

Zinc Transformations in Acidic Soil and Zinc Efficiency on Maize by Adding Six Organic Zinc Complexes

L. M. LÓPEZ-VALDIVIA, M. D. FERNÁNDEZ, A. OBRADOR, AND J. M. ALVAREZ*

Departamento de Química y Análisis Agrícola, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain

Experiments under laboratory and greenhouse conditions were conducted to study the response of maize (*Zea mays* L.) to Zn fertilizer applications (Zn–phenolate, Zn–EDDHA, Zn–EDTA, Zn–lignosulfonate, Zn–polyflavonoid, and Zn–heptagluconate) in an Aquic Haploxeralf soil. The application of Zn complexes significantly increased Zn uptake by the plant compared with that in the control soil. The highest enhancements were obtained in soil treated with Zn–EDTA, Zn–lignosulfonate, and Zn–EDDHA. The highest percentages of Zn taken up by the plants occurred when 20 mg kg⁻¹ Zn was applied as Zn–EDTA fertilizer and 10 mg kg⁻¹ as Zn–lignosulfonate fertilizer. In the greenhouse experiment, Zn speciation in soil after harvesting showed that almost all Zn was found in the residual fraction followed by metal in the water-soluble plus exchangeable fraction and metal bound to organic matter. The most effective fertilizers maintaining Zn in the most labile fractions were Zn–phenolate, Zn–EDTA, and Zn–lignosulfonate. Conversely, in the incubation experiment, only a small percentage of Zn was found in the water-soluble plus exchangeable fraction and no differences in the Zn distribution were observed between the different fertilizer treatments. The micronutrient content in maize was positively correlated with the water-soluble plus exchangeable Zn as well as with the available Zn determined by the diethylenetriaminepentaacetic acid and Mehlich-3 methods, in the greenhouse experiment. Results of this study showed that the incubation experiment in acidic soil is not a suitable tool to establish the different effectiveness of Zn chelates in plants.

KEYWORDS: Acidic soil; available Zn; maize response; soil incubation; speciation; Zn complexes

INTRODUCTION

The interest in the micronutrient zinc (Zn) stems from problems in soil fertility and plant nutrition. Numerous Zn fertilizers are being used to correct Zn deficiencies in crops (1, 2), mainly in maize, which is a very sensitive crop to this microelement (3). These fertilizers vary considerably in physical state, chemical reactivity, cost, and availability to plants (4). The relative efficiency of different Zn carriers with the growth of maize has been studied by several authors (5–13). These studies give conflicting data regarding the effectiveness of the different Zn sources in crop yield and Zn uptake of maize. Several authors have indicated complexed forms to be the major source of Zn in soil available to maize (14–16), and their effectiveness depends on the rate of their disappearance from the soil solution, which is related to their stability (17, 18).

The availability to the plants of Zn applied, in general, decreases owing to processes that have not been defined with precision, presumably due to transformations of Zn chemical forms added to soil (19, 20). Several attempts have been made to fractionate soil Zn into different chemical forms to have a better understanding of their distribution in soils (21, 22).

However, only a very few attempts have been made to determine the relative importance of different Zn fractions in soil to plant uptake (23). The chemical forms in which metals occur are the following: the soluble plus exchangeable fraction, organically complexed fraction, iron or manganese oxide bound fraction, and residual fraction. Soluble and exchangeable forms are considered really available by plants, and metal forms bound to oxide or in residual forms would be at the opposite end. Sequential extraction, through the use of an extractant of increasing strength, has been widely used in determining metal distribution in soils. However, the selectivity of the chemical reagents has been criticized, and back-adsorption phenomena of trace metal during the extraction step have been mentioned (24). Despite some of these limitations, sequential extraction is still considered to be a valuable tool to investigate the various forms of metals in soil. Another approach to determine Zn availability to plants is using extractants, such as diethylenetriaminepentaacetic acid (DTPA) and Mehlich-3. Besides, these extractants have been commonly used to determine the critical Zn levels in soil (25, 26).

The objectives of this study were (a) to characterize the main chemical forms of Zn in an acidic soil treated with six Zn–organic complexes by means of an acidic soil incubation experiment, (b) to compare the effectiveness of the six selected

* To whom correspondence should be addressed. E-mail: jmalvarez@qaa.etsia.upm.es.

Zn-organic complexes in a crop of maize growing in this soil, (c) to determine the Zn status in the soil after the maize is harvested and to study the possible relationship between these metal fractions and the metal content in plants, and (d) to evaluate the applicability of the incubation experiment to assay the effectiveness of the different fertilizers as a source of Zn to maize.

MATERIALS AND METHODS

Soil Characterization. Representative soil was collected from Valdepiélagos in the Region of Madrid (Spain). Surface material was taken from the A_p horizon; samples were air-dried and sieved, and the <2 mm fraction was used for the experiments. Some of the soil properties are the following: texture (USDA), sandy clay loam; concentration of clay, 24%; predominant clays were montmorillonite and illite; pH_w, 6.3 (1:2.5 w/v); concentration of oxidizable organic matter, 1.10%; total N concentration, 0.11%; concentration of extractable P, 12.92 mg kg⁻¹ (Bray-1); cation exchange capacity, 10.4 cmol kg⁻¹; base saturation, 56%. The permeability in the A_p horizon was slow to moderate. The soil profile presented redoximorphic features associated with wetness resulting from the reduction and oxidation of iron and manganese compounds after saturation with water and desaturation, respectively. The soil classification is Aquic Haploxeralf. All of the analysis procedures are described in the *Official Methods of Analysis* manual (27).

Applied Fertilizers. The six Zn complexes used in this study were Zn-phenolate (CE-Zn), Zn-EDDHA (FL-Zn), Zn-EDTA (LU-Zn), Zn-lignosulfonate (MA-Zn), Zn-polyflavonoid (QU-Zn), and Zn-heptagluconate (WE-Zn), the Zn content being 7.0, 7.0, 8.8, 7.5, 5.0, and 6.0% (w/w), respectively. These liquid commercial formulations are commonly added to several crops, and their characteristics are described by Liñán (28).

Extraction Procedures. Zinc fractionation in the soil samples was performed by a modified version of the techniques described by different authors. The Zn soil content was sequentially extracted by 1 M Mg(NO₃)₂, pH 7.0, for 2 h (29) (water-soluble plus exchangeable Zn, WS + EX), two extractions, 30 min in a boiling water bath with 0.7 M NaOCl, pH 8.5 (29) (organically complexed Zn, OC), 0.1 M NH₂OH·HCl, pH 2.0, for 30 min (30) (manganese oxide bound Zn, MnOX), 0.2 M (NH₄)₂C₂O₄ + 0.2 M H₂C₂O₄, pH 3.0, for 4 h in the dark (29) (amorphous iron oxide bound Zn, AFeOX), two extractions, 30 min in a boiling water bath with solution as for previous step plus 0.1 M ascorbic acid (29) (crystalline iron oxide bound Zn, CFeOX), and acid digestion in a microwave oven in four steps (maximum pressure of 120 psi) of the soil residue with a mixture of HCl + HNO₃ + HF (2:12 w/v) (residual Zn, RES). The soil (g):extractant solution (mL) ratio was 5:50 (31). 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 the following equation:

$$\text{mg extracted} = c(5 \text{ g}) - c'm \quad (1)$$

where *c* is the concentration (mg g⁻¹) of Zn in the extracted solution, *c'* is the concentration (mg g⁻¹) of Zn in the solution extracted in the preceding step of the sequence, and *m* is the mass of the solution (g) carried over to the present from the preceding extraction (32). The equation contains a correction for the amount of Zn in the solution entrained in the soil sample after centrifugation that follows each extraction. The speciation of the original soil used for the experiments provided the following Zn fractions (average of three replications) (mg kg⁻¹): WS + EX, 0.23 (0.58%); OC, 0.72 (1.80%); MnOX, 0.25 (0.63%); AFeOX, 0.67 (1.68%); CFeOX, 2.40 (6.01%); RES, 35.62 (89.30%). The total Zn expressed as the sum of the fractions was 39.89 mg kg⁻¹.

The Zn available to the plant was assessed by two different extractions: DTPA-extractable Zn as described by Lindsay and Norwell (33) and Mehlich-3-extractable Zn as described by Mehlich (34). The concentrations of DTPA- and Mehlich-3-extractable Zn in the original soil (average of three replications) were 0.38 mg kg⁻¹ (0.95% of the

total) and 1.16 mg kg⁻¹ (2.91% of the total), respectively. These levels of available Zn can lead to a deficiency of the micronutrient for growing maize in this soil (35).

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 for Zn were prepared for each extraction in a background solution of the extracting agents. The EMPA and BAM standard checks were used for quality control.

Incubation Experiment. Different samples of soil were spiked with aqueous suspensions of the six commercial formulations to give Zn soil concentrations of 0, 10, and 20 mg kg⁻¹. Triplicate samples were incubated for 15, 30, and 60 days at 22 ± 1 °C and field capacity. The 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 and 10 g in 125 mL glass conical flasks for DTPA-extractable Zn. The distribution percentage of applied Zn in different chemical forms was determined according to the following equation:

$$\% \text{ distribution} = (\text{TZn} - \text{UZn}) \times 100 / (\text{TSUM} - \text{USUM}) \quad (2)$$

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 sums of Zn concentrations in all fractions (total Zn) in treated and untreated soil, respectively.

Greenhouse Experiment. The plant used in this study was 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, as urea, super-phosphate, and K₂SO₄, respectively. Additional doses of 37.5 mg kg⁻¹ N were added 7 and 30 days after the seeds were sowed. The soil was spiked with 0, 10, and 20 mg kg⁻¹ Zn applied as Zn-organic chelates. There were three replicates of each treatment. Three seeds of maize were sown in each pot, and the pots were taken to a greenhouse where the temperature varied between 16 and 42 °C. Appropriate amounts of water were added to reach and maintain field capacity moisture conditions with limited drainage. At the end of the maximum plant growth period (45 days after the seeding) the part above ground was cut, washed with tap water, then rinsed with deionized water, and dried in an oven at 65 °C until a constant weight was obtained. Then, they were ground and kept in sealed recipients for later analysis. Plant samples were subjected to wet digestion in a microwave oven (maximum pressure of 170 psi) using an acid mixture (HCl + HNO₃ + HF), 1:14 plant (g):solution (mL). Soil Zn concentrations after the maize crop was cut were studied by means of the sequential, DTPA, and Mehlich-3 extraction procedures.

RESULTS AND DISCUSSION

Incubation Experiment. The chemical forms of Zn in the control soil (untreated Zn) practically did not change during the incubation experiment. The greatest Zn amounts were present in the residual fraction (RES, Zn associated with aluminosilicate minerals), and only minimal Zn amounts were found in the more labile forms (e.g., WS + EX, OC, and DTPA-extractable Zn). Thus, after 60 days of incubation the percentage of the total Zn value in RES was 81.66%.

In treated soils, an initial increase of the Zn concentration in all fractions, particularly in WS + EX and OC, was observed. During the incubation period, concentrations of WS + EX and RES diminished, whereas concentrations of OC, MnOX, AFeOX, and CFeOX increased. At the end of the incubation period, in general, the Zn distribution order among fractions was RES ≫ OC > CFeOX > AFeOX > WS + EX > MnOX. The multifactor variance analysis showed significant differences between the dosage applied and incubation time (*P* < 0.001) in all fractions but not between fertilizer treatments.

Table 1. Concentration of DTPA-Extractable Zn (mg kg^{-1})^a in Acidic Soil with Different Fertilizer Treatments and Incubation Times

treatment	concn of Zn added ^b (mg kg^{-1})			
	15 days	30 days	60 days	
control	0 ^a	0.28	0.28	0.21
CE-Zn	10 ^b	5.64	4.12	3.73
	20 ^e	13.69	11.61	8.83
FL-Zn	10 ^b	5.26	4.16	3.46
	20 ^{d,e}	12.53	10.94	8.40
LU-Zn	10 ^b	5.07	3.72	3.75
	20 ^{c,d}	11.03	9.03	8.75
MA-Zn	10 ^b	6.41	4.95	3.83
	20 ^{c-e}	11.89	10.80	8.56
QU-Zn	10 ^b	6.21	4.12	3.75
	20 ^{c-e}	12.43	10.10	8.24
WE-Zn	10 ^b	5.90	4.41	3.45
	20 ^c	10.23	9.60	8.03

^a Average of three replications. Standard error ranged from 0.78 to 3.53. ^b Values with the same letter are not significantly different according to the LSD test ($P < 0.05$).

The data of DTPA-extractable Zn for the different fertilizer treatments during the 60 days of incubation are presented in **Table 1**. The Zn concentrations available in the control soil would be clearly insufficient for the majority of crops (36, 37), and the application of Zn fertilizers would be necessary. Available Zn in soil treated with organic Zn complexes depended on the experimental time and Zn dosage. With regard to fertilizer treatments, significant differences were obtained only when the formulations were applied at a 20 mg kg^{-1} dose. Although the maximum amount of DTPA-extractable Zn was reached with CE-Zn, in all cases, the available Zn concentration was higher than the concentrations required for plant growth.

Zinc extracted with DTPA decreased during the incubation period, in accordance with Armour et al. (19). These authors found that after incubation the availability of Zn for plants decreases, presumably due to transformations in Zn soil distribution. In the same period, also the differences in DTPA-extractable Zn among different fertilizer treatments diminished. These data indicate that the effectiveness of all fertilizers in maintaining Zn in this available form is similar on a long-term basis.

On the other hand, the distribution percentage of applied Zn in different chemical forms at the end of the incubation period was also studied. The percentages were calculated according to eq 2, and the results are shown in **Table 2**. Significant differences were found between the doses applied, but not between the different Zn fertilizers.

The percentage of Zn applied reached the highest values in the most labile fractions, WS + EX and especially OC, in all fertilizer treatments. In OC, the distribution values were higher at 20 mg kg^{-1} ($47.71\text{--}52.07\%$) than at 10 mg kg^{-1} ($33.30\text{--}38.20\%$), whereas in WS + EX they were higher at a dose of 10 mg kg^{-1} Zn ($24.40\text{--}28.36\%$) than at 20 mg kg^{-1} Zn ($13.44\text{--}15.21\%$) in all cases. These facts indicate that, in general, organic acids are good chelating ligands. An enhancement of the percentage values with a decrease of the dose was observed in MnOX and AFeOX. The opposite occurred in OC and CFeOX. The distribution in RES did not always follow the same trend.

High values of applied Zn in the available form (DTPA-extractable Zn) were obtained corresponding to the high values found in OC and WS + EX. In this case, the highest values occurred with fertilizers at the 20 mg kg^{-1} dose (e.g., MA-Zn, 43.97%) although high percentages were also observed with the application of 10 mg kg^{-1} Zn (e.g., MA-Zn, 37.91%).

Table 2. Distribution (%) of Applied Zn into Chemical Forms in Soil at the End of the Incubation Period As Affected by the Different Fertilizer Treatments^a

treatment	concn of Zn added (mg kg^{-1})	concn of Zn added						
		WS + EX	OC	MnOX	AFeOX	CFeOX	RES	DTPA
CE-Zn	10	27.87	36.87	13.72	15.65	2.04	3.85	37.73
	20	13.63	49.40	9.66	11.87	12.12	3.32	43.36
FL-Zn	10	24.43	34.28	13.07	15.34	7.39	5.49	30.78
	20	15.21	49.21	9.89	9.26	11.05	5.38	43.11
LU-Zn	10	28.36	38.20	9.94	15.98	2.65	4.97	37.46
	20	14.61	51.43	8.38	8.68	11.80	5.10	43.62
MA-Zn	10	26.60	33.30	12.46	16.96	2.72	7.96	37.91
	20	14.17	47.71	8.58	9.69	11.64	8.21	43.97
QU-Zn	10	24.68	33.44	9.70	12.87	10.23	9.08	37.34
	20	13.44	52.07	6.84	10.48	12.30	4.87	39.52
WE-Zn	10	24.40	35.97	10.32	14.18	6.67	8.46	33.79
	20	14.98	50.01	7.57	10.58	11.87	4.99	42.00

^a Values shown were calculated according to eq 2.

Table 3. Dry Matter Yield and Zn Concentration in Maize As Affected by Different Fertilizers^a

treatment	concn of Zn added (mg kg^{-1})	dry matter yield ^b (g per pot)	Zn concn ^b (mg kg^{-1})
control	0	64.72 ± 3.58^a	21.50 ± 0.45^a
CE-Zn	10	67.77 ± 7.94^a	$53.79 \pm 7.24^{b,c}$
	20	74.46 ± 2.64^a	$62.54 \pm 3.02^{c,d}$
FL-Zn	10	69.09 ± 1.50^a	$51.67 \pm 2.13^{b,c}$
	20	70.61 ± 2.37^a	$70.98 \pm 3.76^{d,e}$
LU-Zn	10	66.50 ± 1.35^a	$52.33 \pm 1.67^{b,c}$
	20	69.70 ± 3.99^a	89.41 ± 3.45^f
MA-Zn	10	71.20 ± 2.04^a	$53.20 \pm 1.67^{b,c}$
	20	70.91 ± 1.94^a	$79.42 \pm 7.17^{e,f}$
QU-Zn	10	69.34 ± 5.14^a	42.16 ± 3.27^b
	20	65.92 ± 4.81^a	$60.62 \pm 5.53^{c,d}$
WE-Zn	10	67.39 ± 8.83^a	43.69 ± 3.85^b
	20	71.35 ± 5.16^a	$59.07 \pm 5.28^{c,d}$

^a Data are the mean value for three replications (three plants each) with standard error. ^b In the same column, values with the same letter are not significantly different according to the LSD test ($P < 0.05$).

Greenhouse Experiment. The dry matter yield and Zn concentrations of 45 day old maize are indicated in **Table 3**. The application of fertilizers did not affect the overall growth performance of the maize, since there were no significant dry matter differences ($P < 0.05$) between plants grown in the control and those grown in the treated soils. However, beneficial effects of organic-Zn fertilizers were observed with significant increases of Zn concentration in the plant in comparison to that in the control (**Table 3**). Differences in the Zn concentration in plant samples were observed between both application rates ($P < 0.001$) and between different fertilizer treatments ($P < 0.01$). The highest Zn concentrations in maize were observed at the high Zn dose (20 mg kg^{-1}) of the LU-Zn fertilizer followed by MA-Zn and FL-Zn. When the fertilizers were applied with a dose of 10 mg kg^{-1} , the Zn concentration values in the plants were more than double the values obtained in the control soil. However, in the case of the QU-Zn and WE-Zn, these values did not reach $50 \text{ mg of Zn kg}^{-1}$ of dry matter. This concentration is given by some authors (38, 39) as the minimum Zn concentration necessary to use this plant as feeding fodder for ruminant animals.

The use by the plant of applied Zn (Zn utilization) was maximum when the Zn sources were LU-Zn (3.03%) with a dose value of 20 mg kg^{-1} and MA-Zn (3.00%) with a dose value of 10 mg kg^{-1} (**Figure 1**). Except LU-Zn, all fertilizers

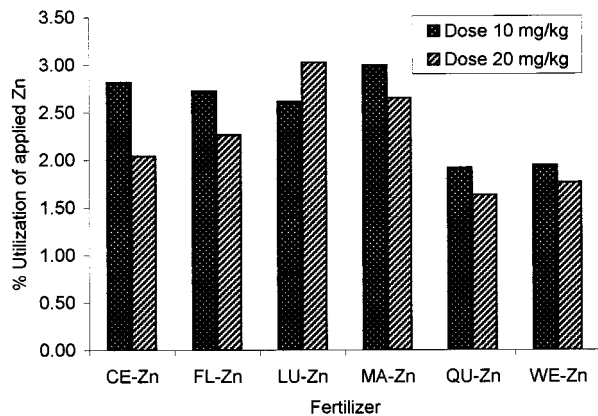


Figure 1. Effect of organic-Zn complexes on the percent utilization of applied Zn in soil by maize plants.

showed a progressive decrease in the use percentage (Zn utilization) with an increase of the Zn dosage. In the literature, Zn-EDTA was found to be a more effective source for maize than Zn-lignosulfonate in acidic soil (40). However, the data described above suggest that the relative effectiveness of these chelates depends on the doses applied.

After harvesting, the availability and distribution of Zn in the soil were studied. In the control soil, no differences in the Zn distribution were observed during the greenhouse experiment. Thus, the order among Zn soil fractions was RES \gg CFeOX $>$ OC $>$ AFeOX $>$ WS + EX $>$ MnOX. In the treated soils, differences were observed between different doses and types

of fertilizers (Figure 2). The micronutrient addition provided a remarkable increase of Zn concentration in the most labile fractions (WS + EX and OC), which are very important for the uptake of Zn in the plants. This effect was especially notable in the treatments at high doses with MA-Zn (WS + EX, 9.00 mg kg⁻¹; OC, 9.17 mg kg⁻¹), LU-Zn (WS + EX, 8.72 mg kg⁻¹; OC 8.16 mg kg⁻¹), and CE-Zn (WS + EX, 7.18 mg kg⁻¹; OC, 7.94 mg kg⁻¹). However, in general the Zn distribution order among fractions was similar in all fertilizer treatments: RES \gg OC \approx WS + EX $>$ CFeOX $>$ AFeOX $>$ MnOX. A noticeable increase of Zn concentration in WS + EX was observed in the soils cultivated with maize (mean value 4.33 at a 10 mg kg⁻¹ dose and 6.98 at a 20 mg kg⁻¹ dose) compared to the soil in the incubation experiment (mean value 2.66 at a 10 mg kg⁻¹ dose and 2.93 at a 20 mg kg⁻¹ dose). Differences had previously been reported between cultivated and not cultivated soils, even between soils cultivated with different plants (41). These differences would be due to the physicochemical changes produced in the soil as a consequence of the crop.

After the maize was cut, Zn availability in the soil was estimated by DTPA and Mehlich-3 extractions. For all of the fertilizers in this study, even at very low dosages, sufficient quantities of available Zn in the soil were left for a new crop, taking into account the critical Zn levels in soils determined by the DTPA and the Mehlich-3 extraction methods. Thus, the worst case in this study showed a concentration more than 4-fold the average critical concentrations (Figure 2) for two extractions: 0.5–1.0 mg of Zn kg⁻¹ by the DTPA method (25, 33)

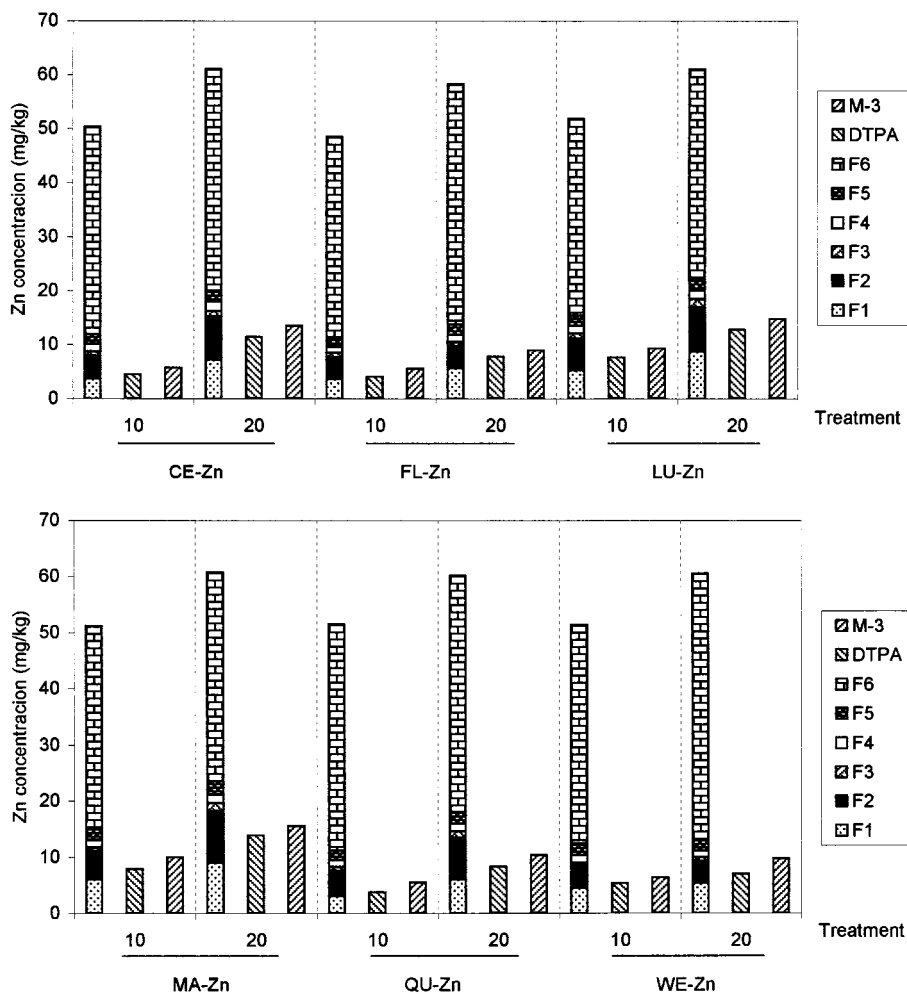


Figure 2. Distribution of Zn fractions and DTPA- and Mehlich-3-extractable Zn in soil at different fertilizer doses after maize cutting.

and 1.2–1.8 mg of Zn kg⁻¹ by the Mehlich-3 method (35). The highest Zn concentration in the available form occurred at the high Zn dose of MA-Zn (DTPA-Zn, 13.85 mg kg⁻¹; Mehlich-3-Zn, 15.53 mg kg⁻¹), LU-Zn (DTPA-Zn, 12.78 mg kg⁻¹; Mehlich-3-Zn, 14.73 mg kg⁻¹), and CE-Zn (DTPA-Zn, 11.45 mg kg⁻¹; Mehlich-3-Zn, 13.47 mg kg⁻¹). These results agree with the highest concentration in the most labile forms, WS + EX and OC, observed with these fertilizers. Besides, values of DTPA-extractable Zn were higher than those obtained in the incubation experiment, similar to the concentration increase observed in WS + EX.

In this study, stability constants of Zn complexes do not explain the effectiveness of fertilizers to maintain Zn in the most labile soil forms since fertilizers with similar stability constants, such as Zn-EDTA (log *K* = 17.4 with ionic strength 0.01 M) and Zn-EDDHA (log *K* = 17.8 with ionic strength 0.01 M) (42), showed important differences in the Zn concentration in the WS + EX and OC fractions, and in the DTPA- and Mehlich-3-extractable Zn (see Figure 2).

The relationships between the Zn plant content and the metal soil fractions and available Zn were analyzed by correlation analysis. A positive correlation (simple linear regression analysis) with high levels of significance (*P* < 0.001) existed between the Zn concentration in the plant and WS + EX (*r* = 0.92), DTPA (*r* = 0.89), and Mehlich-3 (*r* = 0.89). These results indicated that the degree of Zn uptake by the plants is controlled by their chemical forms in soils, and consequently, the effectiveness of organic-Zn complexes in plant uptake depends on their capacity of maintaining the Zn soil content in these labile forms.

An exception to this general behavior is the fertilizer FL-Zn. This fertilizer produced a noticeable increase in metal content in the plant. However, this increase was not accompanied by a similar increase in DTPA-Zn. By contrast, CE-Zn produced an increase in WS + EX and in DTPA-Zn, which was not confirmed by a similar increase in the Zn content in the plant. These results suggest that although DTPA is a good method to determine Zn available to plants, there are other factors that influence the Zn uptake by the plant, such as, for example, the mobility of Zn chelates (43).

On the other hand, in the incubation experiment, soil Zn speciation did not show any differences between fertilizers, and the differences observed in DTPA-extractable Zn correlated poorly with the Zn content in plants obtained in the greenhouse experiment. Besides, as described above, in this acidic soil the maize grown in the soil remarkably affected the Zn distribution in the soil. Consequently, the incubation experiment in acidic soil is not a suitable tool to establish the different effectiveness of Zn chelates in plants.

ACKNOWLEDGMENT

We are grateful to Dr. José L. Marco for his encouragement and support.

LITERATURE CITED

- (1) Shuman, L. M. Micronutrient fertilizers. *J. Crop Prod.* **1998**, *1*, 165–195.
- (2) Anderson, W. B. Zinc in soils and plant nutrition. *Adv. Agron.* **1972**, *24*, 147–186.
- (3) Loué, A. El maíz. In *Los Microelementos en Agricultura*; Mundi-Prensa: Madrid, Spain, 1988; pp 241–260.
- (4) 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 33–44.

- (5) Lindsay, W. L. Zinc in soils and plant nutrition. *Adv. Agron.* **1972**, *24*, 147–186.
- (6) Brown, A. L.; Krantz, B. A. Source and placement of zinc and phosphorus for corn (*Zea mays* L.). *Soil Sci. Soc. Am. Proc.* **1966**, *30*, 86–89.
- (7) Shukla, U. C.; Morris, H. D. Relative efficiency of several zinc sources for corn (*Zea mays* L.). *Agron. J.* **1967**, *59*, 200–202.
- (8) Ganiron, R. B.; Adrian, D. C.; Paulsen, G. M.; Murphy, L. S. Effects of phosphorus carriers and zinc source on phosphorus-zinc interaction in corn. *Soil Sci. Soc. Am. Proc.* **1969**, *33*, 306–309.
- (9) Mortvedt, J. J.; Giordano, P. M. Availability to corn of zinc applied with various macronutrient fertilizers. *Soil Sci.* **1969**, *108*, 180–187.
- (10) Schnappinger, M. G.; Martens, D. C., Jr.; Hawkins, G. W. Response of corn to Zn-EDTA and ZnSO₄ in field investigations. *Agron. J.* **1969**, *61*, 834–836.
- (11) Wallace, A.; Romney, E. M. The effect of Zn sources on micronutrient contents of Golden Cross Bantam corn. *Soil Sci.* **1970**, *109*, 66–67.
- (12) Boawn, L. C. Comparison of zinc sulfate and Zn EDTA as zinc fertilizer sources. *Soil Sci. Soc. Am. Proc.* **1973**, *37*, 111–115.
- (13) Diddowson, J. P.; Watts, H. M. Zinc deficiency in the shallow soils of Niue. III. Response of sweet corn to sources of zinc. *New Zealand J. Exp. Agric.* **1977**, *5*, 241–248.
- (14) Dhillon, K. S.; Dhillon, S. K. Relative efficiency of different chelates in the supply of applied zinc to maize and wheat. *J. Nucl. Agric. Biol.* **1983**, *12*, 93–96.
- (15) Prasad, B.; Sinha, M. K. The relative efficiency of zinc carriers on growth and zinc nutrition of corn. *Plant Soil* **1981**, *62*, 45–52.
- (16) Herget, G. W.; Rehm, G. N.; Wiese, R. A. Field evaluation of zinc sources band applied in ammonium polyphosphate suspension. *Soil Sci. Soc. Am. J.* **1984**, *48*, 1190–1193.
- (17) Lahav, N.; Hochberg, M. Kinetics of fixation of iron and zinc applied as metal chelates. *Soil Sci. Soc. Am. Proc.* **1975**, *39*, 55–58.
- (18) McBride, M. B. Reactions controlling heavy metal solubility in soils. *Advances in Soil Science*; Springer-Verlag: New York, 1989; Vol. 5, pp 1–56.
- (19) Armour, J. D.; Ritchie, G. S. P.; Robson, A. D. Changes with time in the availability of soil applied zinc to navy beans and in the chemical extraction of zinc from soils. *Aust. J. Soil Res.* **1989**, *37*, 277–286.
- (20) Ma, Y. B.; Uren, N. C. The fate and transformations of zinc added to soils. *Aust. J. Soil Res.* **1997**, *35*, 727–738.
- (21) Ma, Y. B.; Uren, N. C. Application of a new fractionation scheme for heavy metals in soils. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 3291–3303.
- (22) Shuman, L. M. Chemical forms of micronutrients in soils. In *Micronutrients in Agriculture*, 2nd ed.; Mortvedt, J. J., et al., Eds.; Soil Science Society of America: Madison, WI, 1991; pp 113–144.
- (23) Thenmozhi, R.; Savithiri, P. Studies on transformation and nutrition of zinc in Alfisol. *J. Indian Soc. Soil Sci.* **1993**, *41*, 106–110.
- (24) Orsini, L.; Bermond, A. Application of a sequential extraction procedure to calcareous soil samples: preliminary studies. *Int. J. Environ. Anal. Chem.* **1993**, *51*, 85–95.
- (25) 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.
- (26) Sistini, K. R.; Mays, D. A.; Taylor, R. W.; Buford, C. Evaluation of four chemical extractants for metal determinations in wetland soils. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 2167–2180.
- (27) Ministerio de Agricultura, Pesca y Alimentación. *Métodos Oficiales de Análisis*; MAPA: Madrid, Spain, 1994; Vol. III.
- (28) Liñán, C. Correctores de carencias simples. In *Vademecum de Productos Fitosanitarios y Nutricionales*; Liñán, C., Ed.; Agro-técnicas: Madrid, Spain, 1996; pp 497–501.

- (29) Shuman, L. M. Fractionation method for soil micronutrients. *Soil Sci.* **1985**, *140*, 11–22.
- (30) 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.
- (31) 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.
- (32) Sposito, G.; Lund, L. J.; Chang, A. C. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases. *Soil Sci. Soc. Am. J.* **1982**, *46*, 260–264.
- (33) 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.
- (34) Mehlich, A. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416.
- (35) Tran, T. S.; Simard, R. R. Mehlich III-extractable elements. In *Soil Sampling and Methods of Analysis*; Carter, M. R., Ed.; Canadian Society of Soil Science; Lewis Publishers: Boca Raton, FL, 1993; pp 43–49.
- (36) Lindsay, L. W.; Cox, F. R. Micronutrient soil testing for the tropics. *Fert. Res.* **1985**, *7*, 169–200.
- (37) Liang, J.; Karamanos, R. E. DTPA-extractable Fe, Mn, Cu and Zn. In *Soil Sampling and Methods of Analysis*, 1st ed.; Carter, M. R., Ed.; Canadian Society of Soil Science; Lewis Publishers: Boca Raton, FL, 1993; pp 87–90.
- (38) Périgaud, S. Les carences en oligo-éléments chez les ruminants en France. Leur diagnostic. Les problèmes soulevés pour l'intensification fourragère. *Ann. Agron.* **1970**, *21*, 635–669.
- (39) McDonald, P.; Edwards, R. A.; Greenhalgh, J. F. D. In *Nutrición animal*; Acribia: Zaragoza, Spain, 1988; p 497.
- (40) Alvarez, J. M.; Obrador, A.; Rico, M. I. Effects of chelated zinc soluble and coated fertilizers on soil zinc status and zinc nutrition of maize. *Commun. Soil Sci. Plant Anal.* **1996**, *27*, 7–19.
- (41) 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.
- (42) Lindsay, W. L. Chelate equilibria. In *Chemical Equilibria in Soils*; John Wiley & Sons: New York, 1979; pp 238–265.
- (43) Alvarez, J. M.; Novillo, J.; Obrador, A.; López-Valdivia, L. M. Mobility and leachability of Zn in two soils treated with six organic Zn complexes. *J. Agric. Food Chem.* **2001**, *49*, 3833–3840.

Received for review July 25, 2001. Revised manuscript received November 16, 2001. Accepted November 26, 2001. This research was financially supported by the Autonomous Community of Madrid, Project 06-033-96.

JF010978V