



Effects of synthetic Zn chelates on flax response and soil Zn status

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Abstract

Throughout the world, flax (*Linum usitatissimum* L.) is often grown in Zn-deficient soils, but appropriate fertilizer management can optimize both crop yield and micronutrient content. A greenhouse experiment was conducted on Typic Haploxeralf (pH 6.1) and Typic Calcixercept (pH 8.1) soils to study the relative efficiency of chelated Zn using two application rates of three different Zn sources [Zn-EDDHSA, ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate of Zn); Zn-HEDTA, N-2-hydroxyethyl-ethylenediaminetriacetate of Zn; and Zn-EDTA, ethylenediaminetetraacetate of Zn]. Dry matter /DM) yield, Zn concentration, chlorophyll content, crude fiber and tensile properties were monitored and the soil-Zn status (available-Zn, Zn-fractions and total-Zn) was assessed. Zinc chelate applications increased the most labile forms of Zn in soils and Zn concentrations in plants. The low rate of Zn generally had a beneficial effect on DM yield and tensile properties. The exception was Zn-EDTA in the weakly acidic soil, where the highest Zn concentrations were observed in leaves and whole shoots; this coincided with the largest concentrations of labile Zn in soil. The most efficient fertilizers were Zn-EDDHSA (in both soils) and Zn-EDTA (in the calcareous soil). The relatively large amounts of labile and available Zn present in both of the soils fertilized with Zn-EDTA points to the applying this chelate at lower rate than 5 mg Zn/kg; this should, in turn, reduce the cost of Zn fertilization and minimize environmental pollution risk.

Additional key words: available Zn; crude fiber; fertilizer; soil Zn speciation; tensile properties.

Abbreviations used: AAS (atomic absorption spectrometry); AB (ammonium bicarbonate); AMOX (amorphous Fe oxide bound Zn); CAR (carbonate bound Zn); CRYOX (crystalline Fe oxide bound Zn); DM (dry matter); DTPA (diethylenetriaminepentaacetate); EDDHSA [ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate)]; EDTA (ethylenediaminetetraacetate); Eh (redox potential); EXC (exchangeable Zn); FM (fresh matter); HEDTA (N-2-hydroxyethyl-ethylenediaminetriacetate); MES (2-(N-morpholino)ethanesulfonic acid); MnOX (Mn oxide bound Zn); OM (organic material and sulfide –oxidizable– bound Zn); RES (residual Zn); TEA (triethanolamine); WS (water soluble Zn).

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Introduction

Flax is an economically important crop which is grown for its fiber and oil (Herdrich, 2001; Mohanty *et al.*, 2005). This plant is also a Zn-deficiency sensitive species with a relatively high Zn requirement (Lonergan, 1951; Moraghan, 1980; Jiao *et al.*, 2007; Storey, 2007). Zinc deficiency is a common, and often important, problem in flax fields throughout the world and especially in well-drained, sandy, acidic soils and in soils that have developed on calcareous rocks (Jeffery & Uren, 1983; Adriano, 2001). In these soils, crop

yields are often reduced due to Zn deficiency (Alloway, 2010). Moraghan (1978) reported that Zn deficiency is often related to a condition known as “chlorotic die-back”. Plants affected by Zn deficiency tend to be pale in color, may sprout new shoots from their lower nodes, often form a type of candelabra appearance, and may suffer delayed maturity.

Although Zn sulphate is the fertilizer most frequently used to correct Zn deficiency, Zn chelates are the most effective sources of Zn for numerous crops, such as maize (*Zea mays* L.), navy beans (*Phaseolus vulgaris* L.), lettuce (*Lactuca sativa* L.) and others main-

ly grown in calcareous soils (Alvarez & Gonzalez, 2006; Gonzalez *et al.*, 2007, 2008). The phytoavailability of Zn depends on soil properties (pH, carbonate content, CEC, organic matter, Fe and Mn oxides, redox conditions-Eh), the nature of the plant, and microbial activity in the rhizosphere (Alloway, 2010). Foliar applications of Zn fertilizers can rapidly correct severe deficiencies, but only offer temporary solutions to the problem. In contrast, soil applications of Zn fertilizers are cheaper and their effects are longer lasting (residual effect). However, in soils with certain characteristics, such as high pH and carbonate content, most of the Zn applied will become unavailable for plant uptake with over time.

Recent studies have suggested that the efficiency of Zn-chelates in sensitive plants such as flax may be associated with either an increased acquisition of this micronutrient from the soil (uptake efficiency) or with an improved utilization of Zn by the plant (utilization efficiency) (Sattelmacher *et al.*, 1994).

A number of researchers have sought to establish a relation between the distribution of Zn and P, Fe, Ca, Mg, Cd, and chlorophyll contents in flax, as well as with a number of disorders of an apparent nutritional origin that often retards the growth of flax plants (Lee *et al.*, 1969; Spratt & Smid, 1978; Moraghan, 1993; Grant *et al.*, 2000; Jiao *et al.*, 2004). Nofal *et al.* (2011) reported that an increase in the Zn fertilization applied to a flax crop caused significant increases in growth, fiber yield, seed yield, and also in length and quality of fiber.

Several cases of Zn toxicity have been reported, affecting various species including: lettuce, onion (*Allium cepa* L.), spinach (*Spinacia oleracea* L.) and maize (*Zea mays* L.) (Vitosh *et al.*, 1994; Kabata & Mukherjee, 2007). According to Paschke *et al.* (2006), toxic levels of Zn in plants may be a result of Zn applications.

Fertilizer management may offer a cost-effective way of meeting crop requirements since appropriate fertilizer management can optimize both crop yield and crop micronutrient content (Chandi & Takkar, 1982; Jiao *et al.*, 2007). More information is therefore needed to determine how Zn fertilizers influence both crop yield and crop micronutrient concentrations, and also the mechanical properties of plants such as flax (Alvarez, 2010). One approach for estimating Zn availability to plants is to use single extractions as with the DTPA-ammonium bicarbonate (DTPA-AB) method (Soltanpour, 1991); this tends to correlate well with metal concentrations in plants (Adriano, 2001; Alvarez, 2007). In addition, according to Schultz *et al.* (2004) the easily leachable Zn could be estimated by the BaCl₂ reagent. Another approach for diagnosing soil-Zn sta-

tus is to use a speciation method (Shuman, 1998). This diagnosis can be used to evaluate what would constitute the most favorable distribution of Zn sources in soil in terms of plant Zn nutrition. Various authors have reported that applying stable organic-Zn fertilizers to soils has a significant effect on Zn content in the most labile and available Zn pools and could have important implications for the nutrition of any subsequent crop (Gonzalez *et al.*, 2008; Alvarez *et al.*, 2009). The effectiveness of different chelating agents (such as EDTA, HEDTA and EDDHSA) as metal carriers in soils depends on their capacity to maintain the metal in its soluble form. The metal displacement within the metal-chelate by other cations from the soil (such as Ca²⁺ or Fe³⁺), with the subsequent metal precipitation and the fixation of either the organic chelate molecule or the free metallic cation on clay colloids, produces a reduction in their effectiveness (Aboulroos, 1981; Shaheen *et al.*, 2013).

To date, only a few Zn-efficient chelates have been compared, and in only a few plant species (Prasad & Sinha, 1981; Paschke *et al.*, 2006; Gonzalez *et al.*, 2007; Alloway, 2010). On the other hand, numerous Zn fertilizers are currently available to farmers, but to select the most suitable fertilizer it is necessary to have more information about their effectiveness. According to Kabata (2004), plant responses to trace elements in the soil can vary and should always be investigated with respect to particular soil-plant systems.

The aims of the present study were to: (i) examine the responses of textile quality flax, including crude fiber content, tensile properties such as tensile strength, Young's modulus and elongation at break, to applications of synthetic Zn chelates; (ii) establish a relationship between plant response and the soil-Zn distribution; and (iii) determine differences among the efficiencies (uptake and utilization) of the Zn application rates when Zn chelates are applied to a weakly acidic soil and to a calcareous soil.

Material and methods

The soils used in this study were surface horizons (A_p horizon) and came from the central region of Spain (Soil I: 40°17' N, 4°01' W; and Soil II: 40°39' N, 3°19' W). Soil I was classified as a Typic Haploxeralf and Soil II as a Typic Calcixerpt (Soil Survey Staff, 2010). Samples of the soils were air-dried and passed through a 2 mm sieve. The sieved fraction was then used in this study. The results of soil analysis are expressed on a dry weight basis (d.w.). The main properties of Soils I and II were (respectively): clay, 100 and 180 g/kg (hydrometric method: Day, 1965); predominant clays: illite and

smectite (X-ray diffraction technique: Schultz, 1964); water-holding capacity at 33 kPa, 66.0 and 205 g H₂O/kg (Richard's membrane-plate extractor: Klute, 1996); pH_w (1:2.5, w/v: soil:water ratio), 6.1 and 8.1; electrical conductivity, 37.2 and 178 µS/cm (1:2.5, w/v) (Chapman & Pratt, 1961); redox potential, 460 and 380 mV (1:2.5, w/v), respectively; oxidizable organic matter, 5.00 and 12.9 g/kg (Walkley-Black procedure: Hesse, 1971); total N, 1.00 and 1.10 g/kg (Kjeldhal digestion: Bremner, 1996); available P, 19.9 and 12.6 mg/kg (Olsen *et al.*, 1954); cation exchange capacity, 4.7 and 23.5 cmol_e/kg (sodium acetate and ammonium acetate extraction procedures: Bower *et al.*, 1952); and Fe (active Fe₂O₃), 141 and 56 mg/kg (dithionite and oxalate extraction method: McKeague & Day, 1966). The total and free carbonate contents measured in Soil II were 13.4 and 3.30% (volumetric methods: Allison & Moodie, 1965; Nijensohn & Pizarro, 1960), respectively. The values presented are means for three replicates.

The liquid fertilizers used were: Zn-EDDHA [Zn-ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate), 21.4 g water-soluble-Zn/L, mass density $\rho = 1.26 \text{ g/cm}^3$, $\log K_c^{0.01} \text{ Zn-EDDHA} \approx 17.4$; Lucena *et al.*, 2005], Zn-HEDTA (Zn-N-2-hydroxyethyl-ethylenediaminetriacetate, 88.3 g water-soluble-Zn/L, $\rho = 1.26 \text{ g/cm}^3$, $\log K_c^{0.01} \text{ Zn-HEDTA} = 15.3$; Lindsay, 1979), Zn-EDTA (Zn-ethylenediaminetetraacetate, 100.0 g water-soluble-Zn/L, $\rho = 1.38 \text{ g/cm}^3$, $\log K_c^{0.01} \text{ Zn-EDTA} = 17.4$; Lindsay, 1979). These three fertilizers are marketed by several different companies (Dabeer, Vegetal Nutrition, Agricultural Atlantic; Spain).

Air-dried soil (14 kg) was placed in polyethylene containers (each with a capacity of 15 L, an internal diameter of 26.5 cm and a height of 25 cm) and kept in a greenhouse. The soil was fertilized with: (i) 100 mg N/kg, which was applied in two separate doses (the first at sowing and the second 45 d after sowing) in the form of urea [(NH₂)₂CO]; (ii) 120 mg P/kg, in the form of KH₂PO₄; and (iii) 150 mg K/kg, in the form of KH₂PO₄ and K₂SO₄. The plant used in this study was the textile-producing cultivar of flax NATASJA (AGROSA, Guadalajara, Spain). One hundred and twenty flax seeds were sown in each container at a depth of 3 cm and after germination seedlings were removed so that only 40 seedlings were left in each container. The control treatment (with no added Zn) and the fertilizer treatments 5 and 10 mg Zn/kg soil (low and high rate, respectively) were replicated 3 times for each soil according to a randomized complete block design (total number of containers: 42). The quantities of Zn added in the experiment were checked three times for each treatment using atomic absorption spectrometry (AAS) (Perkin-Elmer AAnalyst 700). These application rates were selected after considering

the manufacturers' recommendations for different Zn fertilizers. The soils were irrigated with appropriate amounts of potable water to achieve (and/or approximately maintain) conditions equivalent to 75% of field capacity. To evaluate evapotranspiration, the containers were weighed (balance A&D Instruments Ltd., UK, model FG-30 KBM) and the volume of irrigation water required was determined. The experiment was conducted without any leaching. The greenhouse temperature ranged from 10 to 32°C and the relative air humidity ranged from 60 to 85%. The experiment was performed in spring (from 19th March to 17th June) with high natural light intensities and the plants reached heights of approximately 90 and 80 cm, respectively, in Soils I and II.

Soil analysis

The total-Zn concentration in the soils was determined by digestion with HNO₃ (65%) and HF (48%) in Teflon vessels in a microwave oven (CEM Corporation, model-Mars, Matthews, NC, USA); the values obtained for the two original soils were 10.0 and 44.3 mg/kg d.w., respectively.

The concentration of Zn available for plants (mg/kg d.w.) in the soil was determined with DTPA-AB (Soltanpour, 1991). Easily leachable Zn was extracted with the 0.01 mol/L BaCl₂ reagent according to Schultz *et al.* (2004) [the supernatant was filtered by vacuum pump, with a 0.45 µm cellulose acetate membrane filter (Albet 47BL, Barcelona, Spain)]. Soil pH, redox potential [E_h , $p_e = E_h(\text{mV})/59.2$] (ISO 11271, 2002) and electrical conductivity were measured in deionized water at a 1:2.5 (w:v) soil:water ratio. The pH and E_h parameters were also measured for all the different soil treatments in a saturated paste at two different times: 45 d (half-way point) and 90 d (end of experiment) after seeding.

The fractionation of Zn in the soil was performed according to techniques previously proposed by other authors, with only slight modifications (Alvarez, 2010). The Zn fractions were sequentially determined in seven steps for Soil I (carbonate-bound Zn fraction not suitable for non calcareous soil) or in eight steps for Soil II (calcareous) using the following extractants:

- WS: deionized water (water soluble Zn);
- EXC: 1 mol/L Mg(NO₃)₂ (pH 5) (exchangeable Zn);
- CAR: 1 mol/L NaCOOCH₃ (carbonate bound Zn);
- MnOX: 0.1 mol/L NH₂OH·HCl (Mn oxide bound Zn);
- AMOX: 0.2 mol/L (NH₄)₂C₂O₄·H₂O + 0.2 mol/L H₂C₂O₄ (pH 3) (amorphous Fe oxide bound Zn);

- CRYOX: (i) CB: $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ (78.4 g/L) + NaHCO_3 (9.82 g/L) and (ii) $\text{Na}_2\text{S}_2\text{O}_4$ (pH 7) (crystalline Fe oxide bound Zn);
- OM: (i) 0.02 mol/L HNO_3 – 35% H_2O_2 and (ii) 3.2 mol/L $\text{NH}_4\text{COOCH}_3$ (20% v/v HNO_3) (organic material and sulfide –oxidizable– bound Zn);
- RES: residual Zn).

The concentration of residual Zn was calculated by subtracting the other fractions from total Zn. All the Zn concentrations were determined by AAS. The increases with respect to the control in the percentages of Zn with respect to total Zn of the most labile Zn fractions was calculated as:

$$\left(\frac{[\text{Zn-Fraction}]_{\text{Treatment}}}{[\text{Zn-Total}]_{\text{Treatment}}} \times 100 \right) - \left(\frac{[\text{Zn-Fraction}]_{\text{Control}}}{[\text{Zn-Total}]_{\text{Control}}} \times 100 \right) \quad [1]$$

Plant analysis

While the flax was growing in the containers (just before harvest), leaf samples were collected to analyze both the soluble Zn concentration in fresh matter (FM), by means of an extraction with 1 mmol/L MES reagent [2-(*N*-morpholino)ethanesulfonic acid] (at pH = 6.0) (Cakmak & Marschner, 1987; Alvarez, 2010), and the chlorophyll content (AOAC 942.04, 1990). Soluble Zn in FM was determined after collecting 0.5 g of leaves from between leaf layers seven and fifteen in the upper part of the plant. These leaves were macerated in a mortar with 10 mL MES reagent for 5 min. The resulting suspension was then centrifuged (10,000 rpm for 15 min) and filtered through Whatman filter paper No. 41, and Zn was subsequently determined in the solution. The concentration of Zn in the extracted solutions was determined by AAS, and the level of absorbance (A) at each wavelength was measured in a UV-1603 spectrometer (Shimadzu). “Perkin-Elmer Pure” standard checks were used for the Quality Assurance System (certified by NIST-SRM). Standard solutions of Zn were prepared for each extraction in a background solution of the extracting agents.

Ninety days after sowing, and just before seed development, the plants were cut at soil level, washed twice with deionized water, air dried, placed into paper bags and then dried to a constant weight in a forced-draft oven at 60°C. Stems and leaves were separated, weighed and stored in sealed containers for later analysis (including the determination of total Zn content in both tissues and the determination of the plant’s mechanical properties). Subsamples of stems and leaves were subjected to wet digestion in a microwave oven using an acid mixture (HNO_3 and HF). The suspensions were filtered with Whatman n° 41. Zinc concentrations were determined by AAS. Total Zn concentration in

whole shoots was calculated considering dry matter (DM) yield and concentration in stems and leaves. The crude fiber content (% in DM) was determined by the Weende method (AOAC 985.29, 1990), using Fibertec System M2 equipment (Tecator, Höganäs, Sweden).

Plant mechanical properties

Plant mechanical properties including tensile strength, Young’s modulus and elongation at break, were also determined in stored plants. Five stems from each pot were cut to similar lengths (a 12.0 cm length was clipped from the middle of the stem) and their diameters were measured with a slide gauge; they ranged from 1.0 to 2.4 mm. Tensile tests were carried out using an instrument for testing different materials (Texture Analyzer XT2) and tensile properties were determined using Texture Expert Software (Texture Technol. Corp., Scarsdale, NY).

Statistical analysis

Multifactor analyses of variance were performed for all the parameters studied in order to determine the main effects of fertilizer treatment (Zn source \times Zn rate) and experimental repetition. A least significant difference value [LSD ($p \leq 0.05$)] was calculated in order to make comparisons between the six Zn fertilizer treatments considered and the control. All the analyses were performed using Statgraphics Plus-5.1 software (Manugistic Inc., Rockville, MD, USA).

Results

Soil zinc status

The concentrations of Zn extracted from the soils by multi-element extraction methods at the time of the flax harvest are shown in Table 1.

In general, in the soils treated with Zn, the orders of Zn distribution (the mean values of the Zn concentration for all the fertilizers and Zn rates applied) were as follows (mg/kg):

- Soil I: EXC (4.34), RES (3.83), MnOX (3.00), OM (1.68), AMOX (1.43), CRYOX (0.97) and WS (0.92).
- Soil II: RES (37.2), OM (4.86), AMOX (2.84), CAR (2.30), CRYOX (1.80), WS (0.77), MnOX (0.68) and EXC (0.47).

In the weakly acidic soil (soil I), the exchangeable Zn fraction contained a larger amount of Zn than in the

Table 1. Concentration of Zn fractions, and DTPA-ammonium bicarbonate- and BaCl₂-extractable Zn (expressed as mg Zn/kg dry soil) in Soil I (weakly acidic) and Soil II (calcareous) with the different fertilizer treatments at the moment of flax harvest.

Treatment ^[1]	Zn rate (mg/kg)	WS	EXC	CAR	MnOX	AMOX	CRYOX	OM	RES	Total	DTPA-AB	BaCl ₂
Soil I												
Control	0	0.21 a	1.13 a	—	1.13 a	0.97 a	0.80 a	1.50 a	4.21 ab	9.95 a	1.20 a	1.07 a
Zn-EDDHSA	5	0.50 b	3.39 b	—	2.81 b	1.47 b	0.83 ab	1.59 a-c	3.98 ab	14.56 b	4.49 b	2.70 bc
	10	1.23 d	4.87 cd	—	3.07 b	1.48 b	1.07 a-c	1.75 cd	5.42 b	18.88 c	7.63 c	3.93 d
Zn-HEDTA	5	0.61 b	3.53 b	—	2.26 b	1.13 a	0.93 a-c	1.53 ab	4.63 ab	14.63 b	3.66 b	2.43 b
	10	0.98 c	5.77 d	—	4.37 c	1.63 bc	1.17 c	1.72 b-d	4.38 ab	20.01 c	9.00 c	5.38 e
Zn-EDTA	5	0.93 c	4.69 c	—	3.13 b	1.56 bc	0.97 a-c	1.82 d	2.12 a	15.22 b	8.03 c	3.75 cd
	10	1.97 e	7.01 e	—	4.26 c	1.77 c	1.13 bc	1.84 d	1.97 a	19.96 c	13.03 d	8.05 f
<i>LSD</i> _{0.05}		0.24	1.00	—	0.89	0.29	0.30	0.21	3.21	2.41	1.92	1.11
Soil II												
Control	0	0.10 a	0.28 a	0.69 a	0.38 a	1.23 a	1.53 a	3.85 a	36.05 ab	44.12 a	0.45 a	0.13 a
Zn-EDDHSA	5	0.13 a	0.37 ab	1.91 b	0.53 ab	2.31 bc	1.61 a	4.85 ab	38.82 b	50.53 bc	1.81 b	0.23 a
	10	0.27 a	0.46 b	3.20 d	1.07 c	3.87 e	2.10 bc	5.05 ab	38.59 b	54.61 c	4.43 d	0.37 a
Zn-HEDTA	5	0.17 a	0.43 b	1.58 b	0.47 ab	2.03 b	1.70 a	4.79 ab	37.84 b	49.00 b	1.70 b	0.27 a
	10	0.41 a	0.48 b	2.43 c	0.63 b	2.83 cd	1.73 ab	5.57 b	40.54 b	54.63 c	3.29 c	0.43 a
Zn-EDTA	5	1.13 b	0.47 b	2.41 c	0.61 ab	3.03 d	1.73 ab	4.35 ab	35.63 ab	49.36 b	4.00 cd	1.14 b
	10	3.17 c	0.81 c	3.87 e	1.10 c	4.61 f	2.17 c	5.59 b	32.72 a	54.03 c	8.60 e	2.78 c
<i>LSD</i> _{0.05}		0.42	0.11	0.35	0.24	0.72	0.39	1.34	5.05	4.11	0.96	0.33

^[1] EDDHSA, ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate); HEDTA, *N*-2-hydroxyethyl-ethylene-diaminetriacetate; EDTA, ethylenediaminetetraacetate. DTPA-ammonium bicarbonate- (DTPA-AB) and BaCl₂-extractable Zn. Zn fractions are: water soluble (WS), exchangeable (EXC), carbonate bound (CAR), Mn oxide bound (MnOX), amorphous Fe oxide bound (AMOX), crystalline Fe oxide bound (CRYOX), organic material and sulfide-oxidizable-bound (OM) and residual (RES). Mean values within a column were compared using a LSD multiple range test at the 95% level. Homogeneous groups are denoted with the same letter.

others (illite was the predominant clay in this soil; see soil characteristics). On the other hand, in the calcareous soil (soil II), most of the Zn was present as the residual fraction. This was the fraction most closely associated with the mineral portion and most related to aluminosilicate minerals; in other words, it was associated with mineral lattices.

The addition of Zn chelates to the two soils produced different increases in each of the different Zn fractions. Furthermore, the distribution of Zn fractions in soils depended on the type of Zn chelate used for each Zn application rate.

In Soil I, the higher concentrations of Zn in the WS fraction were produced for the treatments Zn-EDTA 10 (9.4 times the control) followed by Zn-EDDHSA 10 (5.9), Zn-HEDTA 10 (4.7) and Zn-EDTA 5 (4.4). For the EXC fraction, the order was similar but with Zn-EDDHSA 10 and Zn-HEDTA 10 exchanging places. With respect to the MnOX and AMOX fractions, Zn-EDTA 10 and Zn-HEDTA 10 produced similar concentrations and higher than that obtained by Zn-EDDHSA 10.

In Soil II, the differences between the effects produced by the different chelates were higher than in soil

I. Only the Zn-EDTA chelate produced significant increases in the concentration of Zn in the WS fraction with respect to the control (31.7 and 11.3 times the control for the rates 10 and 5 mg Zn/kg soil, respectively). With respect to the EXC and CAR fractions, Zn-EDTA 10 also produced the highest Zn concentrations, but followed by Zn-EDDHSA 10, Zn-HEDTA 10 and Zn-EDTA 5 for CAR fraction. In the most residual fraction, the differences between chelates were lower.

In Table 2, which shows the increases with respect to the control in the percentages of Zn with respect to total Zn of the most labile Zn fractions (Eq. [1]), we can observe more clearly the differences listed above.

In Soil I, the Zn chelates produced significant increases in the percentages of Zn associated with the more labile fractions with respect to the control; for example, the increases for WS-Zn (water-soluble Zn) ranged from 1.32% for Zn-EDDHSA 5 to 7.76% for Zn-EDTA 10, while those for EXC-Zn (exchangeable Zn) ranged from 11.93% to 23.76% for the same treatments, respectively. In soil II, only the Zn-EDTA chelate produced significant increases (2.06% and 5.64%

Table 2. Increases (with respect to the control)^[1] in the percentages of Zn (with respect to total Zn) of the most labile Zn fractions^[2] in Soil I (weakly acidic) and Soil II (calcareous) at the moment of flax harvest.

Treatment ^[3]	Zn rate (mg/kg)	Soil I			Soil II			
		WS	EXC	MnOX	WS	EXC	CAR	MnOX
Zn-EDDHSA	5	1.32 a	11.93 a	7.94 a	0.03 a	0.10 a	2.22 a	0.19 a
	10	4.40 c	14.44 a	4.90 a	0.27 a	0.21 a	4.30 c	1.10 b
Zn-HEDTA	5	2.06 ab	12.77 a	4.09 a	0.12 a	0.24 a	1.66 a	0.10 a
	10	2.79 a-c	17.48 ab	10.48 a	0.52 a	0.24 a	2.88 b	0.29 a
Zn-EDTA	5	4.00 bc	19.46 ab	9.21 a	2.06 b	0.32 a	3.32 b	0.37 a
	10	7.76 d	23.76 b	9.99 a	5.64 c	0.86 b	5.60 d	1.17 b
<i>LSD</i> _{0.05}		2.03	9.26	7.50	0.89	0.23	0.71	0.49

^[1]Increase: see Eq. [1]. ^[2,3]See Table 1. Data are the mean value for three replications. Values within a column were compared using a LSD multiple range test at the 95% level. Homogeneous groups are denoted with the same letter.

for the 5 and the 10 mg Zn/kg, respectively) for the WS fraction. The Zn-EDTA 10 also produced the highest increase in the EXC and CAR fraction, while Zn-EDDHSA 10 produced the second highest increase in the CAR fraction and the highest increase with Zn-EDTA 10 in the MnOX fraction.

According to Soltanpour (1991) and Brennan *et al.* (1993), the available Zn concentration (DTPA-AB extractable Zn) in the control treatment for the soil I was close to the critical level for most crops, while that in the control treatment for the soil II was lower than that established as the critical level for crops (0.5-1.0 mg Zn/kg). In contrast, the available Zn concentrations obtained for all the Zn treatments applied to the two soils were higher than for the control (Table 1). The values for DTPA-AB-extractable Zn for the sources applied at 10 mg Zn/kg in Soil I were between 3.3 and 10.9 times greater than in the control and the order was Zn-EDTA 10 > Zn-HEDTA 10 ≈ Zn-EDDHSA 10 ≈ Zn-EDTA 5 > Zn-HEDTA 5 ≈ Zn-EDDHSA 5 > Control, while in Soil II they were from 3.8 to 19.1 times greater than in the control and the order was Zn-EDTA 10 > Zn-EDDHSA 10 ≥ Zn-EDTA 5 ≥ Zn-HEDTA 10 > Zn-EDDHSA 5 ≈ Zn-HEDTA 5 > Control. The Zn concentrations in the calcareous soil (II) were also lower than in Soil I (weakly acidic). Similar behavior was observed with BaCl₂-extractable Zn, but the Zn concentrations estimated as leachable were even lower. The concentrations obtained for this extraction were from 3.7 to 7.5 times greater than the control value in Soil I and from 2.9 to 21.4 times greater in Soil II.

The different Zn treatments did not produce differences in soil pH and pe parameters for a given soil at any of the experimental time points (data not shown). However, there were significant differences $p < 0.001$; $n=84$) between soils for pH and pe parameters and also

between experimental time points for pH. The mean pH values were 6.10 and 7.68 for soils I and II, respectively; and the mean pe values were 9.41 and 8.02 for soils I and II, respectively. Furthermore, the mean pH decreased with time from 7.22 at 45 d to 6.55 at 90 d. On the other hand, at the end of the experiment (90 d), the mean pH value was 5.55 for soil I with values ranging between 5.25 for Zn-EDTA 10 and 5.75 for Zn-EDDHSA 5. For soil II, the mean pH value was 7.55 with values ranging between 7.44 for Zn-HEDTA 10 and 7.67 for Zn-EDDHSA 5. With respect to the pe parameter, the mean value was 9.53 for soil I with values ranging between 9.79 for Zn-HEDTA 10 and 9.44 for Zn-EDTA 10. For soil II, the mean pe value was 8.10 with values ranging between 8.33 for Zn-EDTA 10 and 7.88 for Zn-EDDHSA 10.

Crop response

The response of flax to Zn fertilization with respect to DM yield and, total and soluble-Zn concentration in the plants grown in Soils I and II is shown in Table 3. In general, the values obtained for these parameters were greater for Soil I than for Soil II, but the behavior of the different Zn treatments differed for DM yield and Zn concentrations. In Soil I, only the low application rate of Zn-HEDTA and the high application rate of Zn-EDDHA produced significant increases in yield, with values that were, respectively, 1.4 and 1.3 times greater than that of the control treatment (with no added Zn). In contrast, the high application rate of Zn-EDTA chelate produced a significant decrease in yield, with a value that was approximately only half as high as those observed in the control. This Zn source was associated with the highest total-Zn concentration re-

Table 3. Effect of Zn fertilization with 0, 5 and 10 mg Zn/kg dry soil as Zn-EDDHSA, Zn-HEDTA and Zn-EDTA in the response of the flax crop in Soil I (weakly acidic) and Soil II (calcareous).

Treatment ^[1]	Zn rate (mg/kg)	Soil I			Soil II		
		DM yield (g/pot)	Total Zn conc. DM of whole shoots (mg/kg)	Soluble Zn conc. FM of leaves (mg/kg)	DM yield (g/pot)	Total Zn conc. DM of whole shoots (mg/kg)	Soluble Zn conc. FM of leaves (mg/kg)
Control	0	19.6 bc	42.7 a	6.37 a	12.6 a	23.0 a	4.46 a
Zn-EDDHSA	5	23.7 ce	102 bc	17.3 b	15.9 bc	85.7 c	19.6 cd
	10	26.2 de	143 de	25.9 c	17.8 c	117 d	26.1 e
Zn-HEDTA	5	27.5 e	85.4 b	13.8 ab	15.8 bc	62.1 b	11.8 b
	10	23.0 cd	122 cd	21.5 bc	16.6 bc	100 cd	18.1 c
Zn-EDTA	5	16.6 b	149 e	41.3 d	14.4 ab	103 cd	23.1 de
	10	10.9 a	319 f	86.6 e	14.8 a-c	137 e	43.9 f
<i>LSD</i> _{0.05}		4.3	26.7	7.7	3.1	17.8	4.5

[1] See Table 1. DM = dry matter. FM = fresh matter. Data are the mean value for three replications. Values within a column were compared using a LSD multiple range test at the 95% level. Homogeneous groups are denoted with the same letter.

corded in whole shoots (7.5 times greater than the observed in the control). As usual, the soluble-Zn concentrations in FM corresponding to leaves were smaller than the total-Zn concentrations found in whole shoots; even so, they followed a similar trend. As a result, the Zn-EDTA 10 treatment produced a concentration 13.6 times greater than in the control.

In Soil II, none of the Zn treatments produced a decrease in DM yield; the high rate of Zn-EDDHSA application produced the greatest increase with respect to the control (1.4 times greater), but the values observed were not significantly different from those observed in other treatments. The high application rate of Zn-EDTA produced the highest total Zn concentration in whole shoots (6.0 times greater than that observed in the control). The highest concentrations of soluble Zn in FM from leaves were also associated with the high rate of Zn-EDTA (9.8 times greater than in the control), but all the Zn treatments produced significant increases with respect to the control.

As shown in Fig. 1, all the Zn chelates, applied at both rates, produced increases in the total stem and leaf Zn concentrations, and particularly when Zn-EDTA was applied. In Soil I, the high application rate of Zn-EDTA produced Zn concentrations in stems and leaves that were 6.8 and 7.8 times, respectively, greater than in the control. In Soil II, corresponding concentrations in stems and leaves were 6.2 and 5.2, respectively, times greater than in the control.

The average amounts of Zn uptake from shoot (stem plus leaf) were approximately twice as high in the weakly acidic soil than in the calcareous soil (2.60 and 1.41 mg Zn, respectively; Fig. 2). It was also observed that, for a given Zn source, increasing the application

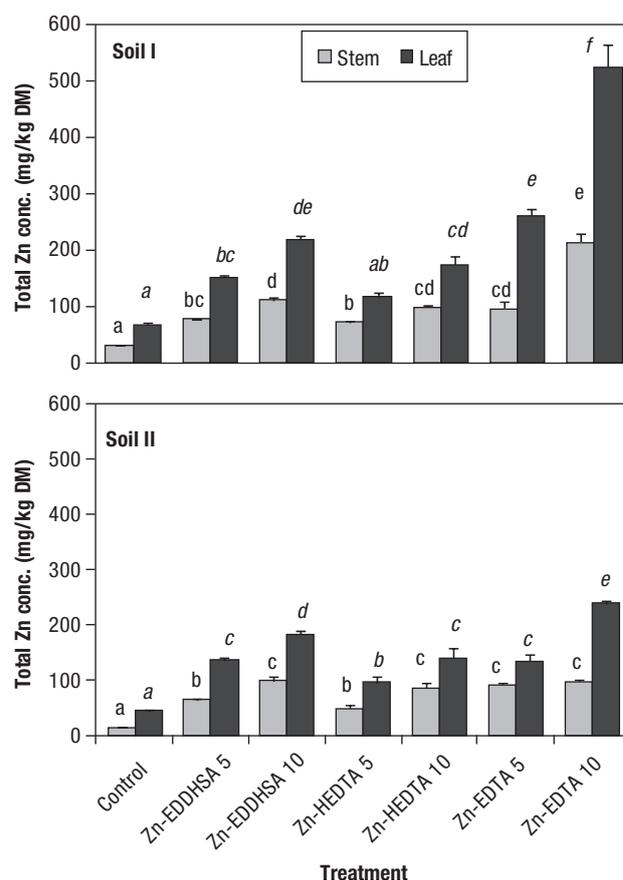


Figure 1. Effect produced by the Zn treatments (0, 5 and 10 mg Zn/kg dry soil) as Zn-EDDHSA, Zn-HEDTA and Zn-EDTA in leaf and stem total Zn concentration (dry weight) in Soil I (weakly acidic) and Soil II (calcareous). Vertical bar at each of the data points represents the standard deviation from the mean. Statistical differences at $p < 0.05$ (LSD test) are presented by different letters for each tissue analyzed (leaf Zn, italic letter; and stem Zn, non-italic letter).

rate produced an increase in shoot Zn content. The highest values for both soils were obtained with the high application rates of Zn-EDDHSA and Zn-EDTA. Furthermore, the Zn content in leaf was only higher than the Zn content in stem with the Zn-EDTA fertilizer in Soil I; this could have been due to the high concentration of Zn observed in the leaves of flax plants.

The total chlorophyll content in flax leaves ranged from 2.08 to 2.48 mg/g in Soil I for the high application rates of Zn-EDTA and Zn-HEDTA, respectively, and chlorophyll content ranged from 1.77 to 2.27 mg/g in Soil II for the control (no Zn addition) and the higher application rate of Zn-EDDHSA, respectively. Even so, there were no significant differences between treatments (data not shown).

The influence of Zn chelates on the crude fiber content and three mechanical stem material properties

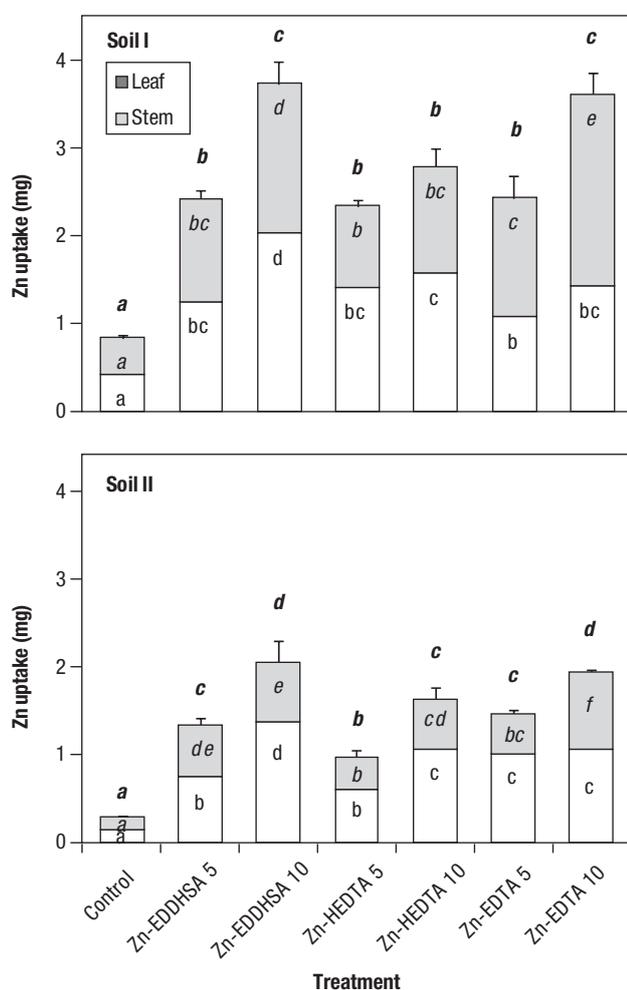


Figure 2. Zinc uptake by flax plants with 5 and 10 mg Zn/kg dry soil from Zn-EDDHSA, Zn-HEDTA and Zn-EDTA fertilizers in Soil I (weakly acidic) and Soil II (calcareous). Vertical bar at each of the data points represents the standard deviation from the mean. Statistical differences at $p < 0.05$ (LSD test) are presented by different letters for each tissue analyzed (leaf Zn, italic letter; stem Zn, non-italic letter; and total Zn, bold letter).

(tensile strength, Young's modulus, and elongation at break) is shown in Fig. 3. In Soil I, Zn chelate applications were not associated with significant increases in crude fiber content with respect to the control; even so, the high application rate of Zn-EDTA produced a significant decrease in crude fiber with respect to the control (8.2%). The highest values observed in both soils were for Zn-HEDTA applied at the low rate (47% and 46% for Soil I and Soil II, respectively), while the lowest values corresponded to the 10 mg/kg application rate of Zn-EDTA (35% and 41% for Soil I and Soil II, respectively); this last value was not significantly different from the control value (42%).

In Soil I, only the low rate of Zn-EDDHSA produced tensile strength values (MPa) that were significantly higher than in the control (1.21 times). In contrast, both rates of Zn-EDTA produced decreases in this parameter (0.76 times with respect to the control value). In Soil II, the effect of Zn treatment showed more significant differences; except in the case of the high rate of Zn-EDTA, all the treatments produced an increase in the tensile strength. For Young's modulus, there were no significant differences among fertilizer treatments in Soil I. In Soil II, the application of Zn was associated with increases in Young's modulus for Zn-EDDHSA and the high application rate of Zn-HEDTA. Finally, when the different values were compared for the property called "elongation at break", it was observed that the application of the high rate of Zn-EDTA produced a reduction in this parameter with respect to the control in Soil I. In Soil II, none of the treatments produced such reductions and, in fact, the low application rate of Zn-EDDHSA and Zn-HEDTA produced significant increases.

Discussion

Soil zinc status

The application of the two Zn rates to Soil I had a significant effect on Zn content in the most labile fractions, mainly in fractions such as WS and EXC which could be considered very important for the Zn nutrition of the plant. The highest increase, with respect to the control, in the percentage of Zn associated with the WS fraction was produced for the high rate of Zn-EDTA, followed by the same rate of Zn-EDDHSA (Table 2). For the EXC fraction, the order was Zn-EDTA 10 \geq Zn-EDTA 5 \approx Zn-HEDTA 10.

In Soil II, for WS-Zn, EXC-Zn, and CAR-Zn fractions, the highest increases were for Zn-EDTA 10; and for CAR fraction the lowest increases were for Zn-HEDTA 5 and Zn-EDDHSA 5 (Table 2). Reed & Mar-

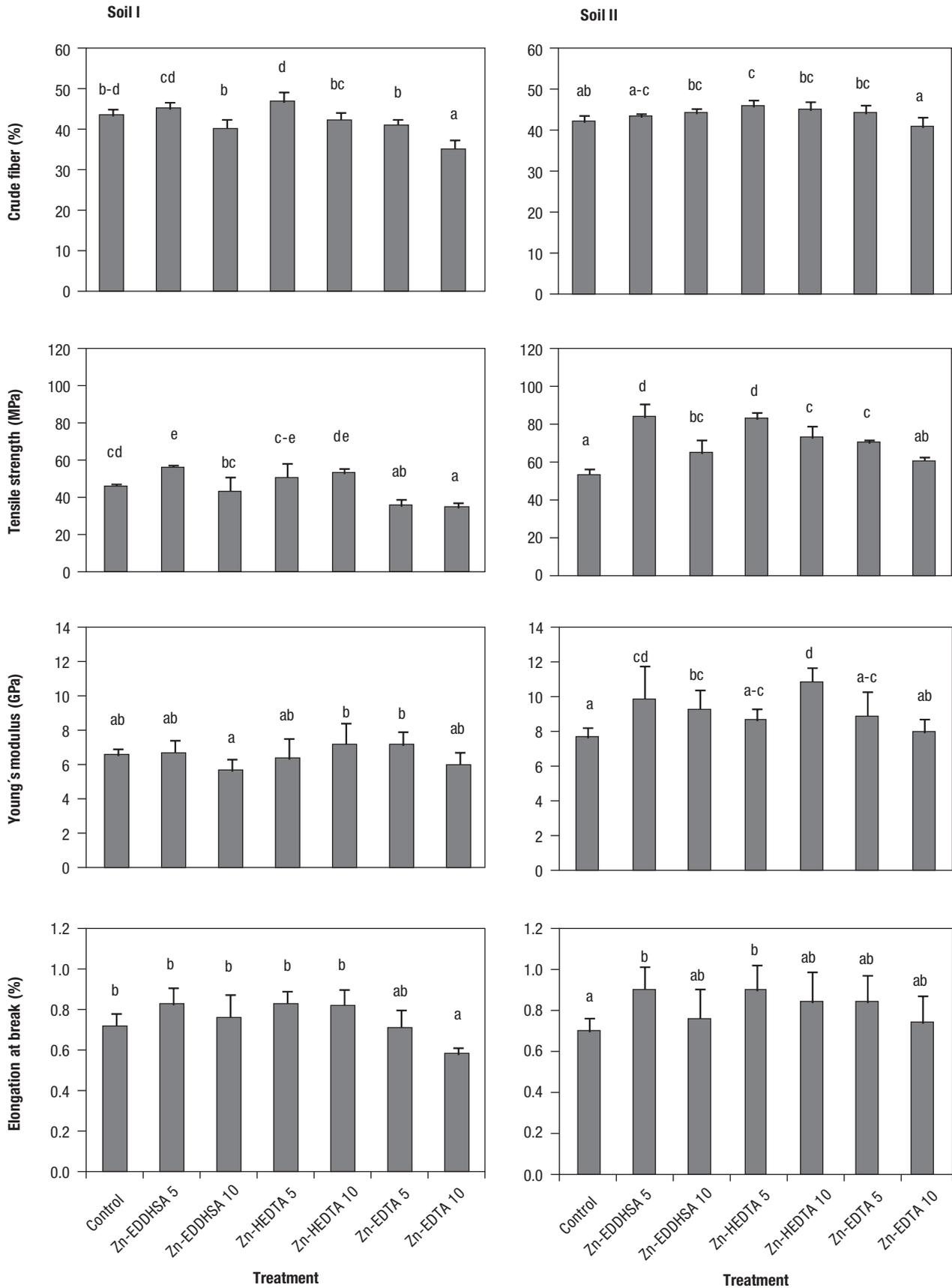


Figure 3. Crude fiber, tensile strength, Young’s modulus and elongation at break of the stems from flax plants in Soil I (weakly acidic) and Soil II (calcareous) for the different fertilizer treatments at the end of experiment (90 d). Vertical bar at each of the data points represents the standard deviation from the mean. Statistical differences at $p < 0.05$ (LSD test) are presented by different letters.

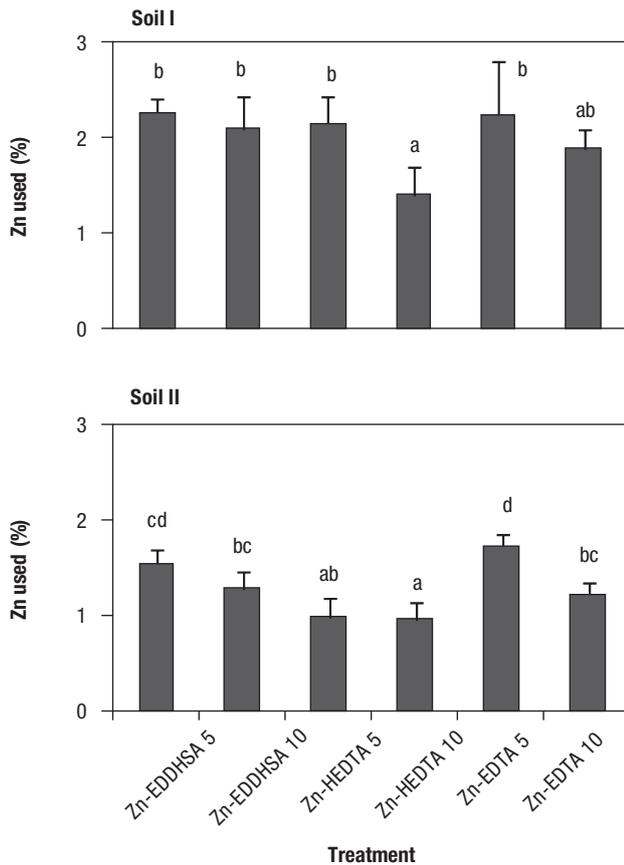


Figure 4. Percentage of Zn used by flax plants with 5 and 10 mg Zn/kg dry soil from Zn-EDDHSa, Zn-HEDTA and Zn-EDTA fertilizers in Soil I (weakly acidic) and Soil II (calcareous). Vertical bar at each of the data points represents the standard deviation from the mean. Statistical differences at $p < 0.05$ (LSD test) are presented by different letters.

tens (1996) and Shuman (1998) reported that high clay and CaCO_3 contents in alkaline soils caused adsorption and the immobilization of added Zn. In this experiment, the Zn-EDTA source was the fertilizer that supplied the largest quantities of Zn in the most labile fractions and the smallest quantities in the most insoluble Zn fractions.

According to Franzen (2004), if soil Zn level (DTPA-TEA-extractable Zn) is less than 1 mg Zn/kg, it is recommendable to apply Zn. Various authors have reported that the amounts of Zn extracted with the DTPA-AB method are greater than those extracted with the DTPA-TEA method (Gonzalez *et al.*, 2008). In this study, and in both soils, all the Zn treatments produced values greater than the recommended level of available Zn (Table 1). The Zn-EDTA treatment produced the largest quantities of available and easily leachable forms of Zn in both soils (Table 1); this was particularly evident at the high Zn application rate in Soil I, where the amount of available Zn in the soil was excessive for the normal growth requirement of flax plants

(Table 3). According to Landon (1991), DTPA-extractable Zn concentration above 10 mg/kg is considered potentially harmful in acidic soils.

Sajwan & Lindsay (1988) and McBride (1989) reported that soil parameters such as pH and pe could influence the behavior of organic Zn complexes and modify their potential bioavailability. In this experiment only the pH values for Soil II (alkaline soil) approached neutrality under conditions of 75% field capacity. According to Patrick *et al.* (1996), under waterlogged conditions the pHs of both acidic and alkaline soils converge on neutrality. The present soils could be classified as “normal” or “oxic” soils, although the conditions of Soil I were slightly acidic and oxidant (mean pH+pe value 15.27) and those of Soil II were slightly alkaline and oxidant (mean pH+pe value 15.48).

Crop response

In Soil I, the flax plants showed different visually-observable responses to the fertilizer treatments in the case of growth to 90 d after sowing. The Zn-EDTA source, which produced high Zn concentrations in the most available fractions and in the available and easy leachable Zn, apparently induced plant Zn toxicity when applied to this soil at both rates. In the present experiment, despite the reduction in DM yield (Table 3), the usual visual symptoms of this toxicity (yellow spots on the bottom leaves) were not observed in plants. In contrast, the Zn-HEDTA chelate, which produced low Zn concentrations in the most available fractions and in the available and easy leachable Zn, produced an improvement in plant growth in this weakly acidic soil when applied at the lower rate. In a field evaluation of Zn sources with a corn crop, Hergert *et al.* (1984) reported that Zn-EDTA was the most effective source at the lowest rate (0.11 kg Zn/ha), but crop yield decreased at the highest Zn rate of this source (3.36 kg Zn/ha).

According to Loneragan (1951) and Storey (2007), tissue analysis values can provide a useful indication of Zn status. These authors reported that for flax tops cultivated in pots until they were 71 d old, the intermediate range of Zn concentrations varied between 32 and 83 mg/kg DM, however they did not indicate a level of toxicity. In the present weakly acidic soil, the Zn-HEDTA chelate applied at the low rate produced a total Zn concentration in whole shoots of 85.4 mg/kg DM and also the maximum DM yield obtained (Table 3). Vitosh *et al.* (1994) reported that the Zn concentration in mature leaf tissue was excessive or even toxic at levels of over 300 mg/kg. In the present

study, the Zn-EDTA source applied at 10 mg/kg produced Zn concentrations that exceeded 300 mg/kg DM (Table 3). Macnicol & Beckett (1985) and Kabata & Mukherjee (2007) reported that the levels that could be considered “sufficient” and “excessive” for a given microelement are variable. This could, for example, be explained by the development of plant resistance to high tissue concentrations of certain microelements.

On the other hand, in Soil II, all of the Zn applications enhanced the growth of the flax plants with respect to the control treatment. Moraghan (1993) reported that, in a greenhouse study with a calcareous soil, applying ZnSO₄ at 8 mg Zn/kg soil advanced the appearance of mature bolls by 15 d and increased the yield of flax seed by 33%. A similar result was reported by Jiao *et al.* (2007), who found that applying of 10 and 20 mg Zn/kg soil in the form of ZnSO₄ enhanced the growth of flax, increased its height, and caused it to mature from 3 to 5 d earlier. Nofal *et al.* (2011), working with a foliar Zn application, also observed that increasing the application rate also caused significant increases for growth, fiber yield and other qualities such fiber length. In this soil, the Zn concentration in leaves remained below 300 mg Zn/kg DM in all the cases studied (Fig. 1). The maximum value was obtained when the Zn-EDTA chelate was applied at the high rate: approximately 240 mg Zn/kg DM (Fig. 1). In contrast, the control treatment produced a Zn concentration that was below the intermediate range reported for flax plants by Loneragan (1951) and Storey (2007).

In both control soils, the values for total-Zn concentration in plant DM were smaller than those recommended by McDonald *et al.* (2002) as the lower limit for Zn in plants used for animal fodder (50 mg/kg DM). In contrast, all the Zn treatments produced total-Zn concentrations that exceeded 50 mg/kg DM.

With respect to Zn uptake, Gangloff *et al.* (2002) reported similar results to those in this study, concluding that Zn-EDTA was more effective than other complexed Zn sources in terms of Zn concentration in plant and its uptake by corn grown in acidic soil. According to Carrillo *et al.* (2006), factors such as high stability constant and net negative charge in metal chelates protect Zn from sorption by soil components and favor their mobility to the root zone and hence metal uptake by plant. In our experiment, the lower stability constant of Zn-HEDTA, and the fact that it had a lower charge than the other metal chelates, could explain its lower Zn uptake by flax plants in Soil II (Fig. 2). However, Zn-EDTA and Zn-EDDHSA have the same charge and probably also have similar stability constant. According to Lucena *et al.* (2005), chelating agents with sulphonic acid groups have similar stability constants to

trace metals containing carboxylic groups. Furthermore, some plant species can take up metals in their ionic form and also in the form of metal chelates, such as Zn-EDTA (Marchner, 1995).

According to Prasad & Sinha (1981), the percentage of Zn used or Zn utilization by the crop [% Zn_{used} = (Zn_{uptake treatment} - Zn_{uptake control}) × 100 / Zn_{added}] is a decisive parameter for the relative effectiveness of any Zn fertilizer application. Zinc utilization varied with soil and fertilizer treatment (for both $P < 0.05$; Fig. 4). In Soil I, the most effective treatments were all the Zn sources applied at the low rate and Zn-EDDHSA applied at the high rate but within a range of between 2.08 and 2.24%. In Soil II, the most effective treatment with respect to this parameter was Zn-EDTA applied at the low rate (1.72%) followed by the same rate of Zn-EDDHSA. However, the low rate of Zn-EDTA apparently induced a small toxic effect in view of the fact that it produced a small reduction in DM yield (Table 3).

The values for tensile properties obtained in our experiment, which was performed in a greenhouse, were lower than those obtained by other authors under other conditions (Joffe *et al.*, 2003; Baley, 2004; Bos *et al.*, 2006). However, considering the diameter of the fiber used to determine the tensile properties, the values obtained should be considered similar (Charlet *et al.*, 2007). The addition of Zn fertilizers containing chelating agents, such as EDTA and applied at the high rate, tended to produce slightly decreases with respect to the control treatment in tensile strength and elongation at break in the weakly acidic soil (Fig. 3). In calcareous soil none of the Zn fertilizer treatments produced reductions in any of the tensile properties. However, the lowest values were also obtained with the high application rate of the Zn-EDTA fertilizer. In spite of the fact that no visual symptoms of toxicity were observed in this experiment, it would be recommendable to limit the application rate of the Zn-EDTA fertilizer and particularly in acidic soils.

Soluble Zn concentration from FM leaves significantly correlated with total Zn in plant DM ($r = 0.96$ and $p < 0.001$). Similar correlations were obtained in other studies which involved the application of Zn fertilizers. Cakmak & Marschner (1987) reported that soluble Zn in leaves provided a good indicator of the nutritional Zn status of a number of plant species, including, maize and grape (*Vitis vinifera* L.).

When considering both soils together, no significant correlation was found between DM yield and any of the single or sequential amounts of extracted Zn (data not shown). Even so, soluble Zn in FM and total Zn in DM were correlated significantly and positively with the soil-extractable Zn for each individual extractant

Table 4. Correlation coefficients between Zn concentrations in soils and parameters of the plants at the moment of flax harvest (n = 14, except for carbonate-bound-Zn or CAR fraction n = 7).

Zn association or parameter ^[1]	Soluble Zn conc. FM of leaves	Total Zn conc. DM of whole shoots	Total Zn conc. DM of leaves	Total Zn conc. DM of stems	Zn uptake	Total chlorophyll
DTPA-AB	0.87***	0.90***	0.90***	0.89***	0.81**	n.s.
BaCl ₂	0.78**	0.85***	0.84**	0.80**	0.81**	n.s.
WS	0.69*	0.62*	0.64*	0.63*	n.s.	n.s.
EXC	0.59*	0.71*	0.69*	0.65*	0.85***	0.59*
CAR	0.95**	0.98**	0.99***	0.94*	0.95***	n.s.
MnOX	0.55*	0.67*	0.65*	0.63*	0.85***	0.66*
AMOX	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CRYOX	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
OM	n.s.	n.s.	n.s.	n.s.	-0.52*	-0.65**
RES	n.s.	n.s.	n.s.	n.s.	-0.63*	-0.69*
pH	n.s.	n.s.	n.s.	n.s.	-0.65*	-0.68*
Eh	n.s.	n.s.	n.s.	n.s.	0.52*	0.61*

[1] See Table 1. Eh (redox potential). ***, **, *; significant at $p < 0.001$, < 0.01 and < 0.05 , respectively; n.s. = not significant.

and also for the first three fractions and for the CAR fraction in soil II (or most labile Zn fractions) that were sequentially performed in the soils. The Zn concentration in the flax plants could therefore be described as a function of the soil-extractable Zn for single reagents and for sequential fractionation. The relationships between the Zn uptake by plant and the Zn extracted with the DTPA-AB, BaCl₂ and EXC, CAR and MnOX fractions were also positive and highly significant (Table 4). Total chlorophyll showed significant correlations with both the Zn extracted concentrations with the EXC and MnOX fractions (positively) and the more residual fractions (negatively) ($p < 0.05$). Zinc uptake and total chlorophyll showed similar correlations: being negative ($p < 0.001$) with soil pH and positive with E_h ($p < 0.05$). Our results agree with those reported by other authors (Kabata & Mukherjee, 2007). They suggest that acidic and oxidizing soil conditions favor Zn solubilization in soil and Zn uptake by plant and also that the effect of pH is more significant than that of E_h .

On the other hand, the crude fiber and tensile properties (tensile strength, Young's modulus and elongation at break) showed significant and negative correlations with the soil-extractable Zn for each single extractant and also for the most labile Zn fractions (data not shown). According to Alvarez (2010), when the natural chelate sources applied produce high concentrations of available Zn in soils, this could cause reductions in the tensile properties of flax.

In conclusion, a balanced micronutrient supply is crucial for obtaining high yields and a good crop quality. It is significant to note that the results obtained when Zn was applied at 5 mg/kg rate (the low rate) and in the form of Zn-EDDHA, Zn-HEDTA and Zn-EDTA fertilizers were similar, in terms of effectiveness (with respect to Zn utilization), in weakly acidic soil.

Applying the Zn-EDDHA and Zn-HEDTA fertilizers to this soil would improve its tensile properties and crude fiber percentage, and would also reduce the easily leachable Zn. The Zn-EDTA and Zn-EDDHA fertilizers were the most effective fertilizers in calcareous soil (with a high clay content, an alkaline pH and the presence of CaCO₃); they produced maximum values of Zn utilization when applied at the low rate, but without causing reductions in any of the other, previously indicated, plant parameters. The relatively large amounts of labile and available Zn present in both of the soils fertilized with the Zn-EDTA source, and the plant Zn toxicity apparently induced by this fertilizer in the weakly acidic soil, would suggest that this fertilizer should best be applied in low doses. This, in turn, should result in less Zn being leached away and in a reduction in the costs of Zn fertilization.

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