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Behavior of Zinc from Six Organic Fertilizers Applied to a Navy Bean Crop Grown in a Calcareous Soil

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The objective of this study was to compare the mobility, leaching, availability, and relative effectiveness of Zn from Zn−polyhydroxyphenylcarboxilate (Zn−PHP), Zn−HEDTA (Zn−*N*-2-hydroxyethyl-ethylenediaminetriacetate), Zn−EDDHSA [Zn−ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate)], Zn−EDTA (Zn−ethylenediaminetetraacetate), Zn−*S*,*S*-EDDS (Zn−ethylenediaminedisuccinate), and Zn−EDTA−HEDTA sources by applying different Zn rates (5 and 10 mg kg^{-1}) to a calcareous soil under greenhouse conditions. A lysimeter experiment was carried out for 60 days and using navy bean (*Phaseolus vulgaris* L.) as an indicator plant. The Zn available to the plant and easily leachable Zn were determined in soil by different single extractions, while the distribution of Zn in the soil was assessed by sequential speciation. The utilization of applied Zn by the navy bean was greatest when the Zn treatments were Zn−EDTA, Zn−EDTA−HEDTA, Zn−HEDTA, and Zn−EDDHSA. Both total Zn in the plants and soluble Zn in the plant dry matter (extracted with 1 mM 2-morpholino-ethanesulfonic acid) were positive and significantly correlated with the following: the amounts of Zn extracted with the three single extractions used to estimate soil available Zn and the amounts of Zn in the water soluble plus exchangeable and organically complexed fractions. The Zn−HEDTA, Zn−EDDHSA, Zn−EDTA−HEDTA, Zn−*S*,*S*-EDDS, and Zn-EDTA sources significantly increased the mobility of micronutrients through the soil with respect to the control and Zn−PHP source. The maximum Zn concentration obtained in the leachate fractions was 65 mg L^{-1} (13% of Zn applied) for the Zn−*S*,*S*-EDDS chelate applied at a rate of 10 mg Zn kg^{-1} soil. In the course of the crop, the soil pH + pe parameter increased significantly with experimental time.

KEYWORDS: Availability; chelates; plant response; sequential speciation; micronutrient

INTRODUCTION

Zinc deficiency is a critical nutritional problem in plants and is responsible for low yield and poor plant quality in some parts of the world (*1*−*3*). Plants are known to differ in their susceptibility to particular mineral deficiencies (*4*), e.g., navy bean (*Phaseolus vulgaris* L.) cultivars are generally susceptible to soil Zn deficiency, which is at least partly due to the poor translocation of Zn from the roots to the tops of plants (*5*, *6*). In contrast, high concentrations of Zn can be toxic for plants and animals and constitute a potential contaminant in soils (*7*−*9*).

Certain soil conditions like high pH (e.g., in calcareous soil), poor aeration, low organic matter content, high clay content, and/or P supply, are known to promote Zn deficiency (*3*, *10*). Such soils therefore need Zn supplements. Inorganic fertilizers (mainly Zn sulfate) have been traditionally used for this purpose, but other organic Zn sources (synthetic chelates and natural complexes) are also commonly used (*11*, *12*). The crop response to Zn fertilization varies according to the Zn fertilizer source (*13*, *14*). The greater mobility of chelated Zn is of agronomic significance in that it suggests that these Zn sources are more likely to move into the root zone and provide feeding sites for the crop. Some studies have reported that applications of chelated forms of Zn to calcareous soils are the most effective for certain crops (*15*, *16*), but column leaching studies have also shown that the addition of some Zn chelates may greatly increase Zn mobility throughout the whole soil column and that important amounts of applied Zn may be lost via leaching (*17*). For example, when fertilizers contain the ethylenediaminetetraacetate (EDTA) chelating agent, the application of micronutrients to the soil supposes a risk to the environment and especially to groundwaters. This deserves consideration in the development of best management practices to reduce the transport of metals from land to water (*9*).

Micronutrient availability to plants can be measured in direct uptake experiments or estimated using a chemical extractant to remove a fraction of the soil micronutrient pool and relate this fraction to plant response (*18*). Chemical sequential extraction techniques are commonly used to fractionate the solid-phase forms of metals found in soils (*19*). These schemes have been applied in environmental solid samples to metal partitioning into

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operationally defined geochemical fractions. The soluble plus exchangeable fraction is considered the most phytoavailable form of metals, while those in the latter stages of the extraction scheme are less available to plants (*20*−*22*). A variety of single soil extractants are also used to evaluate micronutrient availability in soils (*23*). These leaching tests are interesting in order to reduce considerably the experimental task of estimating the available and mobilizable fractions of metals (*15*, *24*). The most widely accepted extractant for micronutrient cations is 0.05 M DTPA (*25*), but other reagents (e.g., dilute acids, chelating agents, neutral salts, *etc.*) are also currently used (*26*−*29*). The nutrient concentrations measured by a specific extractant are a function of both the chemical composition of the extractant in question and the particular properties of the soil tested (*30*). Critical levels for some micronutrients tend to be useful only over a limited range of soils and crops and are closely related to the conditions under which they were determined (*18*).

Although there is evidence that some chemical properties of soils, such as pH and pe (Eh/59.2) (Eh, redox potential), can be affected by the addition of fertilizers to soils (*31*, *32*), there is a general lack of information regarding the effects of fertilization involving organic Zn complexes. To improve predictions of Zn mobility and availability when this type of fertilizer is added to soils, it is therefore interesting to study the redox status of the soil (pH + pe) parameter after fertilizer application and its possible relation with the distribution of various Zn forms.

A greenhouse experiment was conducted to study the behavior of Zn from six organic complexes applied to a Zn deficient calcareous soil hosting a crop of short-growing height navy bean. The objectives of this study were the following: (i) to compare the effectiveness of six commercial Zn fertilizers applied to a navy bean crop (dry matter yield and plant Zn concentration), (ii) to study the mobility and leaching of applied Zn, (iii) to determine the potential availability and chemical forms of soil-applied Zn when the crop was cut and to study its possible relation with plant response, and (iv) to investigate changes in soil pH and potential redox measurements during the experiment.

MATERIALS AND METHODS

Soil Characterization. Surface soil was taken from the A_p horizon (0−20 cm) of an alkaline soil from Torrejón del Rey, Guadalajara, Spain (latitude 40° 39′ N, longitude 3° 20′ W). This was a cultivated soil characterized by a significant calcium carbonate content and lack of organic matter. This type of soil is common in many places in central Spain. The soil was air-dried, and a fraction of less than 2 mm was used in the experiment. A representative subsample was used for its characterization.

Soil properties were the following: texture (USDA), sandy loam; clay, 180 g kg^{-1} (*33*); permeability, moderate to rapid (*34*); water holding capacity, 20.5% w/w (*35*); bulk density, 1.06 g cm^{-3} (*36*); pH 8.1 (1:2.5 w/v); electrical conductivity, 178.2 μS cm^{-1} (1:2.5 w/v); organic matter content, 5.1 g kg^{-1} (*37*); total N, 1.1 g kg^{-1} (*38*); available P, 12.6 mg kg^{-1} (*39*); cation exchange capacity, 235 $mmol^{+}$ kg^{-1} (*40*); base saturation, 86%; total and free $CaCO_3$, 134 g kg^{-1} (calcareous soil) and 33.4 g kg^{-1}, respectively (*41*, *42*); and poor in crystalline (active) oxides, 56 mg Fe kg^{-1} (*43*). The predominant clay in the soil was esmectite (*44*). The soil was a Typic Calcixerept (*45*). Total soil Zn was determined after treating 2 g of dried samples with 10 mL of HNO_3 (65%) and 3 mL of HF (48%) followed by digestion in Teflon bombs in a microwave oven with a rotating tray (CEM, Mars). The total Zn concentration was 43.40 mg kg^{-1} soil. The values presented are the means of three replicates.

Fertilizers Applied. Six liquid fertilizers with different organic Zn sources were selected: Zn−polyhydroxyphenylcarboxylate (Zn−PHP) (3.8 g water-soluble Zn L^{-1} and mass density 1.26 g cm^{-3}), Zn−HEDTA (Zn−*N*-2-hydroxyethyl-ethylenediaminetriacetate) (8.83 g water-soluble Zn L^{-1} and mass density 1.26 g cm^{-3}), Zn−EDDHSA [Zn−ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate)] (4.5 g water-soluble Zn L^{-1} and mass density 1.26 g cm^{-3}), Zn−EDTA−HEDTA (7.7 g water-soluble Zn L^{-1} and mass density 1.29 g cm^{-3}), Zn−*S,S*-EDDS (Zn−ethylenediaminedisuccinate) (8.2 g water-soluble Zn L^{-1} and mass density 1.36 g cm^{-3}) and Zn−EDTA (Zn−ethylenediamine-tetraacetate) (10.0 g water-soluble Zn L^{-1} and mass density 1.38 g cm^{-3}). These organic sources of Zn are produced by different commercial companies (*46*).

Greenhouse Experiment. A navy bean crop was grown in polypropylene lysimeters (capacity, 11 L; internal diameter, 24 cm; and height, 25 cm). A 1.5 cm thick layer of washed gravel was placed in the bottom of each lysimeter to facilitate drainage, and a polyester mesh and 10 kg of soil were placed on top of it. The leachate was collected with a silicone tube leading to a polyethylene bottle. The nutritional condition of the soil for the navy bean crop was assessed using the electroultrafiltration technique (*47*). Taking the results into account, basal fertilization was applied with 50 mg N kg^{-1} [as $(NH_2)_2CO$], 50 mg P kg^{-1} [as $Ca(H_2PO_4)_2$], and 50 mg K kg^{-1} (as K_2SO_4). Three navy bean seeds (*Phaseolus vulgaris* L., variety Garrafal Rabona Enana Esmeralda, Fito S.A., Barcelona, Spain) were cultivated in each lysimeter. The soil was treated with aqueous suspensions of the six liquid fertilizers to obtain samples of each with added Zn concentrations of 0 (control), 5, and 10 mg Zn kg^{-1} soil. The control and treatments were replicated three times in a completely randomized design layout. The lysimeters were placed in a greenhouse, where temperatures varied between 16 and 28 °C and relative air humidity ranged from 60 to 85%. The soils were irrigated at slightly above field capacity moisture (30.8% w/w) to obtain 10 fractions (each one of 200 mL) of leachate. Sixty days after seeding, the plants were cut at soil level, washed in deionized water, and then dried in an oven at 65 °C to a constant weight. Once weighed, they were ground and kept in sealed containers for later analysis. The soils were also dried at room temperature, manually homogenized in order to obtain representative samples, sieved (<2 mm), and stored for further analysis.

Plant, Leachate and Soil Analyses. The total Zn in the plants was determined by wet digestion in a microwave oven (a two-step process with a maximum pressure of 170 psi) using 0.3 g of dried ground samples, 4 mL of HNO_3 (65%), and 2 mL of HF (48%). Soluble Zn in the plant dry matter was extracted by the method proposed by Rahimi and Schropp (*48*) and Cakmak and Marschner (*1*), with slight modifications: 0.25 g of the aerial part of the plant was weighed and its Zn content was extracted with 10 mL of 1 mM 2-morpholinoethanesulfonic acid (MES) at pH 6 (ratio 1:40, w:v).

The leached liquids were collected, and their Zn contents were analyzed. The electrochemical parameters of the soils and leachates were determined by potentiometry analysis using pH and redox (Pt) electrodes. The temperature was automatically compensated by means of a probe connected to the potentiometer in the case of the pH. To calculate the redox potential (Eh), the potential from the reference electrode was added to the measured potential of the cell at the same temperature (*49*). The easily leachable Zn fraction (physical adsorption) in the soil was extracted with $BaCl_2$ 0.01 M (*50*). According to Räisänen et al. (*51*), the $BaCl_2$ reagent only extracts elements which are physically adsorbed on particles.

The relative Zn available to the plant was assessed by extracting it with three commonly used chemical extractants: DTPA−TEA (5 mM DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine, adjusted to pH 7.3) (*25*); DTPA−AB (5 mM DTPA + 1.0 M NH_4HCO_3, adjusted to pH 7.6) (*52*), 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) (*53*).

The Zn distribution in the different soil fractions was determined by the sequential fractionation method proposed by Krishnamurti and Naidu (*54*). The Zn fractions were sequentially determined in seven steps with the following extractants: 1 M NH_4NO_3 pH 7.0 (WSEX, water soluble plus exchangeable); 1 M NaOAc pH 5.0 (CAR, carbonate bound); 0.1 M $Na_4P_2O_7$ pH 10.0 (OC, organically complexed); 0.1 M $NH_2OH{\cdot}HCl$ in 0.01 M HNO_3 (RMO, easily reducible metal oxide bound); 30% H_2O_2 pH 2.0 + 0.02 M HNO_3 and 2 M NH_4NO_3 in 20%

Table 1. Response of Navy Bean in a Calcareous Soil to Different Zn Chelates[a]

treatment	amount of Zn added (mg kg^{-1} soil)	dry matter content (g per lysimeter)[b]	total Zn conc (mg kg^{-1} DM)	total Zn uptake (mg per lysimeter)	soluble Zn conc[c] (mg kg^{-1} DM)
control	0	29.32	19.93 a	0.58 a	16.16 a
Zn–PHP[d]	5	31.42	26.18 ab	0.82 ab	17.54 a
	10	28.24	35.50 cd	1.01 b–d	18.09 a
Zn–HEDTA	5	28.01	46.44 e	1.30 de	25.25 b–d
	10	30.32	51.38 ef	1.55 ef	27.87 c–e
Zn–EDDHSA	5	32.94	36.24 cd	1.20 cd	25.31 b–d
	10	31.20	47.97 e	1.50 ef	29.57 ef
Zn–EDTA–HEDTA	5	29.52	39.24 d	1.16 cd	29.14 d–f
	10	29.77	57.65 f	1.71 f	32.67 f
Zn–*S,S*-EDDS	5	31.97	30.32 bc	0.97 bc	22.87 b
	10	32.73	39.56 d	1.30 de	24.11 bc
Zn–EDTA	5	29.92	51.73 ef	1.55 ef	29.07 d–f
	10	30.32	66.53 g	2.02 g	41.01 g

[a] Values were compared using a Duncan multiple range test at the 0.05 level of probability. Homogeneous groups are denoted with the same letter. [b] No significant differences existed between treatments for dry matter content ($P > 0.05$). [c] Soluble Zn concentration extracted with the MES reagent. [d] Zn–PHP = Zn–polyhydroxyphenylcarboxylate.

HNO_3 (OM, organically bound); 0.2 M $(NH_4)_2C_2O_4$/0.2 M $H_2C_2O_4$ pH 3 in the dark (AMC, amorphous minerals colloids bound); 0.2 M $(NH_4)_2C_2O_4$/0.2 M $H_2C_2O_4$ pH 3 in 0.1 M ascorbic acid (CFeO, crystalline Fe oxide bound). The residual fraction (RES) was calculated as the difference between total Zn and the sum of the other fractions.

All samples were extracted and analyzed in triplicate using each procedure. Zinc concentrations in all extracts were determined by flame atomic absorption spectrophotometry (Perkin-Elmer, AAnalyst 700).

DTPA−TEA extractable Zn in the original soil was 0.44 mg kg^{-1} (0.99% of total Zn). This concentration of available Zn could indicate a deficiency in micronutrient content for growing plants in calcareous soil. On the other hand, the concentrations of the other micronutrients were adequate for normal crop growth since DTPA−TEA extractable Fe, Cu, and Mn were 5.12, 0.43, and 8.57 mg kg^{-1}, respectively.

The sequential extraction of the original soil used for the experiment provided the following Zn fractions (mg kg^{-1}): WSEX, 0.27 (0.62%); CAR, 0.89 (2.05%); OC, 2.71 (6.25%); RMO, 0.17 (0.39%); OM, 1.95 (4.49%); AMC, 1.26 (2.90%); CFeO, 8.64 (19.91%); RES, 27.51 (63.39%).

Data Analysis. Descriptive, simple, and stepwise multiple regression analyses and other statistical studies were made using the Statgraphics-Plus 5.1 software (Manugistic, Inc., Rockville, MD). Multifactor analysis of variance was carried out to determine the main effects and interactions of the different parameters. Multiple comparisons of variables were made using Duncan's separations of means procedures. A probability level of $P \leq 0.05$ was selected to establish statistical significance.

RESULTS AND DISCUSSION

Plant Growth and Zinc Uptake. The effect of fertilizer treatments on 60-day-old navy bean dry matter production, Zn plant concentration (total and soluble), and total Zn uptake is shown in **Table 1**. The fertilizer treatments had negligible effects on navy bean growth since there were no significant differences in dry matter production between plants grown in the control soil and in the Zn-treated soils. It is noticeable that the control (with no Zn addition) exhibited Zn tissue concentrations of almost 20 mg kg^{-1}, which is the normal critical concentration for dried whole shoots (*29*, *55*). However, the application of organic Zn complexes to the soil appreciably increased both the Zn concentration and total Zn uptake and, therefore, improved the nutritional value of the crop with regard to its contribution of Zn to the human diet. In this respect, multifactor variance analyses for total Zn concentration, Zn uptake, and soluble Zn concentration in the plant showed significant differences between treatments ($P < 0.0001$, F-ratio = 35, 18, and 28, respectively) but not between repetitions. The lowest concentrations and total Zn uptake were observed with the two rates of the Zn−PHP source and the low Zn rate of the Zn−*S,S*-EDDS source, both of which are natural compounds. In contrast, the highest concentration and total Zn uptake in navy bean were observed with the high Zn rate of the Zn−EDTA chelate, followed by the low Zn rate of the same fertilizer, the high Zn rate of the Zn−EDTA−HEDTA, and both doses of the Zn−HEDTA product. These treatments produce Zn concentrations that were almost equal to or greater than 50 mg kg^{-1} (dry matter), which is cited by some authors as the amount of Zn required in plants used for animal fodder (*56*). Some authors have reported that the concentrations of soluble Zn in cotton roots and maize, millet, tobacco, sugar beet, and grape leaves provide a good indicator of their Zn nutritional status (*47*, *48*, *57*). In this study, for all fertilizer treatments, the total Zn concentration in plant dry matter and soluble Zn extracted from dry bean tissue with the reactive MES varied in a similar way and were related according the following equation:

$$\text{Zn-soluble} = 5.91 + 0.37 \cdot \text{Zn-total} \quad (R^2 = 0.85, P < 0.0001) \quad (1)$$

In our study, the determination of Zn-soluble MES in dried whole shoots of navy bean can therefore be used to know the nutritional state of the plant with respect to this microelement.

A parameter for studying the relative efficiency of the different fertilizer treatments can be defined as the percentage of Zn used by navy bean plants with respect to that applied, based on the following equation:

$$\text{Zn used (\%)} = [(\text{Zn uptake (treatment)} - \text{Zn uptake (control)} \cdot 100]/\text{Zn added} \quad (2)$$

The most effective treatments were those involving both applied rates of Zn−EDTA (1.94 and 1.44%, for rates of 5 and 10 mg kg^{-1}, respectively) and the Zn−HEDTA applied at a rate of 5 mg kg^{-1} (1.44%), followed by the low rate of Zn−EDTA−HEDTA and Zn−EDDHSA (1.16 and 1.24%, respectively). In contrast, the least effective treatments were those with Zn−PHP (0.48 and 0.43%, at rates of 5 and 10 mg kg^{-1}, respectively) and with the Zn−*S,S*-EDDS (0.78 and 0.72%, at rates of 5 and 10 mg kg^{-1}, respectively).

For the parameters total Zn concentration, total Zn uptake, and soluble Zn concentration, we also performed other multifactor variance analyses to determine the main effects of fertilizer type and the amount of Zn added and their interactions.

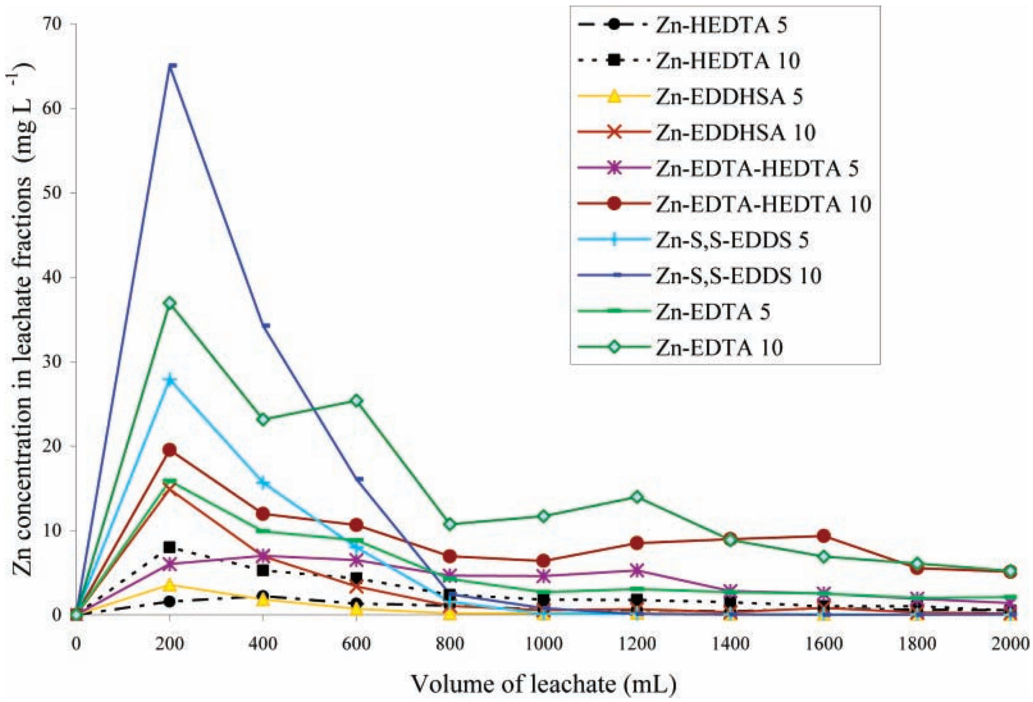

Figure 1. Concentrations of Zn in 10 200 mL leachate fractions from soil lysimeters treated with 5 and 10 mg Zn kg^{-1} soil as Zn–HEDTA, Zn–EDDHSA, Zn–EDTA–HEDTA, Zn–*S*,*S*-EDDS, and Zn–EDTA (coefficients of variation ranged from 0.54 to 12.39%).

These analyses showed significant differences between different fertilizer types (P ranged from 0.001 to 0.0001, and the F-ratio ranged from 12 to 25) and the amount of Zn applied (P ranged from 0.001 to 0.0001, and the F-ratio ranged from 21 to 45). Only one significant interaction was obtained between fertilizer and Zn rate for the parameter Zn soluble concentration ($P < 0.05$, F-ratio $= 4$). The multiple range test (Duncan method) established that the fertilizer Zn−EDTA produced the highest Zn uptake by navy bean (mean value 1.79 mg per lysimeter)

Zinc Leaching. The application of organic Zn complexes to the soil produced very different cumulative quantities of collected Zn in 2 L of leachate (at 60 days), with these depending on the Zn source used. The amount of Zn leached in the soil treated with Zn−PHP (natural complex) was very small for both applied doses (<0.1 mg Zn) and comparable with that leached in the control (no Zn addition). For the other five Zn fertilizers studied, the total amount of Zn leached was significantly higher than in the control and produced a net loss of Zn by leaching which varied between approximately 4% (Zn−HEDTA and Zn−EDDHSA, both at a rate of 5 mg kg^{-1}) and 30% (Zn−EDTA at a rate of 10 mg kg^{-1}) of applied Zn. Some of these values may present a potential risk of contamination to groundwaters. The Zn concentrations in each of the 10 leachates collected showed a generally comparable tendency for these 5 fertilizers, with a peak and maximum Zn concentration in the first 2 leachates (at leaching volumes of 200 and 400 mL), especially in the first case, followed by a decline in subsequent leachate fractions (**Figure 1**). However, the Zn concentration in this first leachate varied considerably according to the fertilizer treatment applied, ranging from 1.55 mg L^{-1} for the Zn−HEDTA fertilizer applied at a rate of 5 mg kg^{-1} to 65.05 mg L^{-1} for the Zn−*S*,*S*-EDDS product applied at a rate of 10 mg kg^{-1} (13% of Zn applied). A variance analysis of the cumulative amounts of Zn leached showed significant differences between the different fertilizers (**Figure 2**). The order of the studied Zn complexes, bearing in mind the mean values of the amounts of total Zn leached, was the same for both rates of application (5 and 10 mg kg^{-1}): Zn−PHP (0.08 mg and 0.08 mg) < Zn−HEDTA (1.9 and 5.5 mg) = Zn−EDDHSA (2.1 and 5.8 mg) < Zn−EDTA−HEDTA (8.5 and 18.6 mg) < Zn−*S*,*S*-EDDS (10.7 and 23.8 mg) ≤ Zn−EDTA (10.8 and 29.8 mg). These results could be related with differences in the stability of Zn-complexes studied under these soil−plant conditions; the most stable complexes maintain greater amounts of Zn in the soil solution, migrate through the soil profile, and are leached (*16*, *58*, *59*). It is noticeable that of the five fertilizers that produced Zn leaching, greater total amounts of Zn were leached when applying the low Zn rate of three of them (Zn−EDTA, Zn−*S*,*S*-EDDS, and Zn−EDTA−HEDTA) than when applying the high Zn rate of the other two sources (Zn−HEDTA and Zn−EDDHSA).

A previous soil column study of Zn leaching without plants, in which Zn was applied in the form of Zn−DTPA−HEDTA−EDTA, revealed considerable movement of Zn in a calcareous soil, but the leaching tendency was quite different (*15*). In that study, the concentration of Zn in the leachates increased with time and did not peak until a leaching volume of 900 mL. This could have been due to the fact that the soil was very calcareous (total carbonate content >20%) and the predominant clay was montmorillonite, which may have retained the Zn applied (*15*).

After the navy bean was harvested, the amount of easily leachable Zn that remained in the soils was estimated using the dilute $BaCl_2$ extraction. The amount per lysimeter of easily leachable Zn in the control soil was 1.33 mg. At the end of the experiment, for all fertilizer treatments, the amount of $BaCl_2$-extractable Zn had significantly increased with respect to the control, but significant differences were found between different treatments. The fertilizer treatments that produced the smallest quantities of $BaCl_2$-extractable Zn involved the two rates of the Zn−PHP complex and the Zn−HEDTA and Zn−EDDHSA compounds applied at a rate of 5 mg kg^{-1}. In contrast, the treatments that produced the largest amounts of easily leachable Zn were Zn−*S*,*S*-EDDS, Zn-EDTA, and, in particular, Zn−EDTA−HEDTA, all of which applied at a rate of 10 mg Zn kg^{-1} (**Figure 3**).

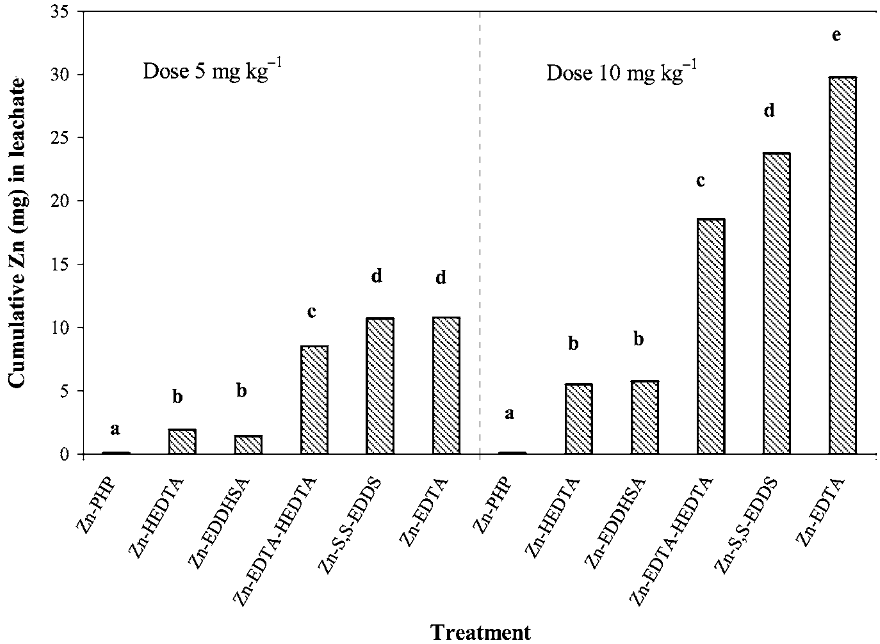

Figure 2. Cumulative amounts of Zn leached from calcareous soil treated with different Zn fertilizer treatments. Statistical differences at $P \leq 0.05$ (Duncan test) are presented by different letters.

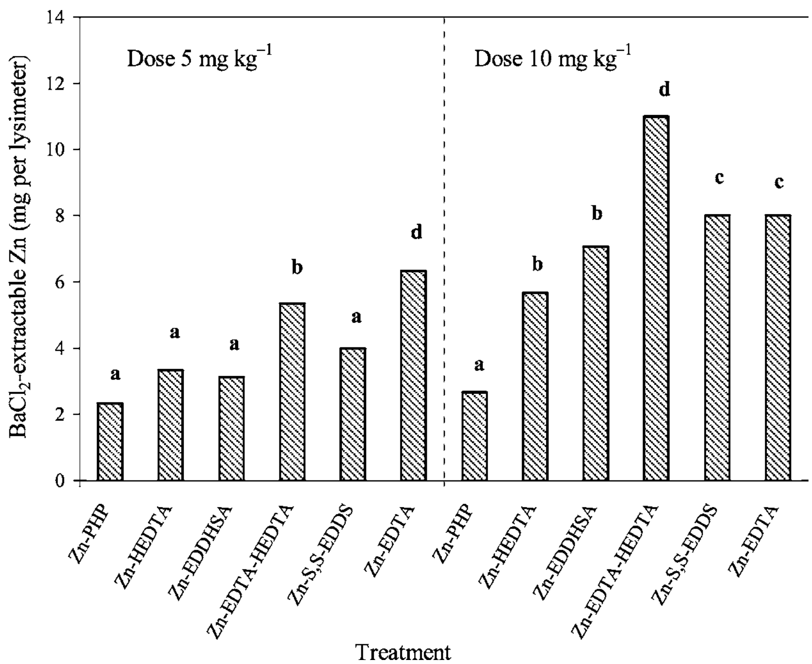

Figure 3. Amounts of $BaCl_2$-extractable Zn (easily leachable Zn) in a calcareous soil treated with different Zn fertilizers. Statistical differences at $P \leq 0.05$ (Duncan test) are presented by different letters.

Potential Availability and Distribution of Applied Zinc in Soil Fractions after Crop Harvest. Values for Zn extracted from soil by each of the three methods used to estimate available Zn (DTPA−TEA, DTPA−AB, and Mehlich-3) are shown in **Figure 4**. The available Zn concentration in the control soil was the following: 0.31 mg kg^{-1} for DTPA−TEA, 0.48 mg kg^{-1} for DTPA−AB, and 1.10 mg kg^{-1} for Mehlich-3. These values for the three extraction methods were lower than those reported as critical levels for most plants in calcareous soils: 1.0 mg kg^{-1} for DTPA−TEA extraction (*60*), 1.2 mg kg^{-1} for DTPA−AB extraction (*53*), and 1.8 mg kg^{-1} for Mehlich-3 extraction (*61*). This soil would therefore require Zn fertilizers providing additional micronutrient for later crops. This could be due to its special physicochemical characteristics: a calcareous soil with a high pH and very low organic matter content. Significant differences were found between the different fertilizer treatments ($P < 0.0001$). Although the concentrations obtained for all of the Zn treatments were higher than those in the control, the available Zn concentration was insufficient when the Zn−PHP source was applied at a rate of 5 mg Zn kg$^{-1.}$ In contrast, application of the other fertilizer treatments produced sufficient available Zn to satisfy the requirements for most crops, although they did not all behave in the same way. The treatments that produced the smallest quantities of available Zn were the Zn−PHP source in doses of 10 mg kg^{-1} and the Zn−EDDHSA applied at a rate of 5 mg kg^{-1}, which showed soil Zn concentrations that were near to critical values. In contrast, the treatment that produced the largest quantities of available Zn

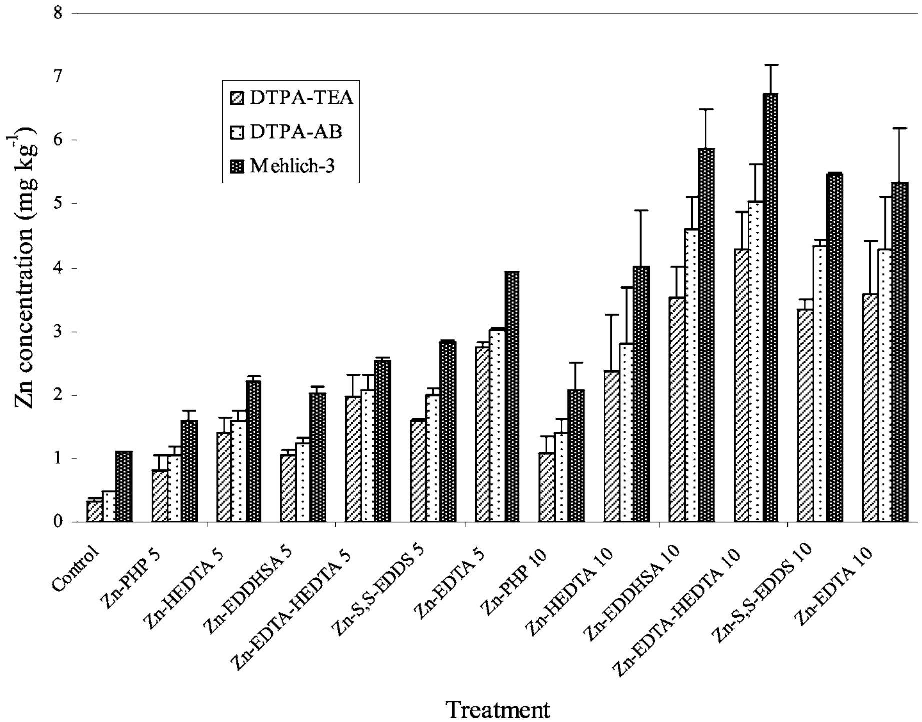

Figure 4. Concentrations of DTPA–TEA-, DTPA–AB-, and Mehlich-3-extractable Zn in calcareous soil treated with different Zn fertilizers. The vertical line at each of the data points represents the standard deviation from the mean.

Table 2. Zinc Fractions (mg kg^{-1} soil) in a Calcareous Soil with the Application of Different Zn Fertilizer Treatments[a]

treatment	amount of Zn added (mg Zn kg^{-1} soil)	WSEX	CAR	OC	RMO	OM	AMC	CFeO	RES
control	0	0.23 a	0.88 a	2.62 a	0.19 a	2.02 a	1.29 a	8.82 a	28.06 bc
Zn–PHP[b]	5	0.38 ab	1.08 ab	2.92 ab	0.38 bc	2.61 a–d	1.82 bc	11.75 b–d	27.12 a–c
	10	0.73 cd	1.65 cd	3.25 a–c	0.42 bc	3.27 de	2.04 b–d	13.42 de	27.45 bc
Zn–HEDTA	5	0.26 a	0.90 a	3.02 ab	0.32 b	2.90 b–e	2.05 b–d	10.00 ab	28.84 c
	10	0.43 ab	1.35 bc	4.33 de	0.35 bc	4.00 fg	2.70 e	12.15 c–e	28.51 c
Zn–EDDHSA	5	0.53 c	1.17 ab	3.23 a–c	0.43 bc	2.62 a–d	1.88 bc	10.99 bc	27.54 bc
	10	0.91 de	2.19 f	5.83 g	0.45 bc	3.45 ef	2.12 cd	13.57 de	25.13 a
Zn–EDTA–HEDTA	5	0.56 bc	1.17 ab	3.84 b–d	0.42 bc	2.47 a–c	2.45 de	11.33 bc	29.19 c
	10	1.78 g	1.83 ef	5.46 fg	0.47 c	4.43 g	2.77 e	12.75 c–e	25.88 ab
Zn–*S*,*S*-EDDS	5	0.42 ab	1.08 ab	4.03 c–e	0.41 bc	2.79 a–e	2.07 b–d	11.08 bc	27.27 a–c
	10	0.75 d	1.83 ef	5.53 fg	0.42 bc	3.18 c–e	2.78 e	13.18 de	26.24 ab
Zn–EDTA	5	1.01 ef	1.35 bc	4.81 ef	0.33 b	2.32 ab	1.65 ab	11.77 b–d	26.12 ab
	10	1.18 f	1.94 ef	5.93 g	0.44 bc	2.79 a–e	1.96 bc	13.83 e	25.12 a

[a] Values were compared using a Duncan multiple range test at the 0.05 level of probability. Homogeneous groups are denoted with the same letter. [b] Zn–PHP = Zn–polyhydroxyphenylcarboxylate.

for subsequent crops was Zn−EDTA−HEDTA applied at a rate of 10 mg Zn kg^{-1} (which produced a concentration that was between 3.7 and 5.0 times greater than the critical concentration, depending on the extraction method employed). This was followed by the treatments involving high rates of Zn−*S*,*S*-EDDS, Zn−EDDHSA, and Zn−EDTA sources (which produced concentrations between 2.9 and 4.6 times greater than the critical concentration, depending on the fertilizer and the extraction method employed). A multifactor variance analysis showed significant differences among the three methods used to estimate available Zn concentration ($P < 0.0001$). The Duncan separation of averages method established the following order (mean value for all repetitions and fertilizer treatments): Mehlich-3 (3.51 mg kg^{-1}) > DTPA−AB (2.60 mg kg^{-1}) > DTPA−TEA (2.16 mg kg^{-1}).

Zinc distributions among the fractions in soil for the different fertilizer treatments are shown in **Table 2**. The Zn concentrations in the different soil fractions depended on the different fertilizer treatments applied. In the control soil, the order for the different Zn fractions was as follows: RES (28.1 mg kg^{-1}) > CFeO (8.82 mg kg^{-1}) > OC (2.62 mg kg^{-1}) > OM (2.02 mg kg^{-1}) > AMC (1.29 mg kg^{-1}) > CAR (0.88 mg kg^{-1}) > WSEX (0.23 mg kg^{-1}) ≈ RMO (0.19 mg kg^{-1}). In this case, the total Zn concentration was 44.11 mg kg^{-1} soil, which was 0.71 mg kg^{-1} greater than for the original soil (see the Materials and Methods section). This behavior was probably due to the fact that the phosphoric fertilizer contained micronutrient impurities.

In the treated soils, although the order of the different Zn fractions remained the same as in the control, higher Zn concentrations were found in treated than in untreated soils for all fractions except RES. For each fraction, multifactor variance analyses showed significant differences between treatments (P ranged from 0.01 to 0.0001, and the F-ratio ranged from 3 to 52) but there were no significant differences between repetitions. The Duncan's separation of averages method was applied for all the soils treated (see **Table 2**) and established the following order (mean value of the Zn concentration for all fertilizers and Zn rates applied): RES (27.0 mg kg^{-1}) > CFeO (12.2 mg kg^{-1})

> OC (4.35 mg kg^{-1}) > OM (3.07 mg kg^{-1}) > AMC (2.19 mg kg^{-1}) > CAR (1.46 mg kg^{-1}) > WSEX (0.75 mg kg^{-1}) ≈ RMO (0.40 mg kg^{-1}). The addition of Zn fertilizers to this soil had a significant effect on the Zn content in the fractions WSEX (0.6−15.6% of added Zn) and OC (6.0−43.8% of applied Zn), which would be very important for the Zn nutrition of any subsequent crop. Zinc concentration values were examined for each fraction using multifactor variance analysis to determine the main effects of fertilizer treatment and repetition. In all fractions, there were significant differences between fertilizer treatments but not between repetitions (see **Table 2**). The biggest Zn concentrations in the most potentially available fraction (WSEX) were associated with the Zn−EDTA−HEDTA (2.4 and 7.7 times greater than in the control soil for application rates of 5 and 10 mg kg^{-1}, respectively) and Zn−EDTA (4.4 and 5.1 times greater than in the control soil for application rates of 5 and 10 mg kg^{-1}, respectively) fertilizers. On the other hand, Zn−HEDTA (1.1 and 1.9 times greater than in the control soil for application rates of 5 and 10 mg kg^{-1}, respectively) and Zn−PHP (1.7 and 3.2 times greater than in the control soil for application rates of 5 and 10 mg kg^{-1}, respectively) were the sources associated with the smallest amounts of Zn in this fraction.

Other multifactor variance analyses were performed to determine the main effects of fertilizer type and the amount of Zn added and their interactions, for each Zn fraction. These analyses showed significant differences between fertilizers and between amounts of Zn for all fractions except for the RMO fraction (P ranged from 0.05 to 0.0001, and the F-ratio ranged from 4 to 52). It is very important to highlight the significant interaction between fertilizer type and the amount of Zn ($P < 0.0001$, and the F-ratio = 22) obtained for the WSEX fraction.

Finally, it is interesting to mention that there were positive and highly significant correlations between the three methods used to estimate available Zn ($P < 0.0001$), which seems to suggest that they could be used in a similar way to predict the availability of Zn for plants. On the other hand, the amount of soil-extractable Zn for all single reagents (DTPA−TEA, DTPA−AB, Mehlich-3) was positively correlated with Zn concentration in almost all sequentially extracted fractions with the exception of RES, with which it was negatively correlated. Furthermore, the correlations between the different Zn fractions were highly significant in several cases.

pH and pe Parameters. The experimental conditions, for example, the application of fertilizers, initially produced a decrease in the electrochemical parameters pH and pe of the soil. These parameters were determined in saturated soils at two crop times: 30 and 60 days (end of the experiment) for all treatment fertilizers. A multifactor variance analysis of the pH + pe parameter values showed significant differences between times ($P < 0.001$) and between fertilizer treatments ($P < 0.005$). The Duncan's separation of averages method established that the pH + pe parameter increased significantly with time in the course of the crop. This enhancement was caused as much by slight increases in soil pH (which varied from 7.1 to 7.3) as by the soil redox potential (which varied from 544 to 550 mV). With respect to fertilizer treatments, the following order was established (mean value of the pH + pe parameter for all repetitions and times): control (16.05) ≤ Zn−EDTA−HEDTA-5 (16.18) ≤ Zn−EDTA−HEDTA-10 (16.19) ≤ Zn−PHP-10 (16.44) ≈ Zn−HEDTA-10 (16.48) ≈ Zn−EDTA-10 (16.52) ≈ Zn−HEDTA-5 (16.53) ≈ Zn−EDDHSA-5 (16.58) ≤ Zn−*S,S*-EDDS-5 (16.61) ≈ Zn−*S,S*-EDDS-10 (16.61) ≤ Zn−EDTA-5 (16.63) ≈ Zn−PHP-5 (16.66) ≈ Zn−EDDHSA-10 (16.80).

At the end of the experiment, the pH and pe parameters of the soils correlated with those determined in the leachates ($P < 0.0001$).

The possible relationships between plant parameters (plant growth and Zn concentration) and Zn concentration in the different soil chemical fractions obtained by single and sequential extraction procedures were analyzed by correlation. No significant correlation was found between dry matter production and any of the single or sequential amounts of Zn extracted. In contrast, plant Zn concentration (total Zn and MES-soluble Zn) was positive and significantly correlated with the amounts of Zn extracted when applying each of the single soil extractants (DTPA−TEA, DTPA−AB, and Mehlich-3). Hence, significant simple linear regression equations ($P < 0.005$, R^2 values were relatively high) could describe Zn concentrations in navy bean plants as a function of soil-extractable Zn for all single reagents. A simple linear correlation analysis between plant metal concentration (total and soluble) and soil sequential extracted fractions also showed significant correlations. The Zn plant concentration (total and soluble) was mainly correlated with the Zn amounts in the WSEX and OC fractions. It was possible to significantly predict the availability of Zn for navy bean using empirical equations with these two soil solid-phase fractions as components. The best fit regression equations describing plant Zn concentrations (mg kg^{-1}) were the following:

$$\text{Zn-total} = 9.64 + 6.62(\text{WSEX} + \text{OC}) \quad (R^2 = 60.97, P < 0.005) \quad (3)$$

$$\text{Zn-soluble} = 9.11 + 3.44(\text{WSEX} + \text{OC}) \quad (R^2 = 61.35, P < 0.005) \quad (4)$$

It therefore seems that the availability of Zn in this calcareous soil and under these growing conditions can be assessed on the basis of absolute metal content in fractions weakly bound to soil components.

The redox status of this calcareous soil (pH + pe parameter) had only a negligible effect on the extractability, leaching, and uptake of Zn by the navy bean.

Finally, it should be noted that the most effective source for the navy bean crop was the Zn−EDTA source but that significant quantities of Zn were leached when this product was applied. Of all the treatments applied, it would be advisable to use Zn−HEDTA at a rate of 5 mg kg^{-1} since this was one of the most effective treatments and the resulting Zn concentration in navy bean dry matter was relatively high (almost 50 mg kg^{-1}). Moreover, both the total amount of Zn leached out during the greenhouse experiment and the total amount of the easily leachable fraction of Zn that remained in the soil after navy bean harvest were quite small. In contrast, the Zn−*S,S*-EDDS fertilizer was one the least effective for this crop and both the total amount of leached Zn and the size of the easily leachable fraction were substantial. The residual Zn produced by the different fertilizer treatments, excluding the Zn−PHP fertilizer applied at a rate of 5 mg kg^{-1}, was available in sufficient quantities to allow subsequent crops to be grown without the need for further micronutrient additions.

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