in the soil solution, is closely related to the permeability of the soil profile. Duchaufour (31) reported the clearly predominant influence of structure, rather than texture, on permeability.

The objectives of this study were to determine (i) the main chemical associations of Zn applied to weakly acidic and neutral soils through a soil incubation experiment involving two Zn fertilizers (Zn-amino acids and Zn-DTPA-HEDTA-EDTA), (ii) the distribution of Zn in the soil profiles and losses of Zn due to leaching through a soil column study, and (iii) the efficiency of plant uptake and availability of applied Zn by maize in a greenhouse experiment.

MATERIALS AND METHODS

Soil Collection and Soil Characteristics. The soil samples used in this study were from two different rural areas of Spain (soil 1, latitude 40°44'N, longitude 3°39'W; and soil 2, latitude 40°32'N, longitude 3°28'W). Samples from the horizons of the two soil profiles were air-dried, and the fraction of less than 2 mm was used for the study. Soil profiles 1 and 2 were, respectively, classified as Aquic Haploxeralf and Calcic Haploxeralf (32). The general properties of the various horizons of the soil profiles are summarized in **Table 1**. The A_p horizons of these soil profiles were weakly acidic for soil 1 and neutral for soil 2. Particle size analysis was carried out by the hydrometer method (33), and the soil pH was measured in water at a 1:2.5 (w:v) soil:water ratio. The textures of the soils (U.S. Department of Agriculture) were sandy-clay-loam and clay-loam, respectively. The predominant clay in the A_p

horizon of soil 1 and in the B_t horizon of soil 2 was illite, whereas in the B_t horizon of soil 1 and in the A_p horizon of soil 2, it was montmorillonite. This last clay determination was carried out using the EUF technique (34). Organic carbon was measured by the Walkley–Black procedure (35), and total N was determined by Kjeldhal digestion (36). Available P in weakly acidic soil was extracted with Bray-1 extractant (37), and the Olsen extraction procedure was used for neutral soil (38). The cation exchange capacity (CEC) was assessed using sodium acetate (NaOAc) and ammonium acetate (NH₄OAc) (39). Total “free” iron oxides were extracted with dithionite–citrate–bicarbonate buffered at pH 7 (40). The B horizons of soils 1 and 2 were argillic (B_t). The colors of the soils were as follows: soil 1, dry 10YR 5/4, wet 10YR 4/4; and soil 2, dry 5YR 4/6, wet 5YR 3/4 (41). According to Monturiol and Alcalá (42), the permeability in the B_t horizon of soil 1 was very slow (<0.1 cm h⁻¹), whereas it was slow to moderate (0.2–0.5 cm h⁻¹) in the other horizons.

Extraction Procedures. Zinc fractionation in the soils was performed employing a modified technique described by different authors (**Table 2**) (43–45). The fractions were determined sequentially in six steps (F1–6) with the following extractants: F1, Mg(NO₃)₂; F2, NaOCl; F3, NH₂OH·HCl; F4, (NH₄)₂C₂O₄ + H₂C₂O₄; F5, solution as in the previous step plus ascorbic acid; and F6, determined by using microwave digestion for the sample remaining from step 6 after air drying and grinding. The speciation of the original soils indicated that the great majority (ranging from 84 to 90% of the total) of Zn was present in F6 (residual fraction) and that the second most abundant form was F5 (ranging from 6 to 9% of the total).

The amount of Zn available to the plant was assessed by two different extractions: DTPA-extractable Zn, as described by Lindsay and Norvell (46), and Mehlich-3-extractable Zn, as described by Mehlich (47). In the original soils, 1 and 2, DTPA- and Mehlich-3-extractable Zn ranged from 0.28 to 0.39 and from 0.79 to 1.28 mg kg⁻¹, respectively. These soil test concentrations are associated commonly with Zn deficiency in maize, especially in neutral soils (48–50).

Standard solutions of Zn were prepared for each extraction in a background solution of the extracting agents. The Zn concentration in the different extracts was determined by flame atomic absorption spectrophotometry (AAS) involving direct aspiration of the aqueous solution by an air–acetylene flame.

Zinc Chelates. The two fertilizers used in this study were as follows: Zn chelated by natural amino acids extracted from vegetable material (Zn-AA) (85 g water-soluble Zn L⁻¹ and mass density, 1.26 mg m⁻³) and Zn chelated with a mixture of three synthetic chelating agents, DTPA-HEDTA-EDTA (Zn-CH) (90 g water-soluble Zn L⁻¹ and mass density, 1.29 mg m⁻³). Liñán (51) described the properties of these liquid commercial formulations. The quantities of Zn added in the three experiments were checked three times for each treatment by means of AAS.

Laboratory Experiments. Different samples of A_p horizons were treated with aqueous suspensions of the two commercial formulations to give Zn rates of 0 (control), 10, and 20 mg kg⁻¹ soil. The soils were physically mixed with the Zn fertilizer treatments. Triplicate samples were incubated for 15, 30, and 60 days at 22 ± 1 °C at field capacity moisture level, under aerobic conditions. The soil moisture was adjusted once every 3 days by weighing. Weighed subsamples were incubated in appropriate containers for direct analysis: 5 g in 100 mL screw-top glass centrifuge tubes for the sequential extraction and 10 g in 125 mL conical glass flasks for DTPA-extractable Zn. Soil samples were treated with aqueous suspensions of the two commercial Zn fertilizers to prepare two different Zn concentrations (10 and 20 mg kg⁻¹).

Transparent plexiglas columns were employed to study the mobility of the two Zn chelates in soils. The soils were packed to a height of 57 cm in 75 cm long columns with an inside diameter of 7.2 cm. Soil columns were built, and gravel and two (Whatman #4) filter papers were placed at the bottom of each column. The packed soils, which had bulk densities of 1.3 g cm⁻³ in their A_p horizons (1.4 kg of soil) and 1.4 g cm⁻³ in their B_t horizons (1.7 kg of soil) were saturated from below with water. The water was added by means of a capillary tube in the center of the column. Excess water was allowed to drain overnight. The soil from the top of each column and down to a depth

Table 2. Sequential Extraction Method for Zn

form/association	solution	soil (g)	solution (mL)	conditions
water soluble plus exchangeable (F1)	1 M Mg(NO ₃) ₂ (pH 7.0) ^a	5	50 ^c	shake for 30 min
organically complexed (F2)	0.7 M NaOCl (pH 8.5) ^a	5	50	boiling water bath, 30 min; stir occasionally (repeat extraction twice)
Mn oxides bound (F3)	0.1 M NH ₂ OH·HCl (pH 2.0) ^b	5	50	shake for 30 min
amorphous Fe oxides bound (F4)	0.2 M (NH ₄) ₂ C ₂ O ₄ + 0.2 M H ₂ C ₂ O ₄ (pH 3.0) ^a	5	50	shake for 4 h in dark
crystalline Fe oxides bound (F5)	solution as for previous step plus 0.1 M ascorbic acid ^a	5	50	boiling water bath, 30 min; stir occasionally (repeat extraction twice)
residual (F6)	HCl, HNO ₃ , and HF in mixture	2	12 ^d	digestion in microwave oven four steps, maximum pressure 120 psi

^a Ref 43. ^b Ref 44. ^c Ref 45. ^d Two HCl, five HNO₃, and five HF.

of 1.5 cm was mixed with the respective amendments. The Zn applied in these treatments was 20 mg kg⁻¹ A_p horizon soil (28 mg of Zn was applied to 1.4 kg of the A_p horizon in each column). Untreated soil columns (control) also were included in the experiment as control treatments. Three replicate columns were installed at room temperature (18–23 °C) for each fertilizer and experimental time (30 and 60 days) combination. Deionized water was added to the top of each column on a daily basis at 30 mL day⁻¹. Leachate was collected in 150 mL fractions, with a total of 1200 mL being collected over an approximately 60 day period. The leachates were acidified with HCl (≈pH 2) and filtered, and then, the concentration of Zn was measured in each leachate portion (150 mL) by AAS.

After leaching, the two halves of the columns were separated along the longitudinal axis. The soil columns were air-dried, divided transversally into various portions, and ground to pass a 2 mm sieve. This study was conducted as described by Rico et al. (52) and Alvarez et al. (53). Six layers of the columns were analyzed separately [sequential extraction, DTPA- and Mehlich-3-extractable Zn, and their weights and depths were as follows: zone a, 364 g (0–7 cm); zone b, 518 g (7–17 cm); zone c, 518 g (17–27 cm); zone d, 566.3 g (27–37 cm); zone e, 566.3 g (37–47 cm); and zone f, 566.3 g (47–57 cm)]. The lengths of zones a, b, and c approximately represent the lengths of the different A_p horizons (0–27 cm) in the soils. The total Zn was determined by digesting 2 g soil samples in 2 mL of HCl + 5 mL of HNO₃ + 5 mL of HF in a microwave oven (four steps; maximum pressure, 120 psi) in Teflon pumps (CEM, Mars).

Greenhouse Pot Experiment. The plant used in this study was short-cycle maize (120 days) of a variety extensively used as fodder (A-33 variety, a double hybrid, Asgrow). Eight kilogram samples of air-dried soil were placed in polyethylene pots, with washed gravel at the bottom of the recipients to facilitate aeration and drainage. Nitrogen, P, and K were applied uniformly to all pots at rates of 75 mg kg⁻¹, in the form of urea, superphosphate, and K₂SO₄, respectively. Additional doses of 37.5 mg kg⁻¹ of N were added 7 and 30 days after sowing the seeds. The upper zone of the soil received 0 (control), 10, and 20 mg kg⁻¹ of Zn applied in the form of the two Zn chelates. Each treatment had three replicates. Three maize seeds were sown in each pot and were taken to a greenhouse in which the temperature varied between 16 and 42 °C. Appropriate amounts of potable water were added to reach and maintain field capacity moisture conditions with limited drainage (36% w/w). At 45 days after seeding, the part above ground was cut, washed with tap water, rinsed with deionized water, and then dried in an oven at 65 °C until constant weight was obtained. These parts of the plants were then ground and kept in sealed recipients for later analysis. Plant samples were subjected to wet digestion in a microwave oven (two steps; maximum pressure, 170 psi) using an acid mixture [(HCl + HNO₃ + HF) 1:14, plant (g):solution (mL)]. Residual Zn concentrations in the soils were determined after harvesting the maize crop as outlined previously.

The experiments were carried out by means of a randomized complete block design and a factorial treatment arrangement structure. Regression analysis and multifactor analysis of variance at a probability level of $P \leq 0.05$ were carried out to determine the main effects and interactions of the different factors. Duncan's multiple range test and

other analyses were performed using Statgraphics Plus software (Statistical Graphics Corp.; Manugistic, Inc.; Rockville, MD).

RESULTS AND DISCUSSION

Incubation Effects on the Chemical Forms of Zinc.

Concentrations of Zn in the DTPA-extractable, F1 (water soluble plus exchangeable Zn), and F2 (organically complexed Zn) fractions from the incubated control soil and from the incubated samples with soil plus fertilizers are shown in **Figure 1**. The concentrations of Zn extracted from the control soils (no Zn added) with DTPA were less than 0.30 mg kg⁻¹ in soils 1 and 2, in all cases. These are considered deficient for most crops in acidic and neutral soils (7, 24, 48). In general, the highest concentrations of available Zn occurred with Zn-AA treatments in soil 1, whereas in soil 2, the Zn-CH treatments produced the highest quantities present. The results, for each rate of Zn application, were subjected to multifactor variance analysis (see the Materials and Methods) to determine the main effects and interactions of the different factors (soil type, incubation time, and type of fertilizer).

For the DTPA-extractable Zn, the analysis did not show any significant differences between either soils or fertilizers for any of the applied Zn rates. During the incubation period, the potential Zn bioavailability decreased in soils treated with both Zn rates (probability less than 0.0001, $P < 0.0001$). Positive and significant interactions existed between soil and fertilizer ($P < 0.01$) and between soil and period of incubation ($P < 0.001$) for the two doses of Zn. Khan and Banwart (54) reported that moist incubation reduces DTPA-extractable Zn in soils with widely varying characteristics including those with the pH of the soils ranging from 4.2 to 9.4, and these decreases in available Zn in soils incubated at field moisture capacity appear to be due to nonmicrobial fixation.

For the F1 fraction, significant differences were observed with respect to all three factors (soil, incubation period, and fertilizer). In soil 2 (neutral), average concentrations in this more labile Zn fraction were lower than in soil 1 (weakly acidic). Furthermore, in the A_p horizon, the CEC of soil 2 was twice as great as in soil 1 (see **Table 1**). Sthl and James (55) reported a gradual decrease in Zn activity as the soil pH increases, which they attributed to increased CEC of the soils. According to McBride and Blasiak (56), a 97% decrease in Zn concentration was observed in acidic soil for each unit increase in soil pH between 5 and 7. Sachdev et al. (57) reported that the Zn²⁺ activity decreased approximately 99% for each unit increase in pH. In the present study, as with available Zn, this Zn fraction (F1) diminished with time in both soils (see **Figure 1**). In soil 1, Zn concentrations in the F1 fraction were similar for both

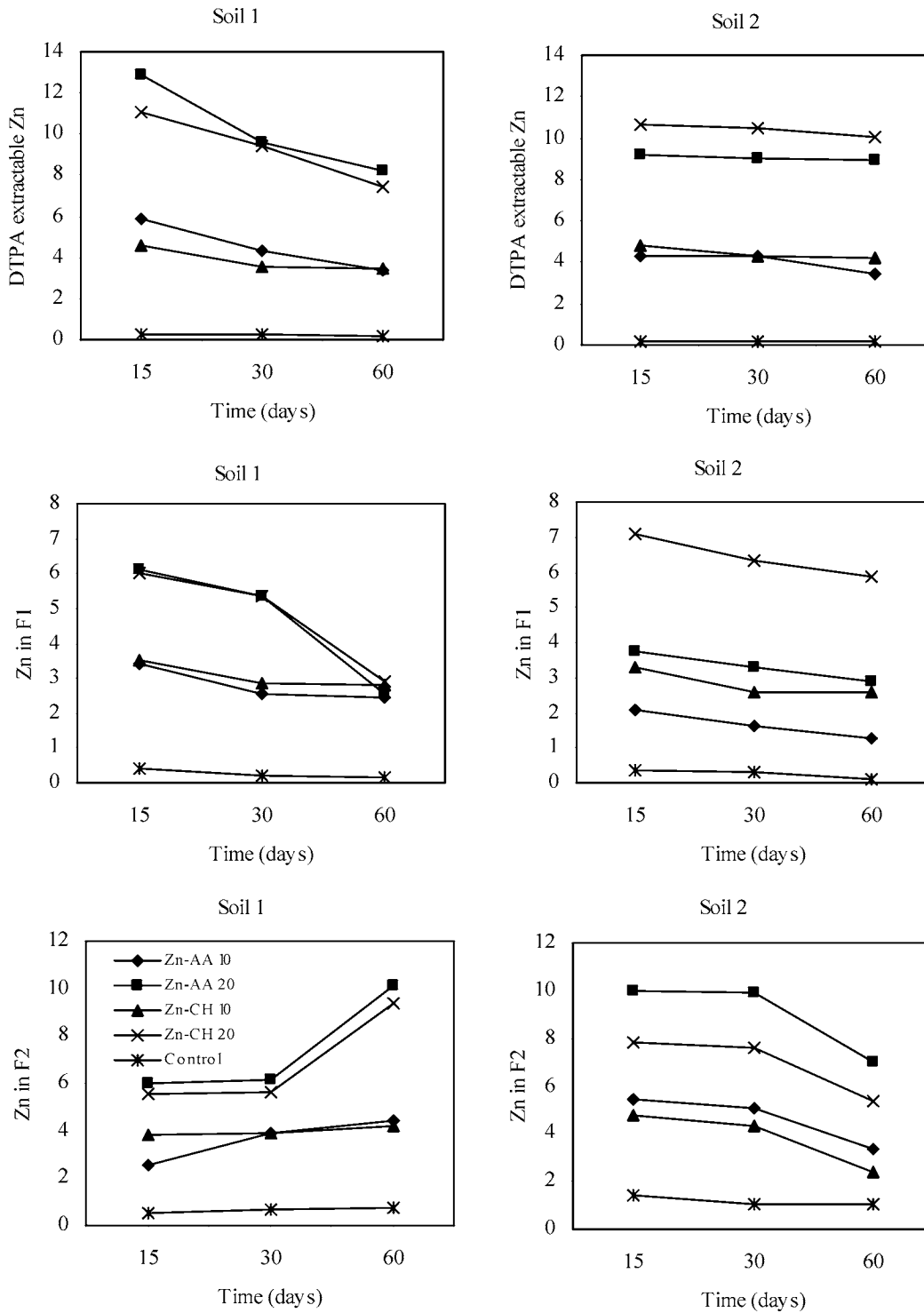


Figure 1. DTPA-extractable Zn and F1 (water soluble plus exchangeable) and F2 (organically complexed) Zn fractions in soils with 0, 10, and 20 mg Zn kg⁻¹ as Zn-amino acids (Zn-AA) and Zn-chelate (Zn-CH) at 15, 30, and 60 days of incubation in soils 1 and 2 (coefficients of variation of DTPA ranged from 0.45 to 7.93%, F1 ranged from 0.79 to 8.23, and F2 ranged from 1.93 to 9.72%).

fertilizers, whereas in soil 2, Zn-CH treatments produced higher quantities. At the higher rate of Zn application (20 mg kg⁻¹), the average concentration of the Zn-CH fertilizer was 1.3 times greater than that of the Zn-AA fertilizer.

In contrast, the concentrations obtained in the F2 fraction for the Zn-AA fertilizer in soil 2 were superior to those obtained for the Zn-CH fertilizer. At the higher rate of Zn application, the average concentration of the Zn-AA fertilizer was 8.20 mg Zn kg⁻¹, whereas for Zn-CH it was 6.89 mg Zn kg⁻¹ (*P* < 0.001). In soil 1, this fraction increased with the incubation

period; on the contrary, in soil 2, it diminished (see **Figure 1**). So, with both F1 and F2, significant interactions existed between the soil and the treatment, although the significance of these interactions varied according to the applied Zn dose. In some cases, differences also existed between soil and time (*P* ranged from 0.02 to 0.0001). For the F1 fraction, a significant interaction was observed between fertilizer and time (*P* < 0.001) when applying 20 mg Zn kg⁻¹.

A similar statistical study was performed with the other fractions (F3–6). In summary, all fractions increased with

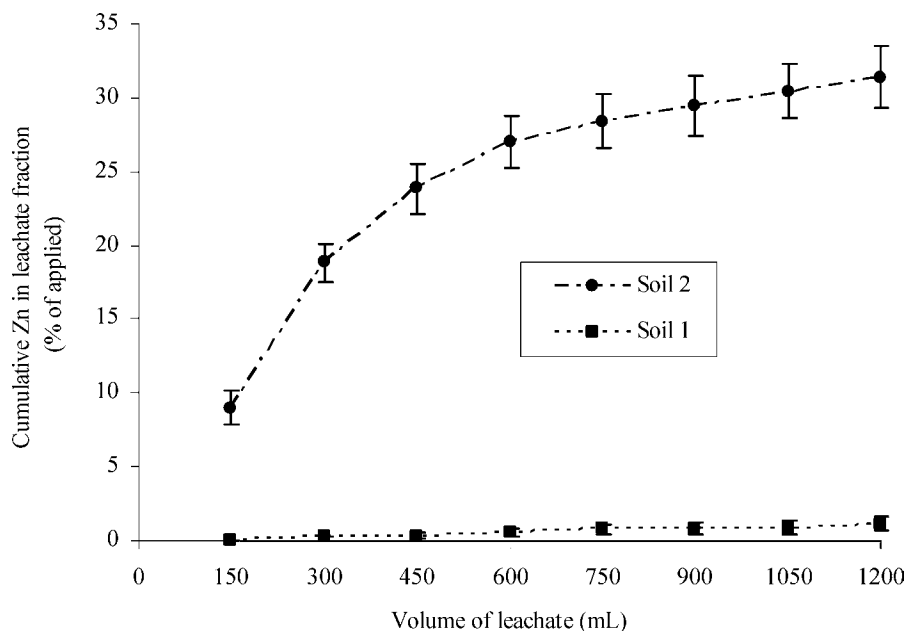


Figure 2. Cumulative percentages of Zn leached when applied as Zn-chelate (Zn-CH) in soils 1 and 2 as a function of leachate volume collected in soil columns. The vertical line at each of the data points represents the standard error of the mean.

incubation time, except for F6 in soil 2, which diminished. Except for the F3 fraction at an application rate of 20 mg Zn kg⁻¹, no significant differences were observed between the fractions with the fertilizers applied. In contrast, the F3 fraction was also the only one that did not present any differences for the soil factor. In this way, the general distribution in fractions F4–6 of the added Zn did not depend on the type of fertilizer applied but rather on the type of soil. Furthermore, significant interactions between soil and time (P ranged from 0.1 to 0.0001) were obtained for fractions F3–6.

Mobility and Leachability of Zinc in the Soil Columns.

The amounts of Zn leached in the control soils and in the original soils treated with Zn-AA were very small in all cases. In the control soils, the cumulative quantities of Zn recovered in 1200 mL of leachates (60 days) were 0.30 mg for soil 1 and 0.11 mg for soil 2. The total amounts of Zn leached from the soil columns amended with Zn-AA were only 0.34 mg for soil 1 and 0.29 mg for soil 2. In these cases, less than 1.21% of the Zn applied was in leachates from any of the Zn-AA treatments applied. With equivalent amounts of Zn applied, the movement of Zn from soil 1 amended with Zn-CH was no greater than that from the untreated soil (0.31 mg of Zn, 1.11% of Zn applied). Although the soil pH of the A_p (6.28) and B_t (6.10) horizons was weakly acidic, permeability in the B_t horizon of this soil was “very slow” (see the Materials and Methods), and most of the Zn applied was retained by the soil components.

In soil 2, amended with Zn-CH fertilizer, the concentrations of Zn in leachate fractions increased considerably with respect to untreated soil and the Zn-AA treatment. Sixty days after the start of the experiment (1.2 L of the leaching volume), the amount of Zn recovered in the leachates was 8.79 mg, which was equal to 31% of Zn applied as Zn chelated by a mixture of the three synthetic chelating agents: DTPA, HEDTA, and EDTA (Figure 2). Most of the Zn leached was obtained between when 0.15 and 0.60 L of the leaching volume that was collected, which represented an experimental time of less than 30 days. Although the heights of the columns used were similar to those of the columns employed with soil 1, the different characteristics of this soil (A and B horizons; see the Materials and Methods) helped to increase the leaching of Zn with respect to soil 1.

Additionally, Li and Shuman (58) concluded that metal movement and leaching with soluble organic compounds in soil columns may represent a long-term process under natural field conditions.

In soil 2, the Zn-CH fertilizer moved down the soil column, and the total amount of Zn leached was considerable. The rate declined with time, in this case being proportional to the amount of Zn still present in the soil column. Zinc leaching could therefore be represented by a first-order kinetics equation. If the nonlixivated percentage of the applied Zn, in time t , as compared to the initial concentration (N_0), is designated as N and the leaching rate constant is k , the equation is as follows: $\ln N = \ln N_0 - kt$, where N_0 must be 100 ($\ln N_0 = 4.6$). By calculating the $\ln N$ value as a function of time t (days) and using linear regression analysis, the values of the slope and ordinate at the origin were obtained with both a high correlation coefficient and a probability level. The following equation was established

$$\ln N = 4.49 - 0.00537t \quad (r = -0.933, P < 0.001)$$

The model explained 86.97% of the variability in $\ln N$, and the standard deviation of the residual was 0.04. This result supported the possibility of establishing the amount of Zn leaching of the soil columns under conditions similar to those in this study.

When Zn organic fertilizers were applied, Zn leaching depended on the stability of the chelate used and the physical and chemical characteristics of the soil. The amounts of applied Zn leached from the soils amended with Zn-AA were negligible. Natural chelates of Zn are less stable under these soil conditions, and the Zn applied to the soil was fixed by the soil components. This ligand agent did not protect the metal from retention by the soil. Furthermore, the amino acids were mineralized rapidly by soil microbes. Over the 60 day experimental period, the only product that moved through this neutral soil was the one containing the synthetic chelating agents. The three ligands of Zn-CH reduced the loss of Zn by precipitation and retention by the soil components, but losses through leaching also occurred. Various authors have noted the greater migration

Table 3. DTPA- and Mehlich-3-Extractable Zn and Total Zn (mg kg⁻¹) in Soil 1 (Weakly Acidic) with 20 mg Zn kg⁻¹ Soil as Zn-Amino Acids (Zn-AA) or Zn-Chelate (Zn-CH) at 30 and 60 Days of a Column Experiment

treatment	horizon	depth	Zn concentration after 30 days			Zn concentration after 60 days		
			DTPA	Mehlich-3	total	DTPA	Mehlich-3	total
Zn-AA	A _p	a	46.25	55.74	101.62	43.11	51.08	99.47
		b	3.03	5.86	43.43	2.77	4.95	44.61
		c	0.41	2.08	39.89	0.68	2.24	40.70
	B _t	d	0.24	0.97	54.67	0.19	1.17	54.34
		e	0.20	0.93	55.53	0.21	1.02	55.48
		f	0.18	0.90	56.03	0.20	0.89	54.91
Zn-CH	A _p	a	24.07	37.51	84.16	19.63	29.48	74.81
		b	11.25	19.73	58.72	13.51	22.57	62.48
		c	3.66	6.50	39.73	3.69	6.53	42.67
	B _t	d	0.55	1.35	55.11	0.30	1.06	55.59
		e	0.28	1.09	54.81	0.23	1.05	56.85
		f	0.33	1.08	55.33	0.29	0.92	55.08

Table 4. DTPA- and Mehlich-3-Extractable Zn and Total Zn (mg kg⁻¹) in Soil 2 (Neutral) with 20 mg Zn kg⁻¹ Soil as Zn-Amino Acids (Zn-AA) or Zn-Chelate (Zn-CH) at 30 and 60 Days of a Column Experiment

treatment	horizon	depth	Zn concentration after 30 days			Zn concentration after 60 days		
			DTPA	Mehlich-3	total	DTPA	Mehlich-3	total
Zn-AA	A _p	a	50.53	58.64	103.22	44.45	55.10	102.38
		b	1.29	2.63	40.14	1.44	2.67	41.74
		c	0.54	1.76	39.17	0.64	2.29	39.72
	B _t	d	0.42	1.37	53.72	0.53	1.68	53.07
		e	0.40	1.42	54.08	0.36	1.19	53.96
		f	0.42	1.49	55.80	0.43	1.45	54.05
Zn-CH	A _p	a	4.15	8.35	48.54	3.89	7.26	48.09
		b	2.32	4.22	45.52	2.44	4.83	42.42
		c	2.09	3.78	42.47	2.38	4.41	44.28
	B _t	d	2.66	5.52	59.92	2.61	4.92	59.11
		e	2.80	5.45	58.97	2.42	4.87	59.00
		f	3.18	6.00	58.80	3.06	5.95	59.32

capacity of Zn-EDTA fertilizer in different soils with respect to Zn-lignosulfonate or ZnSO₄ fertilizers. Zn-EDTA increases Zn mobility by reducing the reaction of Zn with soil colloids (59, 60). Soluble complexes containing metals can be transported downward and possibly reduce groundwater quality (58).

The migration and bioavailability of the Zn applied were studied by determining DTPA- and Mehlich-3-extractable Zn and total Zn. All Zn concentrations for these parameters, for each zone of the soil columns and for 30 and 60 day experimental times, are shown in **Tables 3** (soil 1) and **4** (soil 2). The total concentration of Zn registered in each zone of the columns at the different times showed that there was a great difference between the behaviors of the two fertilizers in the two soils. When Zn-AA was applied to soil 1, most of the added Zn remained in the a zone (depth, 0–7 cm), although Zn concentrations in the b zone (depth, 7–17 cm) also slightly increased. Application of this fertilizer resulted in an accumulation of Zn in the surface layer of the soil. Neither of the Zn-AA treatments resulted in sufficient Zn mobility to distribute Zn throughout the profile and thereby did not transport it to the deepest segments of the column (B_t horizon). This response was due to the retention of Zn by the solid phase of the soil in the top part of the columns. Zinc applied as Zn-CH moved through the a, b, and c zones (A_p horizon) and also, but to a lesser extent, through the d, e, and f zones (B_t horizon). This mobility of the two fertilizers was observed through the measurement of both total Zn and available Zn extracted by DTPA and Mehlich-3. It must be emphasized that a considerable amount of available Zn was in the a zone when Zn-AA was applied. This result was because available Zn remained in the upper part of the soil

and was not as extensively distributed through other layers of the soil column as the Zn-CH treatment.

In soil 2 (see **Table 4**), Zn applied as Zn-AA increased total Zn in the a and b zones of the A_p horizon, but most of the Zn applied remained in the top segment (a zone). Zinc concentrations extracted from the d, e, and f zones of the soil treated with Zn-AA fertilizer were similar to those from the control column and original soil. In contrast, as previously commented, Zn mobility was greatest when Zn-CH was applied. Throughout the treated soil column, the total Zn concentration was higher in similar zones of the control column, but amounts of Zn in the A_p horizon (a, b, and c zones) were smaller than in soil 1, while in the B_t horizon they were greater than in the previously mentioned soil. Part of the Zn applied was lost due to leaching. It was also observed that part of the Zn dissolved in irrigation water was retained in the B_t horizon (d, e, and f zones). The concentrations of Zn estimated by DTPA and Mehlich-3 extractions differed according to the kind of Zn fertilizer applied. Adriano (29) reported that high clay content causes immobilization of Zn through the formation of hydroxides and Zn adsorption on clays. The montmorillonite clay presented strong selectivity for Zn adsorption. The neutral pH induced low solubility to metals and probably played a role in retaining metals in the soil layers. In addition, amino acids are only weak or intermediate ligands for Zn, so there was no significant chelation and Zn was transferred from the amino acids to soil binding sites and complexed Zn was also adsorbed as an intact metal–ligand chelate.

Multifactor variance analysis was carried out for the variables DTPA- and Mehlich-3-extractable Zn and total Zn, using the factors soil, fertilizer, time, and layer depth (**Table 5**). For these

Table 5. Variance Analysis of the DTPA- and Mehlich-3-Extractable Zn and Total Zn with Factors of Soil Type, Fertilizer, Time, and Depth

source of variation	df	F ratio		
		DTPA	Mehlich-3	total Zn
main effects ^a				
A, soil	1	159.6***	214.0***	96.35***
B, fertilizer	1	764.3***	256.6***	76.45***
C, time (day)	1	14.00**	13.25**	0.43 NS
D, depth (zone)	5	5009***	4647***	1215***
positive interactions ^b				
		AB, AD, BC, CD, ABD	AB, AD, BC, CD, ABD	AB, AD, BD, ABD, CD ^c
residual	47			
total (corrected)	143			

^a***, **, and *, significant at 0.01, 0.1, and 5% levels. NS, not significant differences. ^b***, significant at the 0.01% level, except CD. ^c*, significant at the 5% level.

Table 6. Zinc Fractions (mg kg⁻¹) in Weakly Acidic and Neutral Soil Columns Amended with 20 mg Zn kg⁻¹ Soil as Zn Fertilizers after 60 Days in the Depth 0–7 cm (Zone a)

soil	treatment	F1	F2	F3	F4	F5	F6
1 (weakly acidic)	control	0.45 a	1.16 a	0.54 a	1.03 a	2.92 a	32.83 a
	Zn-AA	24.43 c	33.02 c	1.38 b	2.49 b	4.30 b	33.85 a
	Zn-CH	10.83 b	22.51 b	1.38 b	2.16 b	3.91 b	34.02 a
2 (neutral)	control	0.15 a	1.60 a	0.11 a	0.81 a	2.19 a	34.13 a
	Zn-AA	15.55 b	40.05 c	3.41 c	3.68 b	2.59 a	37.10 b
	Zn-CH	1.36 a	6.03 b	0.75 b	1.30 a	2.07 a	36.58 b

^aFor each soil, values were compared using Duncan's multiple range test at the 0.05 level of probability. Homogeneous groups are denoted with the same letter.

three variables, significant differences were obtained between soils ($P < 0.0001$), fertilizers ($P < 0.0001$), time (for DTPA and Mehlich-3, $P < 0.001$), and especially layers or zone depth (see *F* ratio, $P < 0.0001$). This analysis showed different positive interactions between the different factors (see **Table 5**). These results were compatible with Zn leaching, since average Zn concentrations in soil columns were higher in soil 1 than in soil 2, showing higher values for Zn-AA treatments than for Zn-CH. The available Zn diminished with experimental time and fell considerably with layer depth.

The distributions of Zn among the different soil fractions after fertilizer treatment depended on the source of Zn used (**Table 6**). For the Zn-AA fertilizer, the percentage distribution of Zn in the fractions from the a zone showed great differences with respect to both the control soil and the Zn-CH treatment, with the percentage of Zn in the residual fraction (F6) being lowest in the Zn-AA treatments. The percentages of total Zn present in the F1 fraction for the Zn-AA treatments were 25 and 15%, respectively, in soils 1 and 2, and were 14 and 2.9% higher than for the Zn-CH treatments in the respective soils. This fraction is very important for plant nutrition as it potentially represents the most available form of Zn (water-soluble plus exchangeable Zn). Iyengard et al. (3) found that the quantity of exchangeable Zn in soils decreased with increases in soil pH. High percentages of total Zn were also observed in the F2 fraction (organically complexed Zn) for the Zn-AA treatments (soil 1, 33%; and soil 2, 39%) and for Zn-CH in soil 1 (30%). The lowest percentage of total Zn in the OC fraction was found for soil 2 with Zn-CH (13%).

The soil and leachate analyses for each soil column accounted for all of the Zn applied. The percentages of Zn recovered from

Table 7. Response of Maize in Weakly Acidic and Neutral Soils with 0, 10, and 20 mg Zn kg⁻¹ Soil as Zn-Amino Acids (Zn-AA) or Zn-Chelate (Zn-CH)^a

soil	treatment	dry rate (mg kg ⁻¹)	dry matter (g pot ⁻¹)	Zn concn (mg kg ⁻¹)	Zn uptake (mg pot ⁻¹)	Zn utilization (%)
1 (weakly acidic)	control	0	64.72 a	21.50 a	1.39 a	—
	Zn-AA	10	69.89 a	50.50 b	3.53 b	2.68
		20	68.06 a	63.31 c	4.31 c	1.83
	Zn-CH	10	69.03 a	51.07 b	3.52 b	2.66
		20	70.35 a	95.78 d	6.72 d	3.33
	2 (neutral)	control	0	68.94 a	14.80 a	1.02 a
Zn-AA		10	69.99 a	28.54 b	2.00 ab	1.23
		20	75.47 a	29.85 b	2.23 b	0.76
Zn-CH		10	77.07 a	63.89 c	4.92 c	4.88
		20	76.92 a	102.9 d	7.92 d	4.31

^aFor each soil, values were compared using Duncan's multiple range test at the 0.05 level of probability. Homogeneous groups are denoted with the same letter.

the different soil layers and the Zn present in leachates from the different soils accounted for nearly 100% (ranging from 95 to 106%) of initial soil Zn plus added Zn.

Growth and Zinc Uptake in the Greenhouse Pot Experiment. The dry matter yield, Zn concentration, and Zn uptake by plants are indicated in **Table 7**. In both soils, the application of Zn fertilizers did not affect the overall growth performance of the maize; there were no significant differences in dry matter content between plants grown in the controls and those grown in the treated soils.

Beneficial effects of Zn chelates were observed with significant increases in Zn concentrations in plants with respect to the control. Differences in plant Zn concentrations were observed between fertilizer treatments in two different soils ($P < 0.0001$). In soil 1, the highest concentration of Zn in maize was observed with the high Zn rate of the Zn-CH treatment, followed by the high Zn rate of the Zn-AA treatment; in the first case, the concentration was 4.5 times greater than in the control. In soil 2, the highest concentration of Zn was observed with the high Zn rate of the Zn-CH treatment, and this response was followed by the low Zn rate of the same treatment; in the first case, the concentration was 6.9 times greater than in the control. In a previous study with analogous applications of different Zn chelates in a neutral soil (61), involving comparisons with a control of neutral soil, Zn concentrations in plants increased by as much as 689% following the application of Zn-EDTA and by 397% following the application of Zn-EDDHA.

The concentrations for Zn-AA in this neutral soil did not reach 50 mg kg⁻¹ in dry matter, which other authors cite as a convenient minimum for using this plant as feeding fodder for several different animals (62). Furthermore, in this soil, the Zn concentration in the control treatment was lower at 20 mg kg⁻¹, which other authors regard as the critical concentration of Zn in mature leaves (63). From this perspective, in soil 2, the control was deficient judging by established standards, and the Zn-AA treatments produced results similar to what would be considered a deficient level. In soil 1, the Zn concentrations for the control treatment also only slightly exceeded the deficient concentration (20 mg kg⁻¹).

Use of applied Zn by the maize, or Zn utilization, reached maximum levels in soils 1 and 2 when the Zn treatments were, respectively, Zn-CH (3.33%) with a rate of 20 mg kg⁻¹ and Zn-CH (4.88%) with a rate of 10 mg kg⁻¹ (see **Table 7**). According to Fageria et al. (64), as soil pH decreases, Zn uptake

increases. However, in this experiment, this only occurred when the natural chelate, Zn-AA, was applied at both Zn doses. In contrast, when the mixture of chelating agents (DTPA-HEDTA-EDTA) was applied, as soil pH increased, Zn uptake by plants not only remained stable but even increased.

The availability of Zn in the soil was studied after harvesting. Relative Zn recovery from the different DTPA- and Mehlich-3-extractable Zn levels did not vary much with respect to the original soils. Several authors reported differences between cultivated and uncultivated soils with respect to the sequential extraction of different metals. According to Chandi and Takkar (65), even cropping systems can have an influence on the distribution of micronutrients in soil forms due to their effects on soil properties. The two sources of fertilizer employed, including the two applied at very low Zn dosages, left sufficient concentrations of available Zn in the soils for a new crop, taking into account critical Zn soil levels determined by the DTPA and Mehlich-3 methods. In both soils, even the worst case contemplated in this study presented a concentration that was approximately seven times greater than the critical concentration of soil Zn for the two extractions (24, 50, 66).

In conclusion, under a field capacity regimen and aerobic conditions, the incubation of Zn-DTPA-HEDTA-EDTA and Zn-AA in soils influenced their Zn status. The highest concentrations of the most labile forms of Zn occurred with Zn-AA treatments in (weakly acidic) soil 1, whereas in (neutral) soil 2, the Zn-DTPA-HEDTA-EDTA treatments produced the highest quantities registered. Both available Zn (DTPA-extractable Zn) and water-soluble plus exchangeable Zn declined with incubation time. The high mobility of Zn-DTPA-HEDTA-EDTA in soil 2 could increase the effectiveness of its use with maize seedlings under greenhouse conditions with respect to other Zn-AA sources. In this case, the high level of migration of the synthetic Zn chelate could produce a loss of soil metal through leaching in the case of excessive irrigation. The two Zn chelates were shown to be highly effective in soil 1, because they were associated with the largest quantities of labile forms of Zn in the soil. In this way, they could make a major contribution to the appropriate Zn nutrition of maize in this soil. In soil 2, however, only the Zn-DTPA-HEDTA-EDTA treatments produced high Zn concentrations in plants. In this case, the application of synthetic Zn chelate produced noticeable increases (Zn concentration > 50 mg kg⁻¹ of dry matter) in Zn content in maize plants at both rates. Finally, the addition of two Zn chelates to soils 1 and 2 produced high concentrations in available Zn after the maize harvest. These concentrations reached much larger values than those reported as critical for the different soils.

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