

Leaching and Efficiency of Six Organic Zinc Fertilizers Applied to Navy Bean Crop Grown in a Weakly Acidic Soil of Spain

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Zinc contamination of groundwater from fertilizers applied to pulse crops is a potential problem, but the use of different types of organic chelates can minimize the contamination potential while still adequately feeding the crops. The objective of this study was to compare the leaching, distribution in fractions and availability, and relative effectiveness of Zn from six organic Zn fertilizers (zinc–ethylenediaminetetraacetate–*N*-2-hydroxyethylethylenediaminetriacetate (Zn-EDTA-HEDTA), Zn-HEDTA, zinc–*S,S*-ethylenediaminedisuccinate (Zn-*S,S*-EDDS), zinc–polyhydroxyphenylcarboxylate, Zn-EDTA, and zinc–ethylenediaminedi(2-hydroxy-5-sulfophenylacetate) (Zn-EDDHA)) applied to a navy bean (*Phaseolus vulgaris*, L.) crop cultivated by applying different Zn levels, in a weakly acidic soil under greenhouse conditions. Zinc soil behavior was evaluated by diethylenetriaminepentaacetic acid–triethanolamine (DTPA-TEA), DTPA-ammonium bicarbonate (DTPA-AB), Mehlich-3, and BaCl₂ extractions and sequential fractionation. In all the fertilizer treatments, the percentage of labile Zn that remained in the soil was high with respect to the quantity of Zn applied, with values respectively ranging from 42 to 80% for Zn-EDDHA and Zn-EDTA sources. A positive correlation with a high level of significance existed between the micronutrient concentration in the navy bean crop (total and soluble) and labile Zn fractions, available Zn, and easily leachable Zn (*r* ranged from 0.89 to 0.95, *P* < 0.0001). The relatively high quantity of total Zn leached by applying Zn-EDTA and Zn-*S,S*-EDDS sources (11.9 and 6.0%, respectively, for the rate 10 mg of Zn kg⁻¹ of soil) poses a potential pollution risk for neighboring waters. It would seem recommendable to apply Zn-HEDTA or Zn-EDDHA sources, even applied at the low rate (5 mg of Zn kg⁻¹ of soil), because they produced available Zn concentrations in the soil that were above the critical concentration and also produced high Zn concentrations in plants (139 and 106 mg of Zn kg⁻¹ of dry matter, respectively).

KEYWORDS: Acidic soil; available Zn; *Phaseolus vulgaris* L.; lixiviation; speciation; Zn complexes

INTRODUCTION

The correction of Zn deficiency in plants has generally focused on obtaining the maximum improvement in yield (1, 2). Furthermore, the most efficient agronomic practice currently available to farmers, to increase Zn content in food crops and thereby improve their nutritional quality, involves the addition of fertilizers with available forms of Zn. This often implies the application of excess quantities of nutrients that exceed rates required to reach maximum crop yield (3). An appropriate correction can result in a residual effect that benefits subsequent crops, although the benefits will depend on the nature of the soil and crop system. In contrast, inappropriate applications of Zn fertilizers (e.g., high rates, high numbers of applications) may produce environmental pollution and contaminate agricultural products with high levels of heavy metals that could be

harmful to consumer health (4–6). It is also known that soil contamination by heavy metals due to irresponsible agricultural practices can occur within only a few years, whereas natural purification of soils may require hundreds of years (7, 8). Therefore, the challenge of modern agriculture is to safeguard the production of high-quality food in a sustainable natural environment (9). Consequently, it would be advisable to study the effects of new commercial micronutrient fertilizers on soils and plants to optimize fertilization and minimize their environmental impact.

Several Zn sources have been used to correct Zn deficiency in crops (10), including navy beans, which are very sensitive to this micronutrient (11). Anderson (12) reported that the relative effectiveness of different Zn sources on crop growth obeyed the following order (DTPA = diethylenetriaminepentaacetic acid; EDTA = ethylenediaminetetraacetic acid; EDDHA = ethylenediaminedi(2-hydroxy-5-sulfophenylacetate)): Zn-DTPA > Zn-EDTA > Zn-EDDHA > ZnSO₄ > zinc–polyflavonoid.

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Other studies have also indicated that organic sources are more effective fertilizers than inorganic ones (13, 14). Their effectiveness depends on the rate of their disappearance from the soil solution, which is related to their stability (15). Zinc chelates differ in physical state, chemical reactivity, cost, bioavailability, and susceptibility to leaching (14), and, furthermore, some of them may reduce groundwater quality (16). Rico et al. (17) found that fertilization with Zn-EDTA and zinc–lignosulfonate led to increases in corn dry matter yield that were even greater than when the synthetic chelate was applied. The relative efficiencies of different Zn chelates have been studied by several authors (18, 19) growing different plant species in diverse soils. They report contradictory results with respect to the effect of different Zn sources on crop yield and total Zn content in plants. To date, few studies have been conducted using other Zn chelates to grow navy beans. However, the use of fertilizers containing Zn complexes or chelates is increasing, and new commercial fertilizers are continuously being developed (20).

The total concentration of heavy metals in soils does not provide information on their mobility, availability, and reactivity related to their chemical forms (21). The use of sequences of different chemical extractants has therefore become an increasingly popular method for quantifying the amount of metals present in soils, in their different forms. The selectivity of the chemical reagents has been questioned, and the back-adsorption of trace metals has been suggested (22). Despite their severe limitations, chemical speciation provides a better understanding of Zn behavior with respect to the transformation of Zn added to soil (23), and this method has often been used to determine the relative distribution and chemical forms of Zn (24–26). Even so, only a few studies have been made to determine the relative importance of different Zn fractions to the total Zn content in plants (27). Another approach to determine Zn availability to plants involves the use of extractants such as DTPA and EDTA (28). Furthermore, the amount of easily leachable Zn can be determined by means of the BaCl₂ extraction procedure (29).

Metal uptake by plants depends on soil (e.g., source and chemical forms of the metal, pH, pe, oxohydroxides, and organic matter) and plant factors (plant species, age, plant part, and time sampled). The parameter most frequently determined, related with the micronutrient nutritional status of plants, is the total dry matter content, although a better indication is sometimes obtained by simply determining a fraction of the total content, such as the part that is soluble in water or in diluted acids or chelators (30).

The objectives of this greenhouse study were to determine the following: (i) the mobility and leaching of Zn in the form of different organic complexes (natural vs synthetic origin) applied to a weakly acidic soil in which a navy bean crop was cultivated; (ii) the efficiency of six different Zn complexes on the navy bean crop, as exhibited by dry matter yield, and both total and soluble Zn concentrations; (iii) the relative distribution and chemical forms of Zn present in the soil when the crop was harvested and the possible relationship between these metal fractions and the metal content of plants; (iv) the evolution of pH and potential redox in soils and leachates over the experimental period and the estimation of the bioavailability of Zn for subsequent crops.

MATERIALS AND METHODS

Soil Characterization. The selected soil was collected from Navalcarnero (latitude 40° 21'N, longitude 4° 00'W) in the region of Madrid (Spain). It was classified as Typic Haploxeralf (31). Surface material was taken from the Ap horizon (0–26 cm). The soil was air-dried, and a fraction of less than 2 mm was used in the experiment.

Some of the main soil properties were as follows: texture, sandy loam (USDA); clay content, 100 g kg⁻¹ (32); predominant clays, illite and smectite; bulk density, 1.47 g cm⁻³ (33); water-holding capacity (33 kPa), 6.60 g of H₂O 100 g⁻¹ of soil (34); permeability, moderate (2 cm h⁻¹) (35); pH_w, 6.13 (1:2.5 (w/v)); oxidizable organic matter, 2.35 g kg⁻¹ (36); total N, 1.02 g kg⁻¹ (37); extractable P, 19.89 mg kg⁻¹ (38); cation exchange capacity, 4.72 cmol_c kg⁻¹ (39); Fe (active Fe₂O₃), 141 mg kg⁻¹ (40).

Applied Fertilizers. The six liquid commercial products containing complexed Zn used in this study were as follows: Zn-EDTA-HEDTA (Zn-EDTA, zinc–ethylenediaminetetraacetate; Zn-HEDTA, zinc–N-2-hydroxyethylethylenediaminetriacetate; and the ratio EDTA/HEDTA was 1:1), Zn-HEDTA, Zn-S,S-EDDS (zinc–S,S'-ethylenediaminedisuccinate), zinc–polyhydroxyphenylcarboxylate (Zn-PHP), Zn-EDTA, and Zn-EDDHS [zinc–ethylenediaminedi(2-hydroxy-5-sulfophenylacetate)]. The respective Zn contents of the commercial products were as follows: 6.0, 7.0, 6.0, 3.0, 7.3, and 3.6% (w/w). These liquid commercial formulations are commonly added to several crops, and their characteristics have been described by Liñan (20).

EXPERIMENTAL PROCEDURES

A navy bean (*Phaseolus vulgaris*, L.; Garrafal Rabona Enana Esmeralda, Fito S.A., Barcelona, Spain) crop was grown in 10 kg of soil placed in modified polypropylene containers (capacity, 11 L; internal diameter, 24 cm; height, 25 cm), with a 1.5 cm thick layer of washed gravel at the bottom (to facilitate aeration and drainage). The leachate was collected with a silicone tube leading to a polyethylene bottle. Basal fertilization was applied with 50 mg of N kg⁻¹ (as urea), 50 mg of P kg⁻¹ [as Ca(H₂PO₄)₂], and 50 mg of K kg⁻¹ (as K₂SO₄). Three navy bean seeds were cultivated in each container. The soil received 0 (control), 5, and 10 mg of Zn kg⁻¹, which was applied from organic liquid Zn sources. The control and treatments were replicated three times in a completely randomized design layout. The containers were placed in a greenhouse in which temperatures varied between 16 and 28 °C and relative air humidity was between 60 and 85%. The soils were irrigated at slightly above field capacity moisture to obtain 10 fractions of leachate (each of 200 mL). To evaluate the evapotranspiration, the containers were weighed (balance A&D Instruments Ltd., U.K., model FG-30KBM), and we estimated the volume of irrigation water required. Sixty days after seeding and without significant seed development, the plants were cut at soil level, washed in deionized water, and then dried in a forced-draft 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, homogenized, sieved (<2 mm), and stored for further analysis.

Leachate, Plant, and Soil Analyses. The leached liquids were collected, and their Zn contents were analyzed. The electrochemical parameters of the soils and leachates were determined by potentiometry, using pH and redox (Pt) electrodes. Temperature was automatically compensated for by connecting a probe to the potentiometer in the case of pH. To calculate the redox potential (E_h), the potential from the reference electrode was added to the measured potential of the cell (41).

The total Zn in the plant was determined by wet digestion in a microwave oven (a two-step process with a maximum pressure of 1173 × 10³ Pa) using 0.3 g of dried ground samples, 4 mL of HNO₃ (65%), and 2 mL of HF (35%). Soluble Zn in the plant dry matter was extracted by the method proposed by Rahimi and Schropp (42) and Cakmak and Marschner (43), with slight modifications: 0.25 g of the above-ground part of the plant was weighed, and its Zn content was extracted with 10 mL of 1 mM 2-morpholinoethanesulfonic acid (MES), pH 6 (ratio 1:40 (w/v)).

Zinc distribution in the different soil fractions was determined by the sequential fractionation method proposed by Krishnamurti and Naidu (26). This method is described in Table 1 and divides soil Zn into seven fractions (the Zn concentration in the seventh fraction was calculated as the difference between total Zn and the sum of the other six fractions).

Soil-available Zn was assessed by extracting it with DTPA-TEA (5 mM DTPA + 0.01 M CaCl₂ + 0.1 M triethanolamine, adjusted to pH

Table 1. Sequential Extraction Procedure of Zn from Soils

step	fraction	extracting solution	experimental conditions
1	water soluble + exchangeable (WSEX)	10 mL of 1 M NH_4NO_3 (pH 7)	4 h at 25 °C
2	organically complexed (fulvic + humic) (OC)	30 mL of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ (pH 10)	20 h at 25 °C
3	easily reducible metal oxide bound (RMO)	20 mL of 0.1 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ in 0.01 M HNO_3	30 min at 25 °C
4	organically bound (organic) (OM)	5 mL of 30% H_2O_2 (pH 2), 3 mL of 0.02 M HNO_3 3 mL of 30% H_2O_2 (pH 2), 1 mL of 0.02 M HNO_3 cool, add 10 mL of 2 M NH_4NO_3 in 20% HNO_3	2 h at 85 °C 2 h at 85 °C 30 min at 25 °C
5	amorphous minerals colloids bound (AMC)	10 mL of 0.2 M $(\text{NH}_4)_2\text{C}_2\text{O}_4/0.2$ M $\text{H}_2\text{C}_2\text{O}_4$ (pH 3)	4 h at 25 °C (dark)
6	crystalline Fe oxide bound (crystalline) (CFeO)	25 mL of 0.2 M $(\text{NH}_4)_2\text{C}_2\text{O}_4/0.2$ M $\text{H}_2\text{C}_2\text{O}_4$ (pH 3) in 0.1 M ascorbic acid	30 min at 95 °C
7	Al-Si minerals bound (residual) (RES)	differences between the total Zn and the sum of the previous fractions	

7.3) (44), DTPA-AB (5 mM DTPA + 1.0 M NH_4HCO_3 , adjusted to pH 7.6) (45), 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) (46): the easily leachable Zn fraction was extracted with 0.01 M BaCl_2 (29).

Total soil Zn was determined after treating 2 g of dried samples with 14 mL of HNO_3 (65%) and 6 mL of HF (35%) followed by digestion in Teflon bombs in a microwave oven (three steps, maximum pressure of 690×10^3 Pa). The total Zn concentration in the original soil was 9.21 mg kg^{-1} . In all cases, Zn concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer, AAnalyst 700).

Analysis of Data. Correlation analysis and other statistical studies were performed with Statgraphics Plus software, version 5.1 (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 the Duncan's separations of means procedure. To establish statistical significance, a probability level of $P \leq 0.05$ was chosen.

RESULTS AND DISCUSSION

Zinc Leaching, pH and pe Parameters. In the greenhouse experiment, the applied Zn migrated through the soil and leached in amounts that varied according to the source of the Zn in question, the application rate, and the volume of leachate collected (Figure 1). In the control soil (with no Zn addition) the total amount of Zn in the 2 L of leachate collected during the 60 days experiment was 0.24 mg. Zinc concentrations in the different portions collected from the soils fertilized with Zn exhibited maximum levels in the first three portions (see Figure 1a). When Zn-EDTA and Zn-EDTA-HEDTA were applied at a rate of $10 \text{ mg of Zn kg}^{-1}$, an appreciable level of Zn leaching was observed until the eighth portion of the leachate. For the Zn-EDTA source, the Zn concentration in the leachate increased and reached a maximum level in the second (rate: $5 \text{ mg of Zn kg}^{-1}$, $5.6 \text{ mg of Zn L}^{-1}$) or third (rate: $10 \text{ mg of Zn kg}^{-1}$, $16.1 \text{ mg of Zn L}^{-1}$) portions and then declined; Zn-EDTA-HEDTA and Zn-S,S-EDDS (applied at a rate of $10 \text{ mg of Zn kg}^{-1}$) exhibited the greatest concentrations in the first leachate fraction (10.2 and $15.1 \text{ mg of Zn L}^{-1}$, respectively). These two values were greater than those obtained in the first leachate fraction of Zn-EDTA ($4.1 \text{ mg of Zn L}^{-1}$) at the same rate. The values for total Zn leached (milligrams per container) from the different sources (at the different rates expressed as a subindex) were ordered as follows: $\text{Zn-EDTA}_{10} > \text{Zn-EDTA-HEDTA}_{10} > \text{Zn-S,S-EDDS}_{10} > \text{Zn-EDTA}_5 > \text{Zn-EDTA-HEDTA}_5 > \text{Zn-HEDTA}_{10}$ (see Figure 1b; $P < 0.0001$). In the remaining cases, the amounts of Zn leached were very small (<1% of applied Zn) and did not significantly differ from the control. It should be noted that the leaching (and mobility) of Zn-EDTA-HEDTA must have been the result of the presence of EDTA because Zn-HEDTA did not leach.

These results can be partially explained by the relative stability of Zn complexes. Modaihsh (47) indicated that synthetic chelates (such as Zn-EDTA) are more stable than natural ones

(such as Zn-amino acids): they therefore maintain greater amounts of Zn in the soil solution, migrate through the soil profile, and are finally leached. In soil-column leaching studies, Alvarez et al. (48) found that Zn moved through the columns and was leached when Zn-EDTA was applied but remained near the top of the column when the source of the metal was zinc-lignosulfonate (a natural chelate). The results obtained in the present study for the synthetic chelate Zn-EDTA and Zn-PHP of natural origin confirm this tendency. Despite being synthetic chelates, leaching with Zn-HEDTA and Zn-EDDHSa was not significantly different from the control and, behavior was similar to that reported by Alvarez et al. (48) for the synthetic chelate Zn-EDDHA (zinc-ethylenediaminedi-*o*-hydroxyphenylacetate) in calcic Aquic Haploxeralf soil.

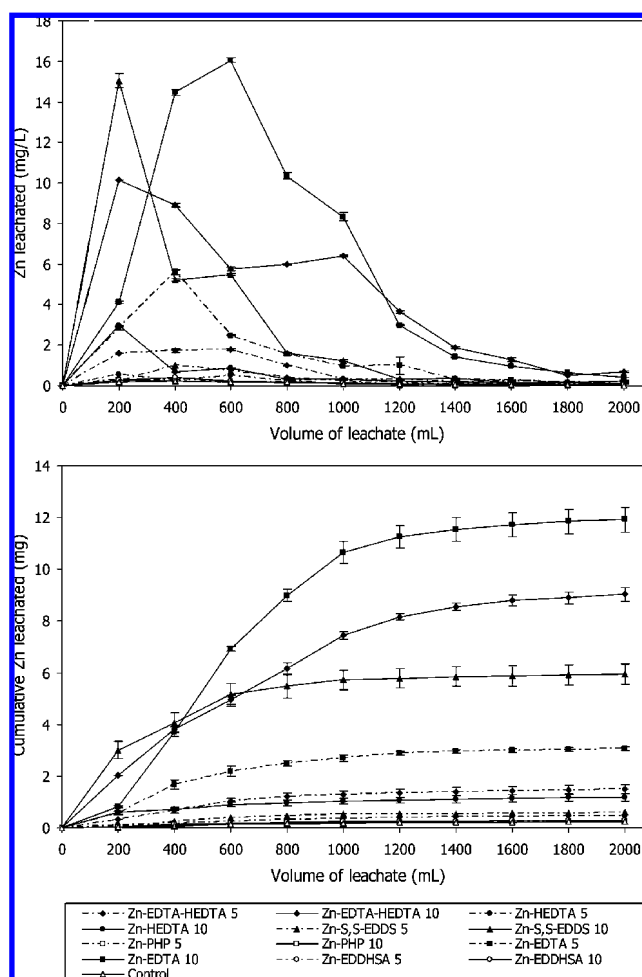


Figure 1. Concentrations (a, top) and cumulative amounts (b, bottom) of Zn in 10 leachate portions as a function of leachate collected from soils fertilized with Zn chelates (Zn-PHP = zinc polyhydroxyphenylcarboxylate). Vertical bars indicate the maximum standard error between treatments for each leachate portion.

Table 2. Response of Navy Bean to Rates and Forms of Zn Chelates in Soil^a

treatment	amount of Zn added (mg kg ⁻¹ soil)	dry matter content (g/container)	total Zn concn (mg kg ⁻¹ DM)	total Zn content (mg/container)	soluble Zn concn (mg kg ⁻¹ DM)
control	0	26.11 a	28.53 a	0.745	17.68 a
Zn-EDTA-HEDTA	5	26.17 a	130.3 c	3.410	58.44 cd
	10	26.11 a	208.9 e	5.455	76.40 f
Zn-HEDTA	5	27.23 a	139.5 c	3.799	60.92 c–e
	10	26.32 a	236.8 f	6.233	95.06 g
Zn-S,S-EDDS	5	26.08 a	132.1 c	3.445	55.35 c
	10	26.17 a	233.1 f	6.104	88.49 g
Zn-PHP ^b	5	26.09 a	98.86 b	2.579	33.12 b
	10	26.04 a	173.5 d	4.517	68.27 d–f
Zn-EDTA	5	31.46 b	129.1 c	4.062	71.35 ef
	10	30.84 b	221.4 ef	6.830	107.9 h
Zn-EDDHSa	5	31.45 b	106.4 b	3.346	42.39 b
	10	28.95 ab	219.9 ef	6.366	71.22 ef

^a Data are the mean value for three replications. Values in the same column were compared using a Duncan multiple range test at the 95% level. Homogeneous groups are denoted with the same letter. ^b Zn-PHP = zinc-polyhydroxyphenylcarboxylate.

In this work, it was observed that, despite its natural origin, Zn-S,S-EDDS was associated with considerable Zn movement through the soil and with appreciable Zn leaching. Tandy et al. (49) reported that, in a wet neutral soil, the agent EDDS extracted larger amounts of metal than the agent EDTA. Moreover, an extensive database compiled by Martell et al. (50) indicated that the log *K* value for the EDDS complexes with the most heavy metals are comparable to log *K* values for benchmark chelators such as EDTA, which could explain the large Zn concentration in the first leachate fraction that was obtained from the soil fertilized with Zn-EDDS. In addition, according to Hauser et al. (51), EDDS biodegraded more quickly than EDTA; this could explain the rapid decline in the concentration of Zn leached when the EDDS chelate was applied (see **Figure 1a**).

The soil selected for this study was weakly acidic with a sandy loam texture (10% clay; 40% smectite and 50% illite, which is associated with the retention of Zn), and there was only significant movement and a distribution of the micronutrient throughout the soil profile when the applied Zn chelates were highly stable (e.g., EDTA). Consequently, the Zn would have been readily available to plants but would also have tended to leach more readily, due to the greater stability of the complex. The relatively large amounts of Zn leached from Zn-EDTA and Zn-S,S-EDDS sources, the only ones that exhibited high levels of mobility in this soil, can lead not only in a loss of micronutrient but also to a potential risk of water contamination.

Electrochemical parameters, pH, and *E_h* were determined for all leached fractions. The mean value of the pH only varied from 6.37 for the first leached fraction to 5.45 for the tenth leached fraction, while *E_h* varied from 370 to 580 mV for these same fractions. The observed variations for *E_h* increased after 1 month of the experiment. This contrasted with what was observed with the Zn concentrations in the leachate, which exhibited smaller decreases for the same periods. Moreover, the values obtained were similar for all of the containers with Zn fertilizers and also for the control (in all cases urea fertilizer was added). According to Stumpe and Vlek(52) and Malhi et al. (53), the changes observed in pH and *E_h* values in the leachates could have been influenced by the evolution of the urea present in soil, bearing in mind that the resulting products are very soluble.

The same electrochemical parameters were also determined for container soils at two different crop times: 30 and 60 days for all treatments. The mean value of the pH only varied from 5.9 to 6.3, while *E_h* varied very little (from 650 to 640 mV).

This behavior contrasted with that observed in the leachates, possibly due to the effect of the added water washing away the acidic products and/or to the buffer capacity of the soil. In addition, the correlation between pH and pe [*p_e* = *E_h*(mV)/59.2] was negative and highly significant (*P* < 0.0001) for both the soil and leachate samples. At the end of the experiment, *p_e* had an average value of 10.85, which was classified as “normal” soil for this parameter (54). After 60 days, or during the 10th leachate, the pH and *p_e* parameters measured in the soil samples were correlated with the pH and *p_e* parameters determined in the leachate samples.

Dry Matter Yield and Total Zn Content in Plants. The dry matter yield, Zn concentration, and total Zn content [(dry matter yield) × (Zn concentration) × 10⁻³, milligrams per container] of 60-day-old navy bean plants are shown in **Table 2**. The increase in rate did not increase the dry matter yield with any of the Zn chelates. Statistical differences were found among fertilizers treatments (*P* < 0.001). The greatest increases in dry matter yield were associated with the application of the Zn-EDTA and Zn-EDDHSa chelates (20.5% with respect to the control for both sources at the rate of 10 mg of Zn kg⁻¹).

The Zn concentration in navy bean dry matter cultivated in control soil (no Zn addition) was 28.53 mg of Zn kg⁻¹. This value is outside the range considered normal in navy bean crop; between 30 and 60 mg of Zn kg⁻¹ (11). Beneficial effects were observed with the application of all Zn fertilizers: there were significant increases in Zn concentration and total Zn content in plant dry matter. All Zn treatments increased micronutrient concentrations to above 50 mg of Zn kg⁻¹ of dry matter. This Zn concentration is important for bean quality since it supposes a greater concentration in the seed, which is an important source of Zn nutrition for certain groups of human population and particularly for those with diets lacking in meat (e.g., vegetarians) who may be risk groups with regard to Zn deficiency (55). Furthermore, the Zn concentration of 50 mg kg⁻¹ is given by some authors (56, 57) as the minimum Zn concentration necessary for using this plant as feeding fodder for various animals. Contrary to what happened with dry matter yield, an increase in the rate applied produced an increase in Zn concentration in plant dry matter of approximately 93 mg of Zn kg⁻¹ (mean value for all Zn sources) when passing from the low to the high rate. Differences in the Zn concentrations in plant samples were observed between different fertilizer treatments (*P* < 0.001). The highest Zn concentrations were obtained by application of the high rate of the following fertilizers, Zn-HEDTA ≈ Zn-S,S-EDDS ≥ Zn-EDTA ≈ Zn-

EDDHSAs; and the lowest Zn concentrations, by application of Zn-PHP at the low rate. Zinc concentration in healthy leaves generally ranges from 15 to 100 mg kg⁻¹ (11); the values obtained were larger, especially at the rate of 10 mg of Zn kg⁻¹, but this did not cause any damage to the plants since their dry matter yield was not affected. These values would be convenient if the plants will be mixed with other components when preparing feeding fodders.

The highest total Zn contents were observed with the high Zn rates of the Zn-EDTA, Zn-EDDHSAs, Zn-HEDTA, and Zn-S,S-EDDS sources (9.2, 8.5, 8.4, and 8.2 times the level of the control treatment, respectively), and the lowest total Zn content was observed with Zn-PHP applied at the lowest rate (3.5 times the control treatment; see **Table 2**).

In a previous study with the same treatments, conducted on a Typic Calcixerept soil (58), it was also observed that the fertilizer treatments did not show any significant differences in dry matter production between navy bean crops grown in Zn-treated soils. It was also observed that applying organic Zn complexes to the soil increased the total Zn content in the plant. The lowest content was also associated with the low Zn rate of the Zn-PHP source, and the highest contents 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 then the two doses of the Zn-HEDTA product. In contrast, in the present study, which was conducted in a weakly acidic Typic Haploxeralf soil, the highest total Zn contents were associated with the application of the highest rate of Zn-HEDTA, Zn-S,S-EDDS, Zn-EDTA, and Zn-EDDHSAs sources: as previously indicated, there were only small differences between these Zn chelates.

The relative efficiency of fertilizers can be estimated by the percentage of their use by the crop (59). In this case, the percentage of Zn used by navy bean plants was defined in the following way:

$$\text{Zn used (\%)} = \left[\frac{\text{total Zn content (treatment)} - \text{total Zn content (control)}}{\text{Zn added}} \right] \times 100 \quad (1)$$

The utilization of Zn by the crop depended on the type of chelate used and the rate of application. For each fertilizer, application at the rate of 5 mg of Zn kg⁻¹ led to a percentage of used Zn that was bigger than when the rate of 10 mg of Zn kg⁻¹ was added. In this weakly acidic soil, the Zn-PHP source showed the lowest level of effectiveness (4.2%, at a rate of 5 mg of Zn kg⁻¹ and 4.0% at a rate of 10 mg of Zn kg⁻¹), and Zn-EDTA, Zn-EDDHSAs, Zn-HEDTA, and Zn-S,S-EDDS fertilizers showed similar levels of effectiveness (percent of Zn used between 5 and 6%).

However, in a previous study in a Typic Calcixerept soil (58), the most effective treatments were those involving both applied rates of Zn-EDTA (2.0 and 1.4%, for rates of 5 and 10 mg of Zn kg⁻¹, respectively) and Zn-HEDTA applied at a rate of 5 mg of Zn kg⁻¹ (1.4%), followed by the low rate of Zn-EDTA-HEDTA and Zn-EDDHSAs (1.2% for both sources). In contrast, the least effective treatments were those with Zn-S,S-EDDS (0.8 and 0.7%, at rates of 5 and 10 mg of Zn kg⁻¹, respectively) and with Zn-PHP (0.5 and 0.4%, at rates of 5 and 10 mg of Zn kg⁻¹, respectively). It is interesting to point out that soil properties (mainly pH, clay content, and carbonate content) condition the effectiveness of the Zn sources. A calcareous soil must be more demanding for the different Zn sources than an acidic soil, because the Zn-EDTA source showed the greatest effectiveness in the calcareous soil, while, in the acidic soil, four sources (including Zn-EDTA) presented similar levels of

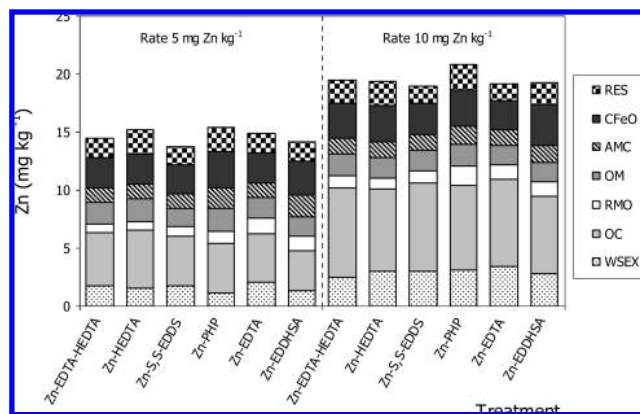


Figure 2. Zinc fractions in soil after navy bean harvest with different fertilizer treatments (rates 5 and 10 mg of Zn kg⁻¹) (Zn-PHP = zinc-polyhydroxyphenylcarboxylate).

effectiveness. Furthermore, the effectiveness of all the Zn chelates was also greater in the acidic soil than in the calcareous soil. The greater effectiveness of Zn chelates at the lower pH is due in part to the fact that Zn alone becomes more available in soils as pH declines. In fact, this may make Zn chelation less important for cropping as soil pH declines. This behavior could particularly occur when soil P levels are not high (1). The percentage of Zn used decreased in the calcareous soil with respect to the acidic soil for all the Zn sources, ranging from 89% for the high rate of Zn-PHP to 71% for the low rate of Zn-EDTA.

Distribution of Zinc Forms in Soil at Navy Bean Harvest.

The results for the distribution of Zn supplied by the fertilizers that remained in the soil at navy bean harvest are presented in **Figure 2**. In the control soil, the order (from highest to lowest) of Zn concentration (mg kg⁻¹) in the fractions was as follows: CFeO, crystalline Fe oxide bound (2.34); OC, organically complexed (1.99); RES, residual (1.81); OM, organically bound (1.36); AMC, amorphous minerals colloids bound (1.26); WSEX, water soluble plus exchangeable (0.67); and RMO, easily reducible metal oxide bound (0.52). In this case, the total Zn concentration was 0.74 mg kg⁻¹ greater than for the original soil (see Materials and Methods). This was probably due to the fact that the phosphoric fertilizer contained micronutrient impurities.

In treated soils, the average Zn concentrations (mg kg⁻¹) in the fractions decreased in the following order: OC (5.81), CFeO (2.85), WSEX (2.28), RES (1.83), OM (1.79), AMC (1.42), and RMO (1.09). The addition of Zn complexes produced a considerable increase in the Zn concentrations in the most labile fractions and particularly in the WSEX and OC fractions, which are the most potentially bioavailable and would therefore be very important for the Zn nutrition of a subsequent crop.

In this study the percentage of labile Zn (WSEX+OC) with respect to total Zn applied was calculated according to the following equation:

$$\text{labile Zn (\%)} = \left[\frac{\text{(WSEX+OC(treatment))} - \text{(WSEX+OC(control))}}{\text{Zn added}} \right] \times 100 \quad (2)$$

It was observed that when Zn-EDTA-HEDTA or Zn-HEDTA was applied (at both rates), approximately 75% of the applied Zn remained in the WSEX and OC fractions. In the case of Zn-S,S-EDDS and Zn-EDTA applied at the rate of 5 mg of Zn kg⁻¹, this percentage was lower (\approx 70%) and for the rate of 10 mg of Zn kg⁻¹, it was higher (\approx 80%). These Zn chelates present high-stability constants: Zn-EDTA (log *K* = 17.4), Zn-HEDTA

Table 3. DTPA-TEA-, DTPA-AB-, Mehlich-3-, and BaCl₂-Extractable Zn (mg kg⁻¹) in Soil after Navy Bean Harvest with Different Fertilizer Treatments^a

treatment	amount of Zn added (mg kg ⁻¹ soil)	amount of Zn added			
		DTPA-TEA	DTPA-AB	Mehlich-3	BaCl ₂
control	0	0.96 a	1.07 a	1.54 a	0.89 a
Zn-EDTA-HEDTA	5	4.07 c	4.78 bcd	5.69 cd	3.87 bcd
	10	10.03 f	10.91 f	11.36 f	7.67 g
Zn-HEDTA	5	5.07 d	5.50 cd	6.18 cd	4.03 cd
	10	7.34 e	8.34 e	8.63 e	6.07 ef
Zn-S,S-EDDS	5	5.00 d	5.11 cd	5.43 cd	4.03 cd
	10	10.27 f	10.46 f	10.92 f	7.03 fg
Zn-PHP ^b	5	3.01 b	3.14 b	3.45 b	2.73 b
	10	9.93 f	10.45 f	10.98 f	6.93 fg
Zn-EDTA	5	5.54 d	6.16 d	6.39 d	4.90 de
	10	10.70 f	11.28 f	11.69 f	8.07 g
Zn-EDDHSa	5	3.65 bc	3.86 bc	4.79 bc	3.27 bc
	10	7.39 e	8.30 e	8.60 e	5.37 e

^a Data are the mean value for three replications. Values in the same column were compared using a Duncan multiple range test at the 95% level. Homogeneous groups are denoted with the same letter. ^b Zn-PHP = zinc polyhydroxyphenyl-carboxylate.

(log $K = 15.35$) (both with ionic strengths of 0.01 M), and Zn-S,S-EDDS (log $K = 13.4$ with an ionic strength of 0.1 M) (50, 54). In contrast, the labile Zn percentages associated with Zn-PHP and Zn-EDDHSa were 55 and 42%, respectively, for the rate of 5 mg of Zn kg⁻¹ and 77 and 68%, respectively, for the rate of 10 mg of Zn kg⁻¹. A previous study involving a calcareous soil (58) showed that these percentages ranged from 59% for Zn-EDTA to 9% for Zn-PHP and Zn-HEDTA, all of which related to application rates of 5 mg of Zn kg⁻¹. As occurred with the percentages of Zn used, the percentages of labile Zn decreased for a calcareous soil with respect to the results obtained for this acidic soil for all Zn sources, with 89% for Zn-HEDTA (85% for Zn-PHP) applied at a rate of 10 mg kg⁻¹ and 17% for Zn-EDTA applied at a rate of 5 mg kg⁻¹.

A simple linear regression analysis between the different Zn fractions present in the soil only produced a highly significant and positive correlation between the WSEX and OC fractions ($P < 0.0001$, $r = 0.93$). Novillo et al. (60), also found a high correlation between WSEX and OC ($r = 0.71$, $P < 0.0001$) in acidic, neutral, and calcareous soils.

Zinc available in the soil was estimated by DTPA-TEA, DTPA-AB, and Mehlich-3 extractions (Table 3). In the control soil (no Zn addition), the Zn concentrations that were extracted by applying the three methods were approximately the same as the average critical concentrations for numerous crops (44, 45, 61). Considering that the Zn concentration in the navy bean dry matter cultivated in the control soil was lower than the range of values considered as normal, the application of Zn fertilizers should have a positive effect on successive crops.

In the fertilized soils, the quantities of available Zn that remained in the soil after harvesting would be sufficient for a subsequent crop since the lowest concentrations obtained were more than twice as large as the average critical concentrations.

Zinc extracted with BaCl₂ (easily leachable Zn) exhibited similar behavior to available Zn; however, the concentrations extracted were smaller than those obtained using the three methods for determining available Zn (see Table 3). This was because the BaCl₂ only extracts elements which are adsorbed on particles (62) while, in contrast, DTPA-TEA, DTPA-AB, and Mehlich-3 also extract more strongly retained Zn (28, 63). The amounts of Zn decreased in the soil extractions in the following order: Mehlich-3, DTPA-AB, DTPA-TEA, and BaCl₂.

Table 4. Simple Correlation Coefficients (R) for Relationships between Plant Parameters and DTPA-TEA-, DTPA-AB-, Mehlich-3-, and BaCl₂-Extractable Zn and Sequentially Extracted Zn Fractions

	dry matter	total Zn concn	soluble Zn MES ^a
WSEX	0.10	0.93 ^b	0.92 ^b
OC	-0.11	0.95 ^b	0.86 ^c
RMO	0.44	0.41	0.41
OM	-0.12	0.29	0.23
AMC	0.25	-0.17	-0.31
CFeO	-0.01	0.39	0.09
RES	-0.43	0.01	-0.17
DTPA-TEA	0.03	0.89 ^b	0.87 ^c
DTPA-AB	0.04	0.91 ^b	0.89 ^c
Mehlich-3	0.05	0.90 ^b	0.88 ^c
BaCl ₂	0.09	0.90 ^b	0.91 ^b

^a MES = 2-morpholinoethanesulfonic acid. ^b Significance at the 0.01% ($n = 13$). ^c Significance at the 0.1% level ($n = 13$).

In all cases, significant differences were found between the amounts of Zn extracted from the control and from the fertilized soils. The lowest available Zn concentrations occurred with the Zn-PHP treatment at the rate of 5 mg of Zn kg⁻¹. This was consistent with the lowest Zn concentrations in the most labile fractions, WSEX and OC, observed with this fertilizer. However, this was not observed when the treatment was applied at a rate of 10 mg kg⁻¹. It was also noticeable that despite the fact that the Zn-EDTA and Zn-S,S-EDDS sources produced the greatest quantities of leached Zn, they also produced high quantities of both, easily (potentially) leachable Zn and available Zn for subsequent crops.

Available and easily leachable Zn methods showed highly significant ($P < 0.0001$, $n = 13$) and positive correlations. The best regression equation obtained was

$$\text{Mehlich-3} = 0.60 + 0.98 \text{ DTPA-AB} \quad (r = 0.998) \quad (3)$$

All of these methods could therefore be used in a similar way to predict the availability of Zn for plants under the conditions of the experiment. Reed and Martens (28) and Lopez-Valdivia et al. (19) obtained similar correlations between Mehlich-3 and DTPA-TEA in acidic soils, and Vocasek and Friedericks (64) reported similar findings for soils with a wide range of chemical properties.

These four methods were also correlated with the most labile fractions (WSEX and OC), and r ranged from 0.94 to 0.96 ($P < 0.0001$), with the Zn associated with these fractions being the type most available to plants.

The relationships between plant parameters (dry matter, total Zn concentration, and soluble Zn concentration) and soil parameters (metal soil fractions, available Zn, and easily leachable Zn) were subject to correlation analysis (Table 4). Dry matter yield did not correlate with any form of Zn in the soil. However, a positive correlation (simple linear regression analysis) with a high level of significance ($P < 0.0001$) existed between the total Zn concentration in the plant and Zn concentrations in the WSEX and OC fractions, and DTPA-TEA, DTPA-AB, Mehlich-3, and BaCl₂ extracted Zn. These results indicated that the total Zn content in plant was influenced by these chemical forms of Zn in the soils and that, consequently, the effectiveness of organic Zn complexes in this parameter depended on their capacity to retain the applied micronutrient in these labile forms. Total Zn concentration and soluble Zn concentration in plant dry matter showed a high degree of correlation with soil available Zn extracted with DTPA-AB, according to the following equations:

$$\text{total Zn} = 36.90 + 17.56 \text{ Zn-DTPA-AB} \\ (r = 0.91, P < 0.0001) \quad (4)$$

$$\text{soluble Zn (MES)} = 19.47 + 6.64 \text{ Zn-DTPA-AB} \\ (r = 0.89, P < 0.001) \quad (5)$$

Furthermore, the concentration of soluble Zn extracted with the reactive MES and total plant Zn concentration exhibited similar behavior (see **Table 4**) and were related according to the following equation:

$$\text{soluble Zn (MES)} = 8.73 + 0.36 (\text{total Zn}) \\ (r = 0.920, P < 0.0001) \quad (6)$$

It would therefore be possible to use the approach to determine soluble Zn MES in navy bean dry matter presented in this study to diagnose the nutritional state of plants with respect to Zn content. Rahimi and Schropp (42) and Cakmak and Marschner (43) reported that the soluble form of Zn reflected the nutritional status of the corn and cotton plants better than total Zn.

It would also be possible to establish several mathematical equations that could be used to relate the parameters determined in the plant and soil. An equation would allow us to forecast the total Zn content (mg) in the navy bean plants as a function of the seven fractions sequentially extracted (milligrams of Zn per kilogram) from the soil ($P < 0.01$). An equation only considering the sum of the WSEX and the OC fractions would, however, be better:

$$\text{total Zn content} = -0.35 + 0.61 \text{ Zn-(WSEX+OC)} \\ (r = 0.94, P < 0.0001) \quad (7)$$

On the other hand, the pH, pe, and pH + pe parameters determined in this weakly acidic soil and in the leachate samples did not correlate significantly with any of the forms of Zn.

Finally, it is interesting to mention that Zn-EDTA-HEDTA, Zn-HEDTA, Zn-S,S-EDDS, Zn-EDTA, and Zn-EDDHSa fertilizers showed similar levels of effectiveness in this weakly acidic soil (between 5 and 6% of the Zn used) and that these levels were greater than those indicated in a previous study conducted in a calcareous soil (with a maximum value of 2.0% for Zn-EDTA). Furthermore, Zn-PHP was shown to be the least effective Zn source. The relatively large amounts of Zn leached in soils fertilized with Zn-EDTA or Zn-S,S-EDDS sources, and the similar levels of effectiveness as associated with other Zn sources, make it more recommendable to use Zn-HEDTA and Zn-EDDHSa sources at their lower application rates in this acidic soil. This would result in less water pollution and also in a reduction in the cost of the fertilization.

The addition of all the Zn complexes resulted in a considerable increase in Zn concentrations in the most labile fractions, while the effectiveness of organic-zinc complexes for total Zn content in the plant depended on their capacity to maintain soil Zn in its labile forms. All the fertilizer treatments produced noticeable increases (Zn concentration $> 50 \text{ mg of Zn kg}^{-1}$ of dry matter) in plant Zn contents. Furthermore, the three methods used to estimate available Zn could also be used in a similar way to predict Zn availability for plants. It should also be stressed that available Zn for subsequent crops was greater than the critical concentration in all Zn fertilized soils. The determination of soluble Zn MES in navy bean dry matter could be used to diagnose the nutritional state of Zn in the plant.

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