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Zinc Transformations in Neutral Soil and Zinc Efficiency in Maize Fertilization

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The effect of six Zn sources (Zn−phenolate, Zn−EDDHA, Zn−EDTA, Zn−lignosulfonate, Zn−polyflavonoid, and Zn−glucoheptonate) was studied by applying different Zn levels to a representative Calcic Haploxeralf neutral soil (the predominant clay is montmorillonite) in incubation and greenhouse experiments. Zinc soil behavior was evaluated by sequential DTPA and Mehlich-3 extraction procedures. In the incubation experiment, the highest percentage recovery values of Zn applied to soil occurred in the water-soluble plus exchangeable fraction (29%) in fertilization with 20 mg of Zn kg^{-1} of Zn−EDTA fertilizer. In the greenhouse experiment with maize (*Zea mays* L.), a comparison of different Zn carriers showed that the application of six fertilizers did not significantly increase the plant dry matter yield among fertilizer treatments. The highest yield occurred when 20 mg of Zn kg^{-1} was applied as Zn−EDDHA fertilizer (79.4 g per pot). The relative effectiveness of the Zn sources in increasing Zn concentration in plants was in the following order: Zn−EDTA (20 mg kg^{-1}) > Zn−EDDHA (20 mg kg^{-1}) ≈ Zn−EDTA (10 mg kg^{-1}) > Zn−EDDHA (10 mg kg^{-1}) ≈ Zn−phenolate (both rates) ≈ Zn−polyflavonoid (both rates) ≈ Zn−lignosulfonate (both rates) ≈ Zn−glucoheptonate (both rates) > untreated Zn. The highest amounts of Zn taken up by the plants occurred when Zn was applied as Zn−EDTA fertilizer (20 mg kg^{-1}, 7.44 mg of Zn per pot; 10 mg kg^{-1} Zn rate, 3.93 mg of Zn per pot) and when Zn was applied as Zn−EDDHA fertilizer (20 mg kg^{-1} Zn rate, 4.66 mg Zn per pot). After the maize crop was harvested, sufficient quantities of available Zn remained in the soil (DTPA- or Mehlich-3-extractable Zn) for another harvest.

KEYWORDS: Neutral soil; maize response; soil incubation; Zn availability; Zn chelates; Zn speciation

INTRODUCTION

Zinc deficiency is a common micronutrient deficiency affecting maize grown in many different parts of the world (*1*). This is one of the most widespread nutritional disorders affecting food quality (*2*). The amount of total Zn in the soil does not determine the nutritional state of the micronutrient (*3*). Zinc availability to plants depends on the chemical reactions in the soil. Studies on Zn have revealed that the extent and nature of its transformation are governed by the reaction time in the soil, the amount of Zn added, the rate of extraction, the type of clay mineral, and the extent of organic matter content in the soil (*4*, *5*). Studies on the rate of reaction of Zn with soils have produced diverse results. Khan and Banwart (*6*) measured the reaction for 1 week and concluded that the decrease in available micronutrients observed was nonmicrobial in nature. According to Tiller et al. (*7*), in a clay soil containing a high proportion of montmorillonite, specific adsorption was still marked after 2 weeks. Continued reaction was less marked for clays dominated by kaolinite or illite. This suggests that there might be marked differences between soils with respect to the rate of reaction. Barrow (*8*) showed that the reaction between added Zn and soil continued for up to 30 days. Adriano (*9*) explained that the Zn applied to soil may be involved in several physical, chemical, and biological reactions which control its concentration in the soil solution. The bioavailability of Zn fertilizers added to soil was particularly dependent on the pH factor. The bioavailability decreased when the pH of the soils increased (*10*).

The degree of metal association with distinct geochemical phases is strongly dependent on the physical-chemical conditions of the soils. Different fractions of soil Zn vary considerably in their chemical reactivity and bioavailability (*4*, *11*). Chemical fractionation has become a common operational approach to bridge the relationship between the bioavailable fraction of metal in soil and its content in plants. It is widely recognized that sequential speciation methods suffer a lack of selectivity and that trace metal redistribution among phases during extraction and attempts to quantitatively predict the bioavailability of metal species generally fail. However, many researchers still consider these methods useful for evaluating the bioavailability of micronutrients in soils (*12*−*15*). Furthermore, the DTPA and Mehlich-3 extractions are procedures usually employed to diagnose Zn bioavailability for plant uptake (*16*).

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The forms of Zn in soils strongly depend on their nature and origin. The response of maize to Zn fertilization varies with the Zn fertilizer source (*17*). The choice of the Zn fertilizer should depend on the relative agronomic effectiveness of the source applied to a given soil. Metal-chelating agents play an important role in transporting Zn from solid phases in soils to the surface of plant roots (*18*). Both synthetic and natural chelates augment the availability of the micronutrient cation to plants from the soil by enhancing both the diffusive and convective flow of the nutrient to the plant surface. Several authors have pointed to complexed forms as the major sources of available Zn to maize (*19*, *20*) and have suggested that their effectiveness depends on their rate of disappearance from the soil solution, which is related to their stability (*5*). In alkaline or calcareous soils, different authors reported the superiority of some Zn sources to others. These studies give conflicting data regarding the effectiveness of the different Zn sources in terms of crop yield and Zn uptake by maize. Prasad and Sinha (*21*), for example, stated that the relative efficiency of the Zn sources for maize (soil pH 8.4) varied in the following order: Zn−DTPA > Zn−fulvate > Zn−EDTA > Zn−citrate > $ZnSO_4$. Goos et al. (*22*) showed that in the long term Zn−EDTA was not a better source for maize than Zn−humate−lignosulfonate and $ZnSO_4$ in calcareous soil. According to Maftoun and Karimian (*23*), Zn−EDTA was generally more effective than $ZnSO_4$ in increasing Zn uptake by maize (soil pH 8.2 and 7.4). However, few studies exist on the use of different Zn chelates in neutral soils.

The objectives of this study were (a) to characterize the main chemical forms of Zn in a neutral soil treated with six Zn chelates by means of a soil incubation experiment, (b) to compare the effectiveness of the six selected Zn chelates in a crop of maize growing in this soil in a greenhouse experiment, and (c) to determine the Zn status in the soil after the maize was harvested and to study the possible relationship between metal forms in soil and the metal content in plants.

MATERIALS AND METHODS

The representative soil was from Daganzo (latitude 40°32′ N, longitude 3°28′ W) in the Madrid region of Spain. Surface soil was taken from the A_p horizon (depth 0−25 cm); samples were air-dried and sieved, and the <2 mm fraction was used for the study. Soil properties were as follows: texture (USDA), clay loam; clay content, 290 g kg^{-1} (*24*); predominant clay, montmorillonite (*25*); permeability, slow to moderate (0.2−2.0 cm h^{-1}) (*26*); pH_w, 7.02 (1:2.5, w/v); oxidizable organic matter content, 5.9 g kg^{-1} (*27*); total N content, 0.80 g kg^{-1} (*28*); available P content, 18.5 mg kg^{-1} (*29*); cation exchange capacity, 21.7 cmol kg^{-1} (*30*); base saturation, 71%; total "free" iron oxide (Fe_2O_3) content, 11.6 g kg^{-1} (*31*). The color of the soil was as follows: dry, 5YR 4/6; wet, 5YR 3/4 (*32*). The soil profile classification was Calcic Haploxeralf (*33*).

Fractionation of Zn in the soil was performed according to techniques proposed by different authors (*13*, *34*−*36*), with slight modifications. The fractions were sequentially determined in six steps with the following extractants: (1) 1 M $Mg(NO_3)_2$, pH 7.0, for 2 h (water-soluble plus exchangeable Zn, WSEX); (2) 0.7 M NaOCl, pH 8.5, two extractions, for 30 min in a boiling water bath (organically complexed Zn, OC); (3) 0.1 M $NH_2OH{\cdot}HCl$, pH 2.0, for 30 min (manganese oxide bound Zn, MnOX); (4) 0.2 M $(NH_4)_2C_2O_4$ + 0.2 M $H_2C_2O_4$, pH 3.0, for 4 h in the dark (amorphous iron oxide bound Zn, AFeOX); (5) solution as in the previous step plus 0.1 M ascorbic acid, two extractions, for 30 min in a boiling water bath (crystalline iron oxide bound Zn, CFeOX); (6) residual Zn (RES) determined by using microwave digestion for the sample remaining from step 5 after air drying and grinding, 1 g of soil residue/6 mL of acid mixture (1 mL of HCl, 3 mL of HNO_3, and 2 mL of HF). The soil (g)/extractant solution (mL) ratio was 5:50. After each extraction, the soil suspension was centrifuged (4000 rpm for 15 min) and the supernatant solution was decanted and filtered. The amount of Zn extracted by a given extracting reagent was calculated according to Sposito et al. (*12*). This calculation contained a correction for the amount of Zn in the solution entrained in the soil sample after the centrifugation that followed each extraction. The sequential extraction of the original soil used for the experiments provided the following Zn fractions (average of three replications) (mg kg^{-1}): WSEX, 0.23 (0.61%); OC, 0.87 (2.30%); MnOX, 0.16 (0.42%); AFeOX, 1.20 (3.17%); CFeOX, 3.47 (9.15%); RES, 31.98 (84.35%). Total Zn expressed as the sum of fractions was 37.91 mg kg^{-1}.

Relative Zn available to the plant was assessed by extracting it with DTPA (5 mM DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine, adjusted to pH 7.30) (*37*) and Mehlich-3 (0.2 M HOAc + 0.25 M NH_4NO_3 + 0.015 M NH_4F + 0.013 M HNO_3 + 1 mM EDTA) (*38*). DTPA- and Mehlich-3-extractable Zn levels in the original soil (average of three replications) were 0.39 mg kg^{-1} (1.03% of the total) and 1.28 mg kg^{-1} (3.38% of the total), respectively. These levels of available Zn could indicate a deficiency in micronutrient content for growing maize in neutral soil (*39*).

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

The six fertilizers used in this study were a liquid solution of Zn−phenolate (70 g of Zn L^{-1}), Zn−EDDHA (ethylenediaminedi-*o*-hydroxyphenylacetate) (70 g of Zn L^{-1}), Zn−EDTA (ethylenediaminetetraacetate) (88 g of Zn L^{-1}), Zn−lignosulfonate (75 g of Zn L^{-1}), Zn−polyflavonoid (50 g of Zn L^{-1}), and Zn−glucoheptonate (60 g of Zn L^{-1}). These organic Zn sources are made by several commercial companies (*40*).

In the incubation experiment, soil samples were treated with aqueous suspensions of the six commercial formulations to prepare two different Zn concentrations (10 and 20 mg of Zn kg^{-1} of soil). The soils were physically mixed with the Zn fertilizers. Triplicate samples were incubated for 15, 30, and 60 days at 22 ± 1 °C at field capacity level, under aerobic conditions. Soil moisture was adjusted every 3 days by weighing. Weighed samples were incubated in appropriate containers for direct analysis: 5 g in 100 mL screw-top glass centrifuge tubes for the sequential extraction analysis and 10 g in 125 mL conical glass flasks for DTPA-extractable Zn. The recovery percentage of added Zn in different chemical forms was determined according to the equation

$$\text{recovery (\%)} = [(\text{TZn} - \text{UZn}) \times 100]/(\text{TSUM} - \text{USUM}) \quad (1)$$

where TZn and UZn are the concentrations of an individual chemical Zn form in treated and untreated soil, respectively, and TSUM and USUM are the sum of Zn concentrations in all fractions (total Zn) in treated and untreated soil, respectively (*14*).

In the greenhouse experiment, the plant used was a short-cycle maize (*Zea mays* L.) of a variety extensively used as fodder (A-33 variety, a double hybrid, ASGROW). Samples of 8 kg of air-dried soil were placed in polyethylene pots with washed gravel at the bottom to facilitate aeration and drainage. N, P, and K were applied uniformly to all pots at rates of 75 mg kg^{-1}, in the form of urea, super-phosphate, and K_2SO_4, respectively. The soil received 0 (control), 10, and 20 mg kg^{-1} Zn applied as Zn organic chelates. Additional doses of 37.5 mg kg^{-1} N were added 7 and 30 days after sowing. There were three replicates for each treatment. Three maize seeds were sown in each plot, and the pots were taken to a greenhouse in which the temperature varied between 16 and 42 °C. Appropriate amounts of water were added to reach and approximately maintain field capacity moisture conditions with limited drainage. At the end of the maximum plant growth period (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 a constant weight was obtained. These parts were then ground and kept in sealed recipients for later analysis. Plant samples were subjected to wet digestion in a microwave oven (maximum pressure 170 psi) using an acid mixture (HCl + HNO_3 + HF) of 1:14 plant (g)/solution (mL). After the maize crop was cut, residual soils were

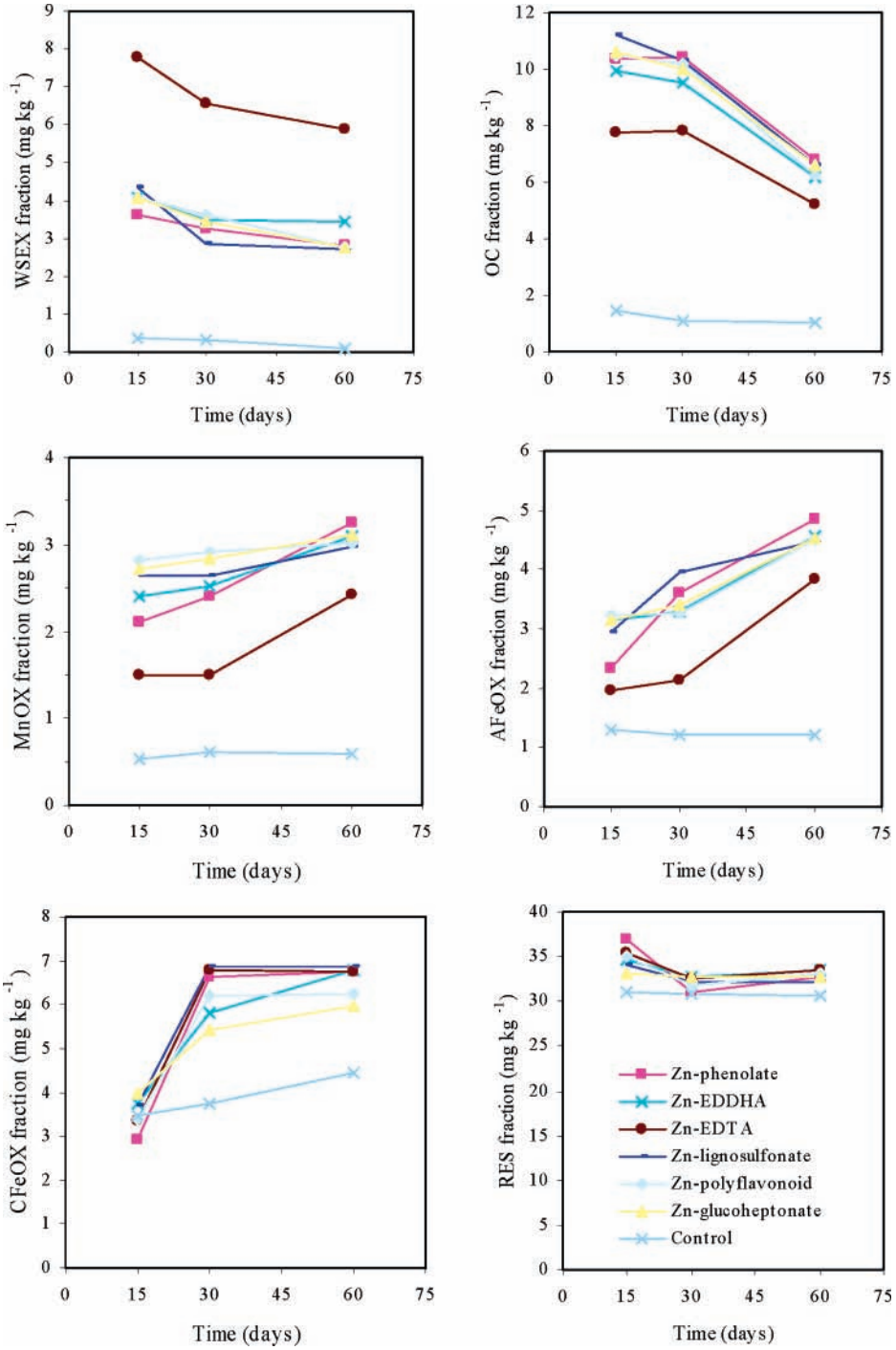

Figure 1. Zinc fraction in neutral soil with different Zn doses (0 and 20 mg of Zn kg^{-1}) of fertilizers and incubation times.

homogenized and residual Zn contents were studied by means of the sequential DTPA and Mehlich-3 extraction procedures.

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

RESULTS AND DISCUSSION

Incubation Experiment. Concentrations of Zn fractions from the incubated untreated soil (control) and from the fertilized samples with 20 mg of Zn kg^{-1} of soil are shown in **Figure 1**. In general, the endogenous Zn distribution in untreated soil followed the same order throughout the incubation period: RES $\gg$ CFeOX > AFeOX $\geq$ OC > MnOX > WSEX. In other words, most of the Zn existed as a residual fraction; this form represents the fraction associated with the mineral portion mostly related to aluminosilicate minerals. Among the nonresidual fractions, the iron oxide fraction contained a larger amount of Zn than the others, probably due to the high stability of iron−zinc oxides (*10*). The addition of Zn complexes led to different increases in each fraction; however, the distribution between fractions of the Zn applied to the soil depended on the type of fertilizer treatment for each rate of Zn application (WSEX to CFeOX, $P < 0.0001$, and RES, $P < 0.01$), and the period of incubation (WSEX to CFeOX, $P < 0.0001$, and RES, $P < 0.001$) (**Table 1**; statistical analysis is only shown for the 20 mg kg^{-1} Zn rate). As time passed, Zn concentrations diminished for WSEX, OC, and RES and increased for MnOX, AFeOX, and CFeOX for both Zn application rates. For most of the

Table 1. Average Concentrations (mg kg^{-1}) of Zn Fractions and DTPA-Extractable Zn with 20 mg of Zn kg^{-1} of Soil as Zn Fertilizers Influenced by the Incubation Period (Days) and Zn Source[a]

source of variation	WSEX	OC	MnOX	AFeOX	CFeOX	RES	DTPA
incubation period							
15	4.04 c	8.82 b	2.10 a	2.58 a	3.51 a	34.36 b	8.45 b
30	3.35 b	8.47 a	2.21 a	2.98 b	5.92 b	31.99 a	7.90 a
60	2.92 a	5.53 a	2.64 b	4.00 c	6.26 b	32.62 a	7.67 a
fertilizer treatment							
control	0.25 a	1.18 a	0.58 a	1.24 a	3.89 a	30.77 a	0.17 a
Zn–phenolate	3.22 b	9.20 c	2.59 c	3.61 c	5.43 bc	33.59 b	8.89 bc
Zn–EDDHA	3.66 c	8.54 c	2.58 cd	3.57 c	5.43 bc	33.64 b	9.33 c
Zn–EDTA	6.72 d	6.92 b	1.81 b	2.64 b	5.68 bc	33.79 b	10.99 d
Zn–lignosulfonate	3.30 bc	9.37 c	2.75 cd	3.79 c	5.81 c	32.82 b	8.36 b
Zn–polyflavonoid	3.48 bc	8.96 c	2.91 d	3.65 c	5.28 bc	33.28 b	8.90 bc
Zn–glucoheptonate	3.42 bc	9.07 c	2.89 d	3.69 c	5.12 b	32.89 b	9.44 c

[a] Values were compared using a Duncan multiple range test at the 95% level. Homogeneous groups are denoted with the same letter.

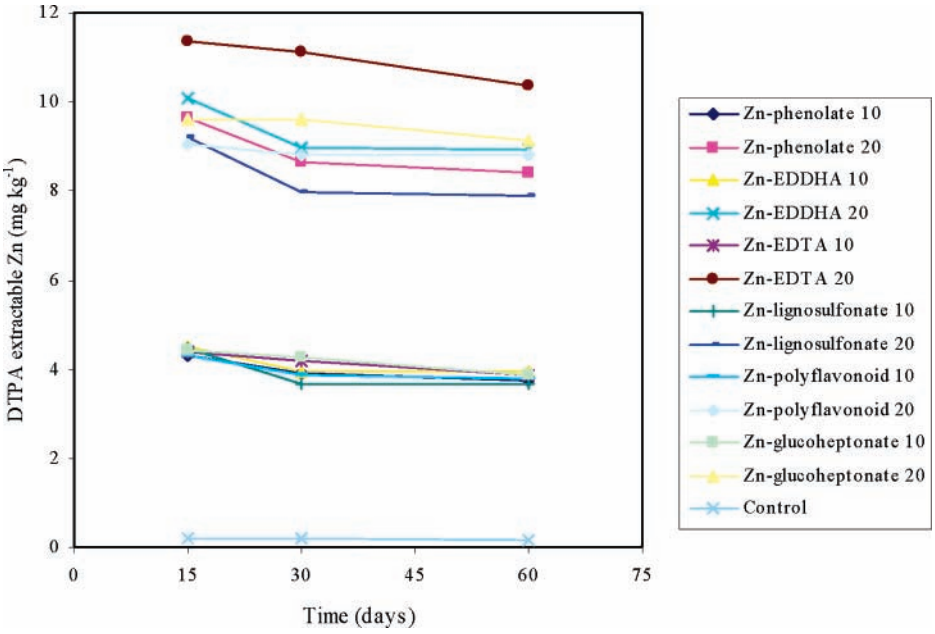

Figure 2. Concentration of DTPA-extractable Zn in neutral soil with different fertilizer treatments (0, 10, and 20 mg of Zn kg^{-1}) and incubation times.

fertilizer treatments, at the end of the incubation period the Zn distribution order between fractions was RES ≫ CFeOX ≥ OC > AFeOX > WSEX > MnOX. For the treatment with Zn−EDTA at the 20 mg kg^{-1} rate the order changed, since the WSEX fraction (6.72 mg kg^{-1}) had a high value, greater than those of CFeOX, AFeOX, and MnOX and similar to that of the OC fraction. In this way, when the addition of the micronutrient to the soil was Zn chelated by the synthetic chelating agent EDTA, the concentration in the most labile forms (WSEX) was greater (homogeneous group c) than for the other five fertilizers. In addition, the synthetic chelate Zn−EDDHA showed the second highest concentration in this fraction. Both chelates showed their greatest stability in aqueous solutions of all six products ($pK_{Zn-EDTA} = -17.4$, $pK_{Zn-EDDHA} = -17.8$, with an ionic strength of 0.01 M; *10*).

Concentrations of Zn extracted with DTPA from untreated and treated soil for the different incubation periods are shown in **Figure 2**. The concentration of Zn extracted from the control soil was affected by incubation and was always less than 0.4 mg kg^{-1}, which is considered deficient in neutral soils for most crops. This concentration is insufficient for maize production (*39*, *41*, *42*) and would require the application of Zn fertilizers. This could be due to the special physicochemical characteristics of this soil: its high montmorillonitic clay content and neutral pH. Potentially available Zn decreased during the incubation period in treated soil for both Zn application rates ($P < 0.001$; see **Table 1** for the 20 mg kg^{-1} Zn rate). Significant differences were found between fertilizer treatments for each Zn rate ($P < 0.0001$). The highest concentration of DTPA-extractable Zn occurred with the Zn−EDTA treatments. Statistical analysis showed that for the 20 mg kg^{-1} rate, there were significant differences between Zn−EDTA (10.99 mg kg^{-1}) and the other five fertilizers. The synthetic chelate Zn−EDTA remains effective for plants in different soil types (*43*). The other five organic ligands also produce a favorable effect on the availability of Zn, although not all of them behave in the same way.

At the end of the incubation period (60 days), the percentage recovery of applied Zn in different chemical fractions and DTPA-extractable was calculated, and the results are shown in **Figure 3**. In most of the fertilizer treatments, the percentage of Zn applied was greatest for the OC fraction. In contrast, for the two rates in the case of Zn−EDTA treatment, the biggest recovery was associated with the WSEX fraction. In some cases, the RES fraction also registered the highest value. In both the OC fraction and the WSEX fraction, the recovery values were higher for the higher rate (20 mg kg^{-1}: OC, 21.14−31.36%; WSEX, 14.02−29.30%) than for the lower one (10 mg kg^{-1}: OC, 14.59−22.50%; WSEX, 10.87−20.21%). This means that

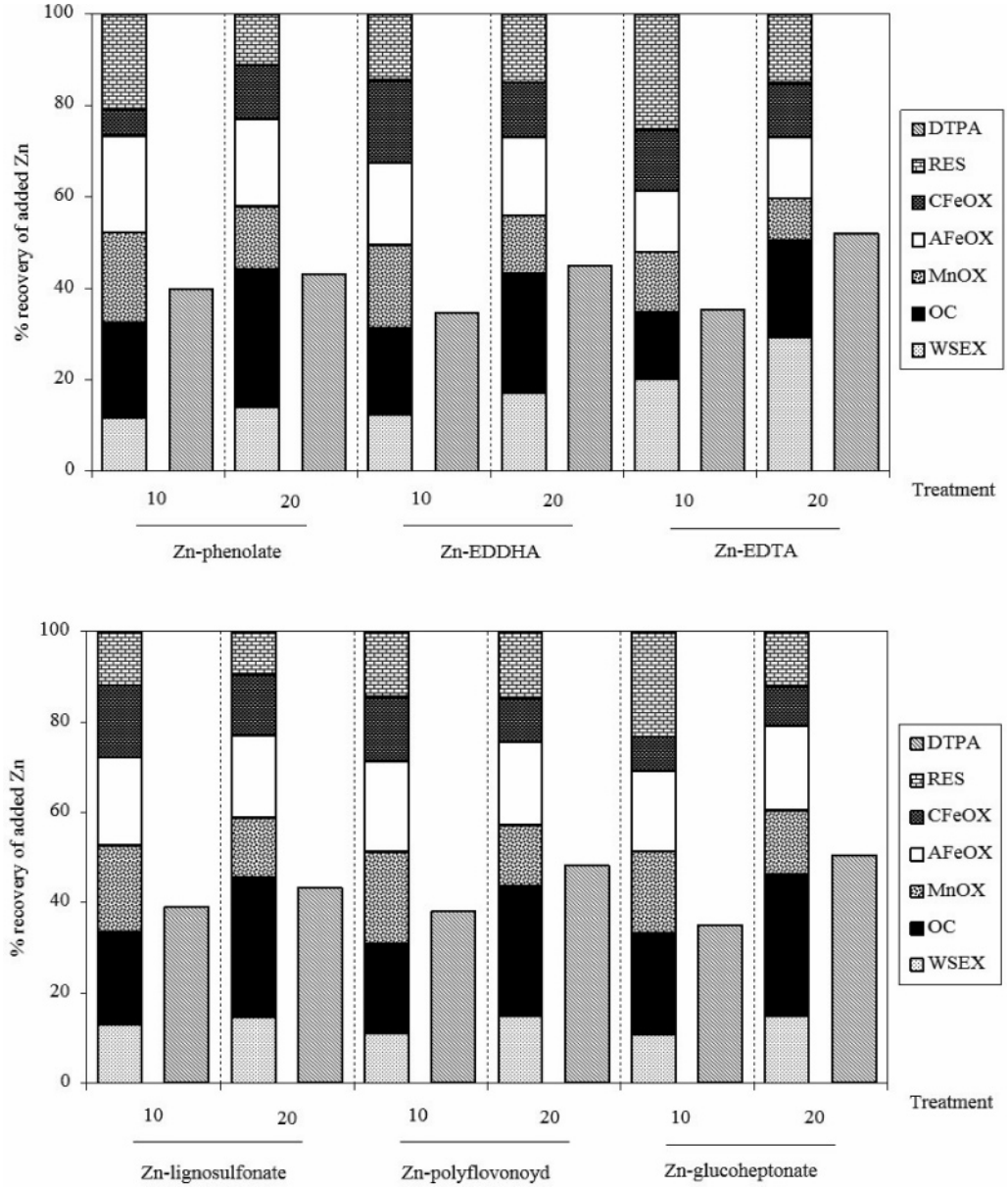

Figure 3. Percentage recovery of added Zn into different forms in neutral soil at the end of the incubation period (60 days) as influenced by fertilizer treatment.

the effect of chelation of the organic acids remains despite the increased rate of Zn application in the two most labile fractions.

According to Lopez-Valdivia et al. (*43*), the distribution values in the organic-complexed fraction in acidic soil (Aquic Haploxeralf soil) were higher at 20 mg of Zn kg^{-1} than at 10 mg of Zn kg^{-1} whereas in the water-soluble plus exchangeable fraction they were higher at a rate of 10 mg of Zn kg^{-1} than at 20 mg of Zn kg^{-1} in all fertilizer treatments.

In comparison, a decrease was observed in percentage recovery values accompanied by an increase in the application rate in MnOX and in general for the AFeOX and RES fractions. The behavior of the CFeOX fraction varied according to the fertilizer treatment. According to several authors, the redistribution of the Zn added to soils is related to the form of the Zn applied (*16*, *44*).

High percentages of applied Zn converted into DTPA-extractable form occurred at both rates of Zn application (see **Figure 3**, 34.64−39.76% for the 10 mg kg^{-1} rate, 42.99−51.85% for the 20 mg kg^{-1} rate). The highest percentages were obtained for the Zn−EDTA and Zn−glucoheptonate treatments at a rate of 20 mg of Zn kg^{-1}.

Greenhouse Experiment. Maize Growth and Zinc Uptake. The experimental results from the evaluation of the effect of six organic Zn complexes in increasing the dry matter yield and Zn concentration of a maize crop in a Zn-deficient neutral soil are presented in **Table 2**. Application of both rates of the different Zn sources resulted in slightly increased yields, producing from 2.86% to 16.01% greater yields with respect to that of the control treatment (untreated Zn); however, according to the Duncan range test, no significant yield increases could be attributed to the application of Zn fertilizers in these cases.

In contrast, in alkaline and calcareous soils, various authors reported significant differences in the dry matter yields for Zn treatments involving $ZnSO_4$, synthetic or natural chelates (*22*, *45*).

Significant differences were observed between fertilizer treatments with respect to the Zn concentration in plant samples ($P < 0.0001$). It is evident that the application of Zn fertilizers enhanced the Zn concentration in the maize. Application of both

Table 2. Dry Matter Yield and Zn Concentration in Maize As Affected by Different Fertilizer Treatments[a]

treatment	amt of Zn added (mg kg^{-1})	dry matter content (g per pot)	Zn concn (mg kg^{-1})
control	0	68.46 a	14.80 a
Zn–phenolate	10	70.42 ab (2.86)[b]	28.48 b (92.43)
	20	75.84 ab (10.78)	29.18 b (97.16)
Zn–EDDHA	10	72.79 ab (6.32)	38.40 c (166.2)
	20	79.42 b (16.01)	58.69 d (296.6)
Zn–EDTA	10	71.64 ab (4.65)	54.92 d (271.1)
	20	72.44 ab (5.81)	102.66 e (593.7)
Zn–lignosulfonate	10	76.89 ab (12.31)	28.27 b (91.01)
	20	76.91 ab (12.34)	28.30 b (91.22)
Zn–polyflavonoid	10	71.67 ab (4.69)	28.01 b (89.26)
	20	71.68 ab (4.70)	28.97 b (95.74)
Zn–glucoheptonate	10	74.86 ab (9.35)	25.36 b (71.35)
	20	76.17 ab (11.26)	26.95 b (82.09)

[a] Data are mean values for three replicates (three plants each). Values were compared using a Duncan multiple range test at the 95% level. Homogeneous groups are denoted with the same letter. [b] Values in parentheses represent percentage increases with respect to the control treatment.

rates of the Zn sources increased the Zn concentration by between 71.4% and 593.7% with respect to the concentration in the control treatment. In comparison with the control treatment, the Zn concentration in the plant was 3.7 and 6.9 times greater for 10 and 20 mg of Zn kg^{-1} as Zn−EDTA fertilizer and 2.6 and 4.0 times greater for 10 and 20 mg Zn kg^{-1} as Zn−EDDHA fertilizer. Furthermore, the influence of the rate applied was only significant for Zn−EDTA and Zn−EDDHA fertilizers (**Table 2**). The rest of the fertilizer treatments produced similar concentrations with no influence on the Zn rate. On the other hand, only the Zn−EDTA (10 mg of Zn kg^{-1} rate, 54.92 mg of Zn kg^{-1}; 20 mg of Zn kg^{-1} rate, 102.6 mg of Zn kg^{-1}) and Zn−EDDHA (20 mg of Zn kg^{-1} rate, 58.69 mg of Zn kg^{-1}) treatments produced sufficient concentration in the plant tissue, with more than 50 mg of Zn kg^{-1} in dry matter, which some authors consider an appropriate minimum for this plant when it is destined for animal fodder (*46*).

To compare fertilizer efficiency, data relating to the effect of applying different Zn sources at varying rates on the total Zn uptake by the plant are presented in **Figure 4**. The results suggest that the relative Zn uptake increased with increases in the rates of Zn applied for Zn−EDTA (10 mg of Zn kg^{-1} rate, 3.93 mg of Zn per pot; 20 mg of Zn kg^{-1} rate, 7.44 mg of Zn per pot) and Zn−EDDHA (10 mg of Zn kg^{-1} rate, 2.87 mg of Zn per pot; 20 mg of Zn kg^{-1} rate, 4.66 mg of Zn per pot) sources. This was particularly due to an increase in Zn concentration in plants (**Table 2**). The treatments involving Zn−phenolate, Zn−lignosulfonate, Zn−polyflavonoid, and Zn−glucoheptonate with two rates of applications only produced an approximate doubling of Zn uptake by plants with respect to the control treatment (1.01 mg of Zn per pot).

Several authors have reported that diffusion is the main mechanism that contributes to Zn nutrition of crops in soils and that the application of very stable sources enhances both the diffusion flow and the uptake of Zn by maize roots. For example, Alvarez et al. (*47*) reported that in a neutral soil the Zn−EDTA chelate migrated, became distributed throughout the soil columns, and produced a loss of Zn due to leaching. On the other hand, when the same six fertilizers were applied to an acidic soil as part of a greenhouse maize growth experiment, the highest percentages of Zn uptake by plants occurred when a rate of 20 mg of Zn kg^{-1} was applied as Zn−EDTA fertilizer and a rate of 10 mg of Zn kg^{-1} was applied as Zn−

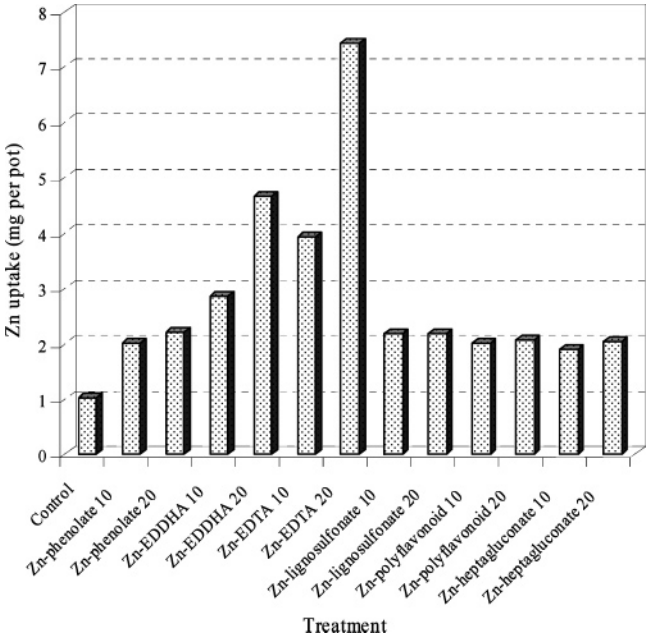

Figure 4. Zinc uptake by maize plants at different fertilizer doses of Zn.

lignosulfonate fertilizer. Furthermore, the percentage of Zn taken up by the plants with Zn−EDDHA was greater than with the other three fertilizers (*43*).

Greenhouse Experiment. Zinc Fractions and the Bioavailability of Zinc in Soil after Maize Harvest. The results of studies of the distribution of the Zn fraction in soils are shown in **Table 3**. In the control soil, differences in Zn distribution were observed in the greenhouse experiment. The order for the different Zn fractions was RES ≫ CFeOX > OC > AFeOX > MnOX > WSEX. In treated soils, the order for the different Zn fractions was RES ≫ OC > AFeOX > CFeOX > MnOX > WSEX. Micronutrient addition produced a significant increase in Zn concentration in the OC fraction ($P < 0.0001$), which is very important for the uptake of Zn in plants. The zinc concentration in the OC fraction reached a value 4.7 times greater than that of the control, in the most favorable case. According to Shuman (*13*), the oxidation of organic matter with NaOCl can dissolve manganese oxide. On the other hand, a noticeable increase in the most labile fraction (WS + EX) occurred in the two Zn−EDTA fertilizer treatments (20 mg of Zn kg^{-1} rate, 0.37 mg of Zn kg^{-1}; 10 mg of Zn kg^{-1} rate, 0.35 mg of Zn kg^{-1}). These results were significantly different from those obtained with the other treatments (two rates of application of Zn−glucoheptonate, 20 mg of Zn kg^{-1} for Zn−lignosulfonate, and two rates for Zn−EDDHA, other treatments with Zn, and the control treatment) ($P < 0.0001$). The concentration of the control treatment was practically negligible. On the other hand, the increase produced for the AFeOX fraction ($P < 0.0001$) with respect to the CFeOX fraction ($P < 0.0001$) was also noticeable. Various authors have reported differences between cultivated and noncultivated soils and even between soils cultivated with different plants. These differences could be due to the physicochemical changes produced in neutral soil as a consequence of growing the maize crop.

After the maize was harvested, Zn bioavailability in the soil was estimated by DTPA and Mehlich-3 extractions (**Table 3**). Even at low Zn rates, sufficient quantities of Zn were left available in the soil for new crops by all six fertilizers, taking into account the critical Zn levels in soils. The concentrations obtained for all the Zn treatments were higher than in the control treatment. The highest Zn concentrations for available micro-

Table 3. Zinc Fractions and DTPA- and Mehlich-3 (M-3)-Extractable Zn (mg kg^{-1}) in Soil after Maize Harvest with Different Fertilizer Treatments[a]

treatment	amt of Zn added (mg kg^{-1})	WSEX	OC	MnOX	AFeOX	CFeOX	RES	DTPA	M-3
control	0	0.01 a	1.97 a	0.45 a	1.26 a	2.21 a	30.99 a	0.47 a	1.30 a
Zn–phenolate	10	0.05 b	5.95 cd	0.92 b–d	3.21 b	2.26 a	35.43 bc	4.72 c	8.61 cd
	20	0.06 b	8.39 fg	1.53 f	3.67 bc	2.42 a	39.18 e	6.72 de	11.28 ef
Zn–EDDHA	10	0.15 c	6.21 d	1.29 ef	2.89 b	2.49 ab	33.83 b	3.64 b	6.60 b
	20	0.16 c	9.32 g	1.48 f	4.33 c–e	2.70 a–d	38.08 de	7.95 g	12.69 fg
Zn–EDTA	10	0.35 e	4.96 bc	0.68 ab	3.97 b–d	3.37 de	33.61 b	4.51 c	7.45 bc
	20	0.37 f	8.01 f	0.79 bc	4.40 c–e	3.40 e	38.29 e	7.02 e	12.07 fg
Zn–lignosulfonate	10	0.05 b	5.97 cd	0.74 b	4.54 c–e	3.11 b–e	33.64 b	4.25 bc	7.47 bc
	20	0.20 d	9.33 g	1.12 de	5.26 ef	3.28 c–e	36.34 cd	7.70 g	13.63 g
Zn–polyflavonoid	10	0.05 b	4.89 b	0.77 bc	3.93 b–d	2.79 a–d	34.63 bc	3.73 b	6.30 b
	20	0.06 b	7.37 ef	0.81 bc	5.97 f	3.31 de	37.36 de	6.22 d	9.88 de
Zn–glucoheptonate	10	0.19 d	6.63 de	0.89 b–d	2.99 b	2.69 a–c	33.84 b	4.07 bc	7.54 bc
	20	0.20 d	8.11 f	1.05 c–e	4.99 d–f	3.05 a–e	38.33 ab	7.10 ef	12.19 fg

[a] Values were compared using a Duncan multiple range test at the 95% level. Homogeneous groups are denoted with the same letter.

nutrient were associated with the application of high Zn rates of Zn−EDDHA and Zn−lignosulfonate. The worst case observed in this study showed a concentration of more than 3.5 times the average critical concentration for the two extraction methods: 0.5−1.0 mg of Zn kg^{-1} by DTPA extraction and 1.2−1.8 mg of Zn kg^{-1} by Mehlich-3 extraction (*39*, *41*, *48*).

The relationship between dry matter yield and Zn plant content and the metal forms present in soils was analyzed by correlation. A simple linear regression analysis of the two extraction methods used to determine the available Zn established a very high correlation. The equation for the fitted model was [Mehlich-3 Zn] = 0.62 + 1.60[DTPA Zn] (r = 0.99%, P < 0.0001). A positive correlation with high levels of significance existed between the dry matter yield and the OC fraction (r = 0.78, P < 0.01), DTPA-extractable Zn (r = 0.69, P < 0.01), and Mehlich-3-extractable Zn (r = 0.69, P < 0.01). A significant correlation was observed between the concentration of Zn in the plant and the WS + EX fraction (r = 0.76, P < 0.01). The relationship between Zn uptake by the plant (mg of Zn per pot) and the WSEX fraction was highly significant. The following regression equation was obtained:

$$\text{Zn uptake} = 1.21 + 10.89[\text{WSEX Zn}] \ (r = 0.75, P < 0.01)$$

These results indicated that the degree of Zn uptake by the maize was controlled by the water-soluble plus exchangeable fraction in neutral soil, and consequently, the effectiveness of organic Zn complexes in plant uptake depends on their capacity to maintain the Zn soil content in this labile form.

In contrast, the equations obtained by multiple regression analysis between the zinc uptake by maize and the fractions of zinc in the soil are not sufficiently significant.

Finally, the incubation experiment provided information about fertilizer bioavailability that complemented the one obtained from the greenhouse experiment. The incubation of Zn chelates in a neutral soil, in a field capacity regimen and under aerobic conditions, influenced the distribution of Zn in its different chemical forms. The presence of the most bioavailable forms decreased as the incubation period increased. This experiment showed that when Zn−EDTA was applied, Zn remained more labile and available than with the other five fertilizers. Even so, among these other forms, the Zn−EDDHA chelate was the one that contained the greatest quantity of water-soluble and exchangeable Zn. The improved response of the maize, with respect to the concentration of Zn in the plant, to the treatment with Zn−EDTA (and to a lesser extent, with Zn−EDDHA) might be attributed to the greater stability of the molecule in neutral soil and the ability of this fertilizer source to provide a more appropriate distribution of Zn within the soil. These Zn chelates of synthetic origin are shown to be more effective than the others because they present the biggest quantities of labile forms of Zn in the soil and in this way make a large contribution to the appropriate Zn nutrition of maize in neutral soil. These results were verified by the Zn uptake by maize plants under the reported greenhouse conditions. The differences observed in the water-soluble plus exchangeable fraction in the soil correlated with the Zn uptake by maize in the greenhouse experiment. Only the Zn−EDTA (10 and 20 mg of Zn kg^{-1}) and Zn−EDDHA (20 mg of Zn kg^{-1}) sources produced noticeable increases (Zn concentration >50 mg kg^{-1} of dry matter) in the Zn content in plants.

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