Residual effects of natural Zn chelates on navy bean response, Zn leaching and soil Zn status

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Abstract A greenhouse experiment was conducted on weakly acidic and calcareous soils to evaluate the aging and residual effects of three natural organic Zn chelates [Zn-ethylenediaminedisuccinate (Zn-EDDS), Zn-poly-hydroxyphenylcarboxylate and Zn-aminelignosulfonate] each administered in a single application to a first navy bean (*Phaseolus vulgaris* L.) crop at several different Zn application rates. In a second navy bean crop, we determined the following parameters: the extent of Zn leaching, the amount of available Zn remaining in soils, the amount of easily leachable Zn, the size of Zn fractions in soils, the pH and redox potential, the dry matter yield, and the soluble and total Zn concentrations in plants. The residual effect after 2 years of Zn fertilization mainly depended on the aging effect of Zn chelates and losses due to Zn leaching. The data relating to the evolution from the first to the second crop showed that the aging effect was noticeable in the calcareous soil. In the latter soil, the Zn-S,S-EDDS treatments showed greater decreases in the Zn uptake by plants than the other Zn treatments and the greatest Zn uptake by plants occurred when Zn was applied as Zn-aminelignosulfonate (10 mg Zn kg^{-1} rate, 6.85 mg Zn per lysimeter; 5 mg Zn kg^{-1} rate, 3.36 mg Zn per lysimeter). In contrast, in the calcareous soil, the maximum amount of Zn uptake, for the three chelates was 0.82 mg Zn per lysimeter. Consequently, a further application of Zn would be needed to prevent Zn deficiencies in the plants of a subsequent crop. The behaviour of the pH and Eh parameters in the soils and leachates did not depend on the natural Zn sources applied. In this study, the easily leachable Zn estimated by BaCl$_2$ extraction was not adequate to predict Zn leaching from the soils in subsequent crops.

Abbreviations

AB	ammonium bicarbonate
AMC	amorphous minerals colloids bound
CAR	carbonate bound
CFeO	crystalline Fe oxide bound
DTPA	diethylenetriaminepentaacetate
OC	organically complexed
OM	organically bound
RES	residual fraction
RMO	easily reducible metal oxide bound
TEA	triethanolamine
WSEX	water soluble plus exchangeable
Zn-AML	Zn-aminelignosulfonate
Zn-PHP	Zn-polyhydroxyphenylcarboxylate
Zn-S,S-EDDS	Zn-ethylenediaminedisuccinate

Introduction

Zinc fertilizers are commonly applied to many crops around the word (Alloway 2008; Cakmak 2008; Prasad and Sinha 1981). The primary soil factors controlling the potential bioavailability of metals are soil pH, the accessibility and character of sorption sites on soil surfaces, the contents of iron and aluminium oxyhydroxides, soil organic matter, and clay mineral. However, according to US EPA (2003), the first two of these factors are the most important for controlling the release of metals to pore water and their subsequent bioavailability. One way to determine the availability of micronutrients to plants is to measure direct uptake in plant experiments; however, availability can also be estimated by extracting a portion of the soil micronutrient content with a chemical reagent and relating this portion to plant response (White and Zasoski 1999). Several extraction schemes have been developed to determine the distribution of metals among operationally defined fractions (e.g., Chandi and Takkar 1982; Krishnamurti and Naidu 2002). However, sequential extraction methods do not cleanly distinguish the occurrence and speciation of different forms of metals in soils (Alvarez et al. 2006; Ure 1995). A variety of single soil extractants can also be used to evaluate micronutrient availability in soils. These soil tests are interesting as they offer the possibility of considerably simplifying the task of estimating the available and mobilizable fractions of metals (Li and Shuman 1997; Obrador et al. 2003). The most widely accepted extractant for micronutrient cations is 0.05 M DTPA (Hossain et al. 2008; Lindsay and Norvell 1978; Lopez-Valdivia et al. 2002), although other reagents (e.g., dilute acids, chelating agents and neutral salts) are also currently used (Jones 2001; Narwal and Singh 1998; Sims and Johnson 1991). Critical levels for some micronutrients are only normally useful for a limited range of soils and crops and are closely related to the conditions under which they were determined (White and Zasoski 1999).

Some studies have indicated that organic sources are more effective fertilizers than inorganic ones. Their effectiveness depends on the rate of their disappearance from the soil solution, which is related to their stability (Alloway 2008). Zinc chelates differ in physical state, chemical reactivity, cost, bioavailability, and susceptibility to leaching. The chelating agents DTPA, HEDTA and EDTA are some of the strongest synthetic chelating agents; in combination with Zn, they form much stronger chelates than naturally occurring organic ligands (Mortvedt and Gilkes 1993).

Although there is evidence to suggest that some chemical properties of soils, such as pH and pe [pe=Eh (mV)/59.2] (being pe the negative logarithm of the free electron activity and Eh the redox potential), can be affected by the addition of fertilizers to soils (Thind and Rowell 1999), there is a general lack of information regarding the effects of fertilization involving organic Zn complexes. To improve predictions of Zn mobility and availability when this type of fertilizer is added to soils, it is therefore interesting to study the evolution of the pH and pe parameters after fertilizer applications and their possible relation with the distribution of various Zn forms.

After applying Zn fertilizers, the activity and extractability of Zn added to soils in water soluble forms continually and slowly decreases, and Zn changes to more stable forms through slow reactions with soil constituents (Barrow 1986; Shuman 1991). This process is referred to by some authors as aging (Hossain et al. 2008; Ma and Uren 2006; McLaughlin 2001). The slow reactions are attributed to micropore diffusion (Ma and Uren 1997), occlusion in solid phase by co-precipitation and co-flocculation, cavity entrapment (Ma and Uren 1998), and solid solution interface formation (Davis and Kent 1990; Sparks 2003).

After Zn fertilization, there is normally a period of several years in which the residual effect provides an adequate supply of Zn to successive crops. The length of this period will depend on the nature of the soil in question and the crop system applied (e. g., fertilizer type and micronutrient rate added; Hossain et al. 2008). Using an experimental field on a highly Zn-deficient soil in Western Australia, Brennan (2001) found that the effectiveness of the ZnO treatment for wheat (*Triticum aestivum* L.) decreased by 50% over a period of 13 years. In Turkey, on a highly deficient calcareous soil in Anatolia, Cakmak et al. (1999) found that 28 kg Zn ha^{-1} as $ZnSO_4$ was sufficient to correct a Zn deficiency in wheat for between 4 and 7 years. Most of the studies into the residual effects of Zn have been carried out using sewage sludge, due to the relationship between its residual effect and its potential for pollution (Basta et al. 2005), or inorganic

Zn fertilizers (oxides, sulphates, oxisulphates and others Zn salts) since, as with most crops, the normal way of correcting Zn deficiencies in soils is to apply these fertilizers (Brennan and Bolland 2006; Ma and Uren 1997). Until some years ago, chelated forms of Zn were mainly used in foliar applications as a rescue treatment for a current deficient crop and, they do not have much residual value for following crops. Now, however, in some countries, including Spain, fertilizers that contain Zn complexes or chelates are being added to soils in order to correct Zn deficiencies (Liñán 2007).

Although some studies have been conducted to discover more about aging and long-term transformations in soils to which inorganic sources of Zn were added, only limited data are available concerning the aging and residual effects of Zn added as organic complexes. It would therefore be advisable to study the residual effects of new commercial micronutrient fertilizers on soils and plants in order to optimize fertilization (and save the expense of unnecessary Zn applications) and minimize its environmental impact (and ensure that the element does not accumulate at undesirably high concentrations in soils, plants and/or neighbouring waters).

In light of what has been previously outlined, it was expected the various natural chelating agents would have different residual effects on a subsequent crop and that these effects would depend on the type of soil in question. To verify this hypothesis, a greenhouse experiment was designed in which three organic complexes of natural origin were applied to a first navy bean crop in two soils (acidic and calcareous) in which a second crop was later cultivated. The operational objectives were to study the: (1) leaching of the Zn applied, (2) availability and chemical forms of soil-applied Zn, (3) pH and redox potential of the soil and (4) effectiveness of three commercial Zn fertilizers on the navy bean's dry matter yield and its total and soluble Zn concentrations.

Materials and methods

Soil characteristics

The Ap horizons of two representative soils were collected from rural areas of Spain [weakly acidic soil (acidic soil): latitude 40° 21′ N, longitude 4° 00′ W; calcareous soil: latitude 40° 39′ N, longitude 3° 20′ W].

The acidic soil was classified as a Typic Haploxeralf and the calcareous soil as a Typic Calcixerept (Soil Survey Staff 2006). The main soil properties, which were based on means of three replicates, are reported in Table 1. Both were soils commonly used to cultivate cereals and characterized by their low organic matter contents and low Zn availability. The clay content was higher in calcareous soil and the X-ray diffraction technique (Schultz 1964) showed that the predominant clay in acidic soil was illite and that in calcareous soil it was smectite.

Greenhouse experiments

Soil preparation

The soils used in this study were second-cropped soils from an experiment carried out the previous year that had involved growing a navy bean crop in lysimeters (Gonzalez et al. 2007, 2008a, b). The original soils were air-dried and fractions of less than 2 mm were used in the experiment. The soils were only treated with aqueous suspensions of three liquid fertilizers of natural origin: Zn-ethylenediaminedisuccinate (Zn-S,S-EDDS; 82 g water-soluble-Zn L^{-1} and mass density 1.36 g cm^{-3}), Zn-polyhydroxyphenylcarboxylate (Zn-PHP; 38 g water-soluble-Zn L^{-1} and mass density 1.26 g cm^{-3}) and Zn-aminelignosulfonate (Zn-AML; 59 g water-soluble-Zn L^{-1} and mass density 1.20 g cm^{-3}) at the beginning of the first experiment. These Zn sources were produced by commercial companies (Liñán 2007). The control (no Zn addition) and fertilizer treatments (5 and 10 mg Zn kg^{-1}) were replicated three times in a completely randomized design layout.

After the navy bean harvest, the soil was manually removed from each lysimeter and homogenized and then 9.75 kg of soils was placed in the polypropylene lysimeters (total number, 42; capacity, 11 L; internal diameter, 24 cm; and height, 25 cm). A polyester mesh and a 1.5 cm-thick layer of washed gravel were also placed in the bottom of each lysimeter to facilitate drainage.

Navy bean crop

In the second year, a new navy bean crop was grown without Zn fertilization. The leachate was collected with a silicone tube leading to a polyethylene bottle.

Table 1 Selected properties of the original soils and analytical procedures used in the experiment

Soil properties		Acidic soil	Calcareous soil	References
Clay (g kg^{-1})		100±6[a]	180±5	Day (1965)
Texture (USDA)		Sandy loam	Loamy sand	–
Bulk density (g cm^{-3})		1.4±0.1	1.1±0.1	MAPA (1994)
WHC (33 kPa; g H$_2$O 100 g^{-1} soil)		6.60±0.21	20.5±0.7	Klute (1996)
Permeability		moderate	moderate to rapid	Monturiol and Alcalá (1990)
pH$_{w(1:2.5)}$		6.13±0.09	8.13±0.03	Chapman and Pratt (1961)
EC (µS cm^{-1})		37.2±1.6	178±7	Chapman and Pratt (1961)
Extractable P (mg kg^{-1})		19.89±0.29	12.58±0.48	Bray and Kurtz (1945), Olsen et al. (1954)
Organic mater (g kg^{-1})		2.35±0.13	5.10±0.23	Hesse (1971)
N (g kg^{-1})		1.02±0.11	1.10±0.05	Bremner (1996)
Total CaCO$_3$ (g kg^{-1})		–	134±6	Allison and Moodie (1965)
Free CaCO$_3$ (g kg^{-1})		–	33.4±1.9	Nijensohn and Pizarro (1960)
CEC (cmol$_c$ kg^{-1})		4.72±0.12	23.5±1.0	Bower et al. (1952)
Fe (mg kg^{-1}; active Fe$_2$O$_3$)		141±2	56.0±1.1	McKeague and Day (1966)
Total Zn (mg kg^{-1})		9.21±0.75	43.40±1.54	–
Extractable Zn (mg kg^{-1})	DTPA-TEA	0.73±0.06	0.44±0.04	Lindsay and Norvell 1978
	DTPA-AB	0.83±0.04	0.60±0.03	Soltanpour 1991
	Mehlich-3	1.33±0.07	0.96±0.05	Mehlich 1984
	BaCl$_2$	0.90±0.05	0.30±0.03	Schultz et al. 2004

WHC water-holding capacity, *EC* electrical conductivity, *CEC* cation exchange capacity, *DTPA-TEA* diethylenetriaminepentaacetate-triethanolamine, *DTPA-AB* diethylenetriaminepentaacetate-ammonium bicarbonate

[a] Standard deviation

The nutritional condition of the soil for the navy bean crop was assessed using the electroultrafiltration technique (Wiklicky and Nemeth 1981). Taking previous results into account, basal fertilization was applied with 50 mg N kg^{-1} [as (NH$_2$)$_2$CO], 50 mg P kg^{-1} [as Ca(H$_2$PO$_4$)$_2$] and 50 mg K kg^{-1} (as K$_2$SO$_4$). Three navy bean seeds (*Phaseolus vulgaris* L., variety Garrafal oro velez enrame, Fito S.A., Barcelona, Spain) were cultivated in each lysimeter. The lysimeters were placed in a greenhouse in which temperatures ranged from 10°C to 32°C and relative air humidity ranged from 60% to 85%. The soils were irrigated at slightly above field capacity moisture to obtain ten portions (each of 200 mL) of leachate for a total of 2,000 mL (acidic soil, 0.623 pore volume; calcareous soil, 0.391 pore volume). To evaluate evapotranspiration, the containers were weighed (balance A&D Instruments Ltd., UK, model FG-30 KBM) and we estimated the volume of irrigation water required.

Sixty days after seeding, samples of fresh leaves were collected from between the third and fourth leaves of the upper parts of the plants. The plants were then cut at soil level, washed in deionized water and 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. Soil samples were also collected and stored for further analysis.

Chemical analysis

The soluble Zn in fresh matter was determined in a sample of 2 g of young leaves. The samples were manually homogenized for 5 min in a mortar with 10 mL of 1 mM morpholino acid (MES: 2-morpholino-ethanesulfonic acid, C$_6$H$_{13}$NO$_4$S·H$_2$O) 10^{-3} M to pH 6 (ratio 1:5, *w:v*). After each extraction, the plant suspension was centrifuged (10,000 rpm for 15 min) and the supernatant solution was decanted and filtered through Whatman no. 41 filter paper.

Soluble Zn in plant dry matter was extracted by the method proposed by Rahimi and Schropp (1984) and Cakmak and Marschner (1987), with modifications: 0.25 g of the aerial part of the plant was weighed and its Zn content was extracted with 10 mL of MES at pH 6 (ratio 1:40, *w:v*).

Total Zn in plant dry matter was extracted by wet digestion in a microwave oven (CEM Corporation, model-Mars, Matthews, NC, USA). This involved a two-step process with a maximum pressure of 170 psi,

using 0.3 g of dried ground samples, 4 mL of HNO_3 (65%) and 2 mL of HF (48%). The leached liquids were collected and their Zn contents were analyzed. The pH and redox potential (Eh) parameters of the soils and leachates were determined by potentiometry, using pH and redox (Pt) electrodes (they were determined at two crop times: 30 and 60 days after germination, for all treatments). For soils, the procedure consisted of making a hole 2 mm larger than the diameter of the electrode by inserting a stainless steel bar into previously humidified soil. The electrode was then inserted to a depth of more than 2 cm. The temperature probe was also inserted into soil. Measurements were taken after between 10 and 15 min, once values had become stable, and three replicates were performed. In the case of leachates, the electrodes were submerged in the solutions collected from each lysimeter. Temperature was automatically compensated by connecting a probe to the potentiometer in the case of pH. To calculate the redox potential (Eh), the potential from the reference electrode was added to the measured potential of the cell (ISO 11271 2002). Two standard redox checks (212 and 468 mV, Crison Instruments, Barcelona, Spain) were used when measuring the redox potential. The amount of Zn available to the plant was assessed by extraction using three common used chemical extractants: DTPA-TEA, DTPA-AB, and Mehlich-3. The easily leachable Zn fraction was estimated by extraction with 0.01 M $BaCl_2$ (Schultz et al. 2004). Zinc distribution in the different soil fractions was determined by the sequential fractionation method proposed by Krishnamurti and Naidu (2002). The Zn fractions were sequentially determined in seven steps (using a 1 g soil sample) with the following extractants: 10 mL of 1 M NH_4NO_3 pH 7.0 for 4 h (WSEX, water soluble plus exchangeable); 25 mL of 1 M NaOAc pH 5.0 for 6 h (CAR, carbonate bound); 30 mL of 0.1 M $Na_4P_2O_7$ pH 10.0 for 20 h (OC, organically complexed); 20 mL of 0.1 M $NH_2OH \cdot HCl$ in 0.01 M HNO_3 for 30 min. (RMO, easily reducible metal oxide bound); 30% H_2O_2 pH 2.0 + 0.02 M HNO_3 and 10 mL of 2 M NH_4NO_3 in 20% HNO_3 for 30 min. (OM, organically bound); 10 mL of 0.2 M $(NH_4)_2C_2O_4$/0.2 M $H_2C_2O_4$ pH 3 for 4 h in the dark (AMC, amorphous minerals colloids bound); 25 mL of 0.2 M $(NH_4)_2C_2O_4$/0.2 M $H_2C_2O_4$ pH 3 in 0.1 M ascorbic acid for 30 min. (CFeO, crystalline Fe oxide bound). The residual fraction (RES) was calculated as the difference

between total Zn, extracted by wet acidic digestion in a microwave oven, and the sum of the other fractions. All samples were extracted and analyzed in triplicate using each of the procedures.

The "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. In all cases, Zn concentrations were determined by flame atomic absorption spectrophotometry (Perkin-Elmer AAnalyst 700).

Statistical analysis

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

Results

Metal leaching study

In the acidic soil, the residual effect produced by the Zn treatments applied in the first crop with respect to the amount of Zn leached (mg per lysimeter) during the second crop is shown in Fig. 1. The amounts of Zn leached in the control (no Zn addition) and in the soil treated with Zn-*S,S*-EDDS at a rate of 5 mg kg^{-1} were small. The cumulative quantity of Zn collected in 2 L of leachate (at 60 days) from the control soil was 0.38 mg, and the quantities from soils treated with Zn-*S,S*-EDDS were 0.53 mg (1.1% of applied Zn) and 1.61 mg (1.6%) for rates 5 and 10 mg Zn kg^{-1} soil, respectively. However, in soils amended with Zn-AML, the total amounts of leached Zn were respectively 1.67 (3.3%) and 3.17 mg (3.2%) for the two rates, while in soil amended with Zn-PHP, they were 1.71 (3.4%) and 1.88 mg (1.9%) for the same rates.
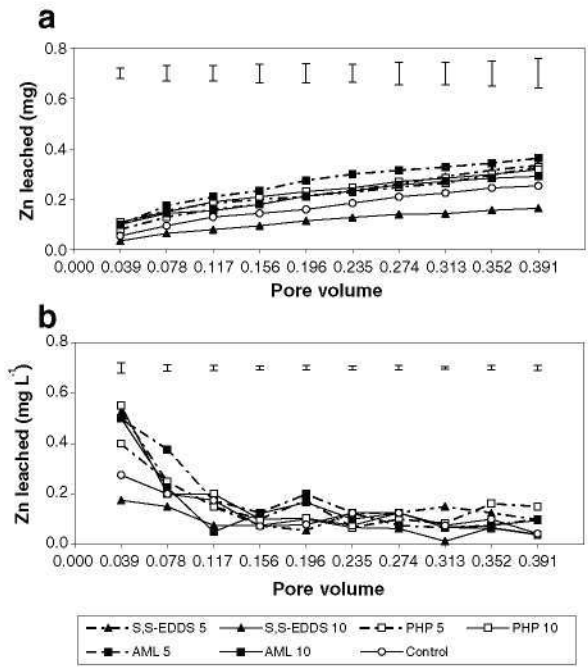
In the calcareous soil, the control and all the Zn treatments produced a very small amount of leached Zn (Fig. 2a), with values ranging between 0.17 (0.2%) and 0.36 mg (0.7%) and, the maximum Zn
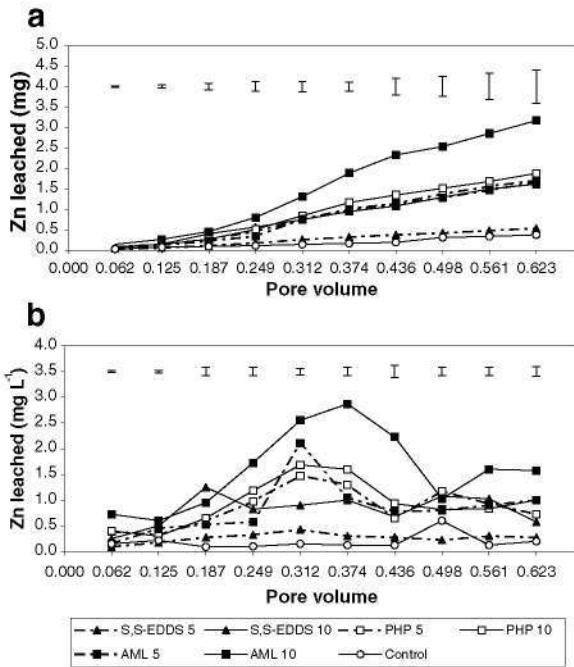
Fig. 1 Residual effect produced by the Zn treatments [0 (control), 5 and 10 mg Zn kg^{-1} soil as Zn-S,S-EDDS, Zn-polyhydroxyphenylcarboxylate (Zn-PHP) and Zn-aminelignosulfonate (Zn-AML)] in acidic soil with respect to the cumulative quantities (**a**, *top*) and concentrations (**b**, *bottom*) of Zn leached. *Vertical bar* indicates the maximum standard error from the means for each leachate portion

Fig. 2 Residual effect produced by the Zn treatments [0 (control), 5 and 10 mg Zn kg^{-1} soil as Zn-S,S-EDDS, Zn-polyhydroxyphenylcarboxylate (Zn-PHP) and Zn-aminelignosulfonate (Zn-AML)] in calcareous soil with respect to the cumulative quantities (**a**, *top*) and concentrations (**b**, *bottom*) of Zn leached. *Vertical bar* indicates the maximum standard error from the means for each leachate portion

concentration being associated with the first leachate portion for all treatments (Fig. 2b).

pH and redox potential (Eh) were determined for all leached portions. In the acidic soil, the mean pH value (for all Zn treatments and the control) ranged from 6.62, for the first leached portion, to 5.70, for the tenth, while Eh varied from 452 to 582 mV for these same portions. In the calcareous soil, the mean pH value only ranged from 7.37 for the first leached portion to 7.66 for the tenth, while Eh varied from 432 to 483 mV for these same portions. In general, the values obtained were similar in each soil for all of the lysimeters fertilized with Zn and also for the control. Zinc complexes of natural origin did not, therefore, exhibit any variation with respect to the control treatment. Any possible changes that the natural complexes would produce with respect to the control would not be observed due to the fact that any leachates in contact with the ambient atmosphere oxidation would immediately modify the redox potential.

Potential availability and distribution of zinc fractions in second-cropped soils

The values for the concentrations of Zn extracted from the two soils by each of the three methods used to estimate available Zn (DTPA-TEA, DTPA-AB and Mehlich-3) at the time of the second navy bean harvest are shown in Table 2.

In the acidic soil, the available Zn concentrations in the control soil for the three extraction methods were near what constitute critical levels for most plants (Lindsay and Norvell 1978; Soltampour 1991; Tran and Simard 1993). The concentrations obtained for all Zn treatments (applied only in the first crop) were higher than those in the control and ranged between 4.4 and 18.2 times the control values, while significant differences were found between fertilizer treatments ($P<0.0001$). The treatments that produced the smallest quantities of available Zn were Zn-S,S-EDDS and Zn-PHP sources applied at a rate of 5 mg Zn kg^{-1}. In contrast, the treatment that produced the largest

Table 2 Residual effect of Zn fertilization with 0 (control), 5 and 10 mg Zn kg^{-1} soil as Zn-S,S-EDDS, Zn-polyhydroxyphenylcarboxylate (Zn-PHP) and Zn-aminelignosulfonate (Zn-AML) in the extractable Zn (mg kg^{-1}) in soils at the time of the second navy bean harvest

Extraction method	Control	Zn-S,S-EDDS		Zn-PHP		Zn-AML	
		Rate 5	Rate 10	Rate 5	Rate 10	Rate 5	Rate 10
Acidic soil							
DTPA-TEA	0.56a	4.95b	7.70c	5.24b	8.33c	8.51c	10.15d
DTPA-AB	0.68a	6.10b	9.80c	6.15b	9.83c	9.15c	12.35d
M-3	1.55a	6.80b	10.70c	8.15b	11.77cd	10.95c	13.85d
Calcareous soil							
DTPA-TEA	0.33a	2.12b	2.68b	2.10b	5.44c	3.12b	4.49c
DTPA-AB	0.38a	2.43b	3.06b	2.35b	6.65c	3.92b	5.72c
M-3	1.00a	4.25b	5.45bc	4.70bc	11.10d	6.25c	9.75d

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

quantities of available Zn for subsequent crops was the Zn-AML source applied at a rate of 10 mg Zn kg^{-1}. The lower rate of this source produced similar quantities of available Zn to the higher rate of the other two Zn sources.

In the calcareous soil, the available Zn concentrations in the control soil were lower than those reported as critical for most plants in calcareous soils. However, the concentrations obtained for all of the Zn treatments were higher than these critical levels (between 4.2 and 17.5 times greater than the control values), although not all treatments behaved in the same way. The 10 mg Zn kg^{-1} application rate of both the Zn-PHP and Zn-AML chelates produced the largest quantities of available Zn for subsequent crops.

The distribution of Zn fractions in both of the second-cropped soils at the time of the second navy bean harvest are shown in Fig. 3. In the acidic soil, the Zn concentrations (mg kg^{-1}) in the fractions of the control treatment decreased in the following order: CFeO (2.50), OM (2.20), OC (1.89), RES (1.64), AMC (1.12), RMO (0.45) and WSEX (0.17). Zinc concentration values were examined for each fraction using multifactor variance analysis to determine the main effects of fertilizer treatments and repetition. Significant differences were found between treatments (P values ranged between 0.0001 and 0.05) in the WSEX, OC, RMO, and CFeO fractions. The Zn fertilizers generally produced a significant increase in the Zn content in fractions such as WSEX (from 17.3% to 31.2% of applied Zn) and OC (from 36.2% to 78.8%), which could be considered very important for

the Zn nutrition of any subsequent crop. The biggest Zn concentrations in the most labile fraction (WSEX) were associated with Zn-AML followed by applications of Zn-PHP fertilizer, both at a rate of 10 mg Zn kg^{-1} (respectively, 17.2 and 13.3 times greater than in the control soil). Zn-S,S-EDDS was the source associated with the smallest amounts of Zn in this fraction (7.0 and 10.8 times greater than in the control soil for application rates of 5 and 10 mg kg^{-1}, respectively).

In the calcareous soil, the Zn concentrations (mg kg^{-1}) in the fractions of the control treatment decreased in the following order: RES (21.05), CFeO (10.63), OC (3.40), AMC (2.90), OM (2.35), CAR (1.38), RMO (0.28) and WSEX (0.18). Significant differences were found between treatments in all Zn fractions (with similar probability levels to those for acidic soil). The Zn fertilizers generally produced a significant increase in Zn content in the first three fractions: WSEX (from 0.2% to 2.2% of applied Zn), CAR (from 7.5% to 38.7%) and OC (from 7.0% to 79.0%) of the second-cropped soil. The biggest Zn concentrations in the WSEX fraction were associated with Zn-AML (2.2 times greater than in the control soil for the application rate of 10 mg Zn kg^{-1}). Zn-PHP was the source associated with the smallest amount of Zn in this fraction (only 1.1 times greater than in the control soil for the application rate of 10 mg Zn kg^{-1}).

pH and redox potential (Eh) parameters were determined in both of the second-cropped soils at two crop times: after 30 and 60 days (end of the experiment) for all treatments. In the acidic soil, the Duncan's separation of averages method established that the pH increased with

Fig. 3 Distribution of Zn fractions and DTPA-TEA extractable Zn in second-cropped soils (acidic and calcareous) at the time of the second navy bean harvest. *Vertical bar* indicates the standard error from the mean of the total Zn

Acidic soil
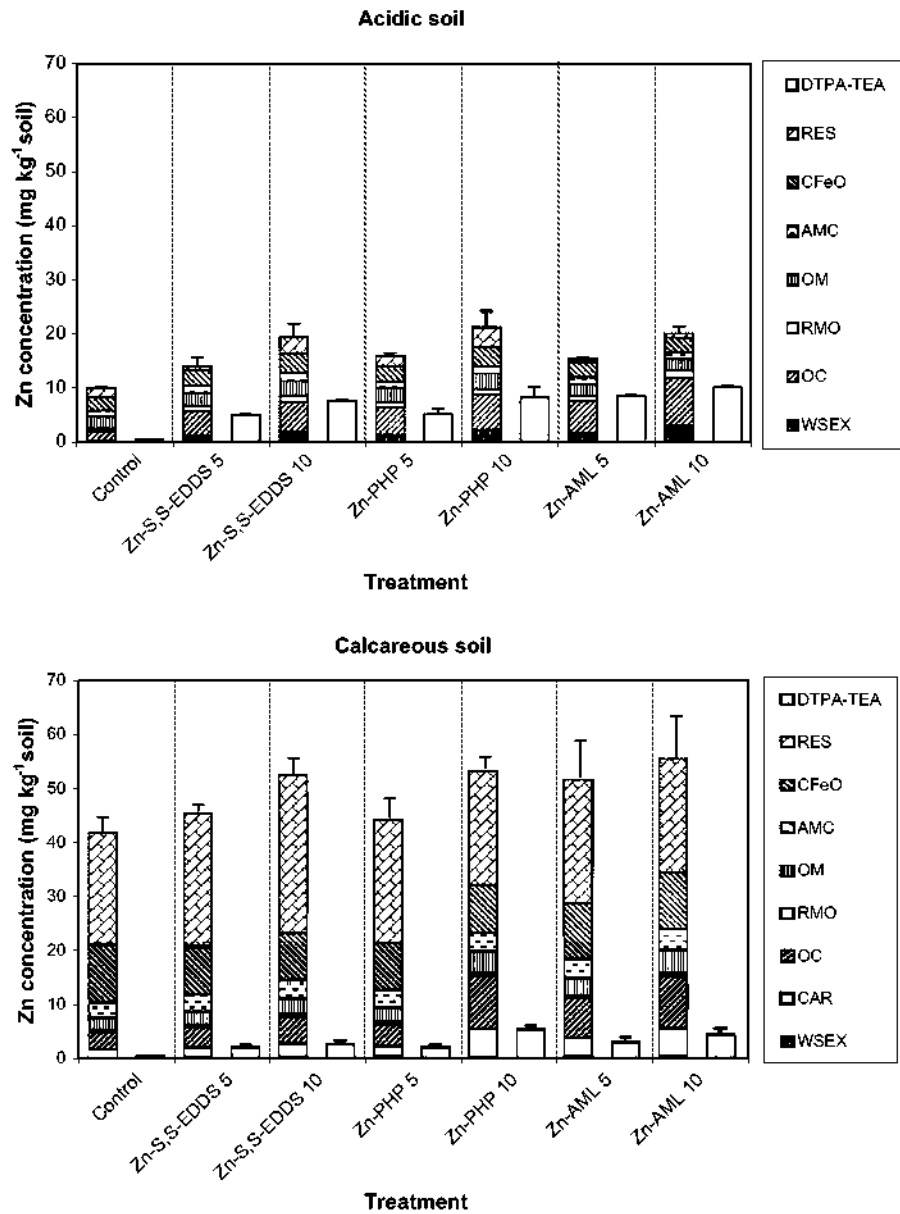
Calcareous soil

time ($P<0.0001$) in the course of crop development (from 5.84 to 6.52), while Eh significantly diminished ($P<0.0001$; from 621 to 572 mV). In the calcareous soil, both parameters significantly diminished with time in the course of crop development ($P<0.0001$; from 7.73 to 7.34 for pH, and from 519 to 477 mV for Eh).

Navy bean response to zinc fertilization

The residual effect of Zn fertilizer treatments on navy bean dry matter yield and Zn concentration (soluble and total) in second harvest plants is shown in Table 3. The concentrations of soluble Zn in fresh matter from leaves for all Zn treatments were greater in acidic soil than in calcareous soil. In the acidic soil, the high rate of Zn-AML chelate application was associated with the highest concentration in fresh matter (5.44 mg Zn kg^{-1}). However, in the calcareous soil the highest concentration (3.62 mg Zn kg^{-1}) was associated with the Zn-S,S-EDDS source applied at the high rate.

In the acidic soil, all fertilizer treatments produced increases in dry matter yield and Zn concentration in

Table 3 Residual effect of Zn fertilization with 0 (control), 5 and 10 mg Zn kg⁻¹ soil as Zn-S,S-EDDS, Zn-polyhydroxyphenylcarboxylate (Zn-PHP) and Zn-aminelignosulfonate (Zn-AML) in the response of the second navy bean crop

Extraction method	Control	Zn-S,S-EDDS		Zn-PHP		Zn-AML	
		Rate 5	Rate 10	Rate 5	Rate 10	Rate 5	Rate 10
Acidic soil							
Soluble Zn conc. FM (mg kg⁻¹)	2.02a	2.31ab	3.28bc	2.07a	3.08a-c	3.96c	5.44d
Dry matter yield (g per lysimeter)	14.4a	18.8b	20.2b	18.0b	17.8b	26.3c	25.4c
Total Zn conc. DM (mg kg⁻¹)	27.9a	103b	201d	118bc	234e	128c	269f
Soluble Zn conc. DM (mg kg⁻¹)	17.2a	37.6b	54.5c	38.5b	58.0c	51.1c	94.7d
Calcareous soil							
Soluble Zn conc. FM (mg kg⁻¹)	1.92a	2.92d	3.62e	2.61cd	2.08ab	2.39bc	2.34bc
Dry matter yield (g per lysimeter)	15.2a	25.6b	30.8c	27.6b	30.5c	25.6b	27.5b
Total Zn conc. DM (mg kg⁻¹)	19.9ab	26.3d	26.5d	18.1a	19.4ab	21.6bc	24.0cd
Soluble Zn conc. DM (mg kg⁻¹)	15.1a	19.5bc	22.0c	15.0a	14.5a	17.3ab	18.2a-c

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

FM fresh matter of leaves, DM dry matter of whole shoots

plant dry matter with respect to the control. For both parameters, the highest increase was produced by the Zn-AML chelate. With respect to the control treatment, the high application rate of this source increased both dry matter yield (11.0 g per lysimeter) and Zn concentration (241 mg Zn kg⁻¹ DM). For all the Zn treatments, the values of Zn concentration in plant dry mater, were greater than that cited by McDonald et al. (1981) as the amount of Zn required by plants used for animal fodder (50 mg kg⁻¹). However, there were no significant differences among the application rates of each Zn source with respect to dry matter yield.

In the calcareous soil, all the Zn treatments applied in the first crop increased dry matter yield in the second crop, and increasing the application rate of each Zn source produced an increase in dry matter yield, except for the Zn-AML source. It was noticeable that the control exhibited Zn tissue concentrations of almost 20 mg kg⁻¹, which is cited by some authors (Alloway 2008; Jones 2001) as the critical concentration for dried whole shoots of navy beans. However, only direct application to the soil of the Zn-S,S-EDDS chelate (at both rates) and Zn-AML chelate (at the high rate) increased Zn concentrations in plant dry matter. Even so, at the very most, this increase amounted to about 7 mg Zn kg⁻¹. All treatments therefore produced Zn concentrations of less than 50 mg Zn kg⁻¹ and none of the treatments improved the nutritional value of the second navy bean harvest.

Discussion

Metal leaching study

The application of organic Zn complexes to the soil produced different cumulative quantities of collected Zn in the leachates. These quantities depended on the Zn source used, the soil type and the length of time after Zn was last applied.

In previous papers (see Gonzalez et al. 2007, 2008a, b), only the Zn-S,S-EDDS chelate produced a significant amounts of total leached Zn for both soils in the first crop. These amounts were higher in calcareous soil than in acidic soil, despite calcareous soil presenting some physical and chemical characteristics that favoured the retention of Zn (its alkaline pH, high smectite clay and free carbonate content), this soil was more permeable than acidic soil (see Table 1).

In contrast, in the second crop (see Figs. 1 and 2), leaching was greater in the acidic soil than in the calcareous soil. In the acidic soil, the homogenization of the soils after the first harvest produced a redistribution of micronutrient throughout the soil, which favoured Zn leaching of the less mobile sources (Zn-AML and Zn-PHP) and facilitate the lixiviation of the Zn applied as Zn-S,S-EDDS, at a rate of 10 mg kg⁻¹, that was not leached in the first crop year. According to Hauser et al. (2005) and Luo et al. (2006), the Zn-S,S-EDDS chelate would also have biodegraded in the soil with its Zn being

retained by soil components. This could also explain the smaller amounts of Zn leached in the second crop, particularly at the lowest Zn rate. Furthermore, leached Zn from the Zn-AML and Zn-PHP sources showed maximum Zn concentrations between the fifth and seventh leachate portions (see Fig. 1b), i.e. with in the 1,000–1,400 mL interval of leached volumes (0.312 and 0.436 pore volumes, respectively). After that, Zn was retained by the soil and Zn leaching was limited. This could be explained by recovery of the soil structure (Duchaufour 1987) after these portions had been leached, as reported by Alvarez et al. (2001) for a similar soil in a column experiment.

In the calcareous soil, the change in the total amount of leached Zn from the first to the second crop could be explained by losses of Zn from the soil during the first crop year and—according to Lu et al. (2005), Ma and Uren (1998) and Obrador et al. (2002)—by the aging effect that changes Zn from labile pools to more residual pools.

The amount of Zn estimated to be easily leachable was determined by the $BaCl_2$ extraction procedure, after the first harvest. Estimated amounts of easily leachable Zn were much greater than the amount of Zn actually leached during the second crop year in the control and in all the Zn treatments involving both soils (Fig. 4). In this study with Zn chelates of natural origin, the amount of Zn estimated as being easily leachable and the amount of Zn actually leached did

not correlate by linear regression analysis, although the experimental conditions did favour Zn leaching, because a sufficient number of irrigations were performed in order to collect ten leachate portions (each of 200 mL). These results indicated that this extraction (Schultz et al. 2004) was not appropriate for predicting Zn leaching in subsequent crops to which natural chelates with limited mobility were applied.

Potential availability and distribution of zinc fractions in second-cropped soils

DTPA-TEA-extractable Zn in the control soils slightly decreased in the second crop with respect to the original soils (see Tables 1 and 2). For both soils and crop years, the order (from highest to lowest) of available Zn concentrations extracted using the three different methods (mean values for all repetitions and fertilizer treatments) were: Mehlich-3, DTPA-AB and DTPA-TEA. It is also interesting to note that in soils fertilized with the higher rate of the Zn-S,S-EDDS chelate, as in the control treatments, the available Zn slightly decreased during the second crop year. In contrast, in both soils fertilized with the Zn-PHP and Zn-AML chelates, the available Zn increased during the same period. This could have been due to the fact that the Zn-S,S-EDDS chelate is biodegraded in the soil and that the aging effect produced a reduction in the availability of Zn. According to Nörtemann

Fig. 4 Amounts of easily leachable Zn after the first harvest and Zn really leached during the second crop year in the control and in all the Zn treatments involving both soils. *Vertical bar* at each of the data points represents the standard error from the mean
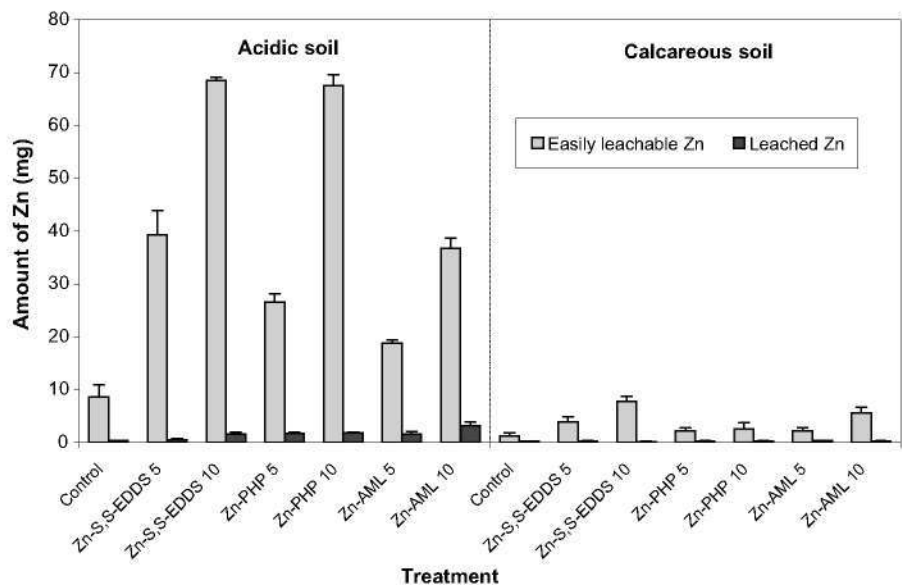
(2005), the aminopolycarboxylate chelating agents (e.g. EDDS) generally form complexes with relatively low or moderately high stability constants which are readily degradable. However, the Zn-PHP and Zn-AML chelates are immobile sources of Zn and they did not exhibit any significant leaching during the first crop year and therefore were not lost and were able to provide available Zn to the second crop. For a typical inorganic Zn fertilizer, such as $ZnSO_4$, a rapid decline in DTPA extractable Zn has been reported during the first year after application (Boawn 1974). In contrast, in our study, Zn-PHP and Zn-AML organic fertilizers of natural origin exhibited the opposite behaviour, since available Zn slightly increased during the second crop year.

The distribution of Zn fractions depended on the soil type and fertilizer treatments applied (see Fig. 3). It is interesting to note that during the second crop on the acidic soil only Zn-AML at application rates of 5 and 10 mg kg^{-1} and Zn-PHP at the lower application rate produced an increase in Zn content in the WSEX and OC fractions. However, in the calcareous soil the Zn content in the WSEX decreased from the first to the second crop in all the fertilized soil as well as in the control soil. In contrast, the Zn content in the CAR fraction increased and the greatest increase was associated with the Zn-AML and Zn-PHP sources at both application rates. These sources also produced a greater increase in the OC fraction. This confirms that Zn added to soils (in various forms) was slowly transferred from labile fractions (such as WSEX) to more residual fractions (such as CAR and OC) and that this aging effect was more important in the calcareous soil than in the acidic soil. Following an incubation experiment in calcareous soils involving the addition of $ZnCl_2$, Jalali and Khanlari (2008) observed that the metal content associated with the most weakly bound fraction tended to decrease, while there were corresponding increases in other more strongly bound fractions during incubation. Xiang et al. (1995), also observed a more rapid transformation of labile fractions to more stable Zn fractions in calcareous than in acidic and neutral soils.

According to Ure (1995), the extractants DTPA and EDTA could be used to extract elements from: water soluble, exchangeable, sorbed and organically bound pools and also from bound pools occluded in oxides and secondary clay minerals. This could possibly explain why, despite the observed decrease in WSEX fraction in the calcareous soil in all the Zn fertilizer treatments and in the control soil, available Zn generally increased in soils fertilized with the Zn-PHP and Zn-AML chelates.

It is also interesting that there were positive and highly significant correlations between the three methods used to estimate available Zn (r ranged from 0.95 to 0.99; $P<0.0001$). This seems to suggest that they could be used in a similar way to predict the availability of Zn for plants. The amount of soil-extractable Zn for three DTPA-TEA and DTPA-AB extractions was also positively correlated with Zn concentration in the WSEX and RMO extracted fractions (r ranged from 0.90 to 0.92; $P<0.0001$). Furthermore, Zn concentrations in the WSEX and RMO fractions were negatively correlated with the Zn concentrations in the most residual forms (AMC, CFeO and RES fractions; r ranged from −0.73 to −0.81; *P ranged from* <0.05 to <0.001) which were also intercorrelated.

In this experiment, the evolution of the pH and Eh parameters in soils was not clearly related to the source of Zn used and was probably influenced by the amount of water drained and the urea added as basal fertilization. According to Haynes and Swift (1987) and Barak et al. (1997), the urea added to the soil produces ammonium and consumes protons in the first phase, this increases the soil pH, and the subsequent nitrification of NH_4^+ cations produces acidification of the soil due to the production of protons. This last process is slow in acidic soils and this would explain the increase in pH of 0.7 observed in the acidic soil in this experiment. As the NH_4^+ cations produced are very soluble in soils (Malhi et al. 2000), they can be readily leached, causing an appreciable decrease in the pHs of leachate. The nitrification process is fast in calcareous soils, which would explain the decrease in the pH of this soil (by 0.4). This relatively small decrease in pH, despite the great production of protons associated with the nitrification process, was probably due to the buffer effect of the calcareous soil. This would also explain the very limited variation observed in the pHs of the leachates.

The observed variations in Eh in the soils and leachates could have been partly due to the washing effect of adding water to ionic species. In addition, as previously was indicated, a slight increase in the Eh of leachates could be due to the ambient atmosphere oxidation. In our study, the evolution over time of each pH and Eh parameter showed opposite behav-

iour for soils and leachates. At the end of the experiment, the pH and Eh parameters of the two soils jointly correlated with those determined for the leachates ($P<0.0001$). Values of soil pH and Eh corresponded to normal (oxic) soils [pH+pe>14, Patrick et al. (1996)]; however acidic soil exhibited more oxidant conditions than calcareous soil. The experimental conditions associated with the crop produced a decrease in the oxidant status of both soils.

Navy bean response to zinc fertilization

The Zn uptake varied with soil type and fertilizer treatment. In the acidic soil, the Zn-AML produced the highest levels of Zn uptake, with respective increases for the 5 and 10 mg Zn kg^{-1} rates of 2.97 and 6.44 mg Zn per lysimeter with respect to the control treatment. The other two Zn fertilizers provided similar increases in Zn uptake which ranged from 1.55 to 3.76 mg Zn per lysimeter for the same Zn application rates. In the calcareous soil, however, there were only small differences in Zn uptake by plants between the control treatment and all the other Zn treatments and the increases ranged from 0.20 for the 5 mg Zn kg^{-1} rate of Zn-PHP to 0.51 mg Zn per lysimeter for the 10 mg Zn kg^{-1} rate of Zn-S,S-EDDS. It is interesting to note that an increase in the rate of fertilizer application (from 5 to 10 mg Zn kg^{-1}) produced an increase in Zn uptake, although this increase was much smaller in calcareous soil.

For each soil, multifactor variance analysis showed that the crop year significantly influenced the Zn uptake by navy bean plants. For all the fertilized soils, both dry matter yield and Zn concentration in the navy bean crop decreased from the first (see Gonzalez et al. 2007, 2008a, b) to the second harvest (in both soils $P<0.0001$). Furthermore significant interactions were obtained between crop-year and treatment factors ($P<0.01$). In the acidic soil, the Zn-S,S-EDDS chelate produced the greatest decrease in Zn uptake (with decreases for the 5 and 10 mg Zn kg^{-1} rates of 1.50 and 2.04 mg Zn per lysimeter, respectively). In the calcareous soil, however, the greatest decreases occurred with the high application rates of the Zn-S-S-EDDS (0.48 mg Zn per lysimeter) and Zn-AML (0.73 mg Zn per lysimeter).

The observed behaviour of the S,S-EDDS chelate was probably due to the greater mobility, which produced greater losses of Zn due to leaching than the Zn-PHP and Zn-AML chelates. The homogenization of the soil before the second harvest may have redistributed Zn throughout the soil, thereby improving the interception of Zn by plant roots. This could also have made Zn from less mobile sources more accessible to plants (e.g. Zn-PHP and Zn-AML sources). However, the aging effect reduced the Zn uptake from all Zn chelates in the second harvest and new applications of all Zn fertilizers would therefore probably be required each year to prevent Zn deficiencies in calcareous soil. In the acidic soils, however, new applications of Zn fertilizers would not be necessary for successive crops for several years. These results also indicate that calcareous soil, which had a free carbonate content, higher pH and greater clay content than the acidic soil, suffered a more pronounced aging effect.

In addition, the amounts of Zn removed by crop uptake and leaching during the first harvest were therefore significant in some cases and could partially explain the variations observed in the availability, leaching and uptake of Zn by plants in the second harvest. In this second crop year, Zn mass balances were performed considering the amounts of total Zn initially present in soils and recovered from the leachates (see Figs. 1 and 2) and plants (see Table 3), and total Zn in soils after crop (see Fig. 3). In the acidic soil, the Zn percentages recovered ranged from 101% for the control treatment to 106% for the 10 mg Zn kg^{-1} rate of Zn-AML, and in the calcareous soil, they ranged from 98% for the control treatment to 103% for the 5 mg Zn kg^{-1} rate of Zn-PHP. Therefore, in the second crop year the mass balance showed percentages of recovered Zn of around 100%, as also occurred in the first crop year (Gonzalez et al. 2007, 2008a, b).

Significant correlations and positive coefficients were observed between Zn uptake and the WSEX and RMO Zn fractions ($P<0.0001$, $n=14$). It is interesting to highlight the relationship between the Zn uptake by the plant and the more labile Zn fraction:

$$\mathrm{Zn-uptake} = -0.02 + 2.00 \times \mathrm{Zn-WSEX}$$

$$\left(R^2 = 0.96\right)$$

In our study, it was therefore possible to predict the Zn uptake by the navy bean crop with a significant level of accuracy by determining the water soluble plus exchangeable fraction in the soil at the moment

of the harvest. High correlations were also obtained between Zn uptake and DTPA-TEA-, DTPA-AB- and Mehlich-3-extractable Zn (with respective probabilities of less than 0.0001, 0.0001 and 0.01). Soluble Zn, in both dry and fresh matter and total Zn concentration in plant dry matter showed similar tendencies in the correlations that the previously presented for the Zn uptake. However correlations for soluble Zn in fresh matter exhibited a lower degree of significance or even showed $P > 0.05$.

In this experiment, the relationship between total Zn concentration and soluble Zn concentration (extracted with reactive MES) from dry matter could the expressed by the equations:

$$\text{Total-Zn} = -34.47 + 3.63 \times \text{Soluble-Zn}$$

$$\left(R^2 = 0.93, \; P < 0.0001\right)$$

Therefore, the Zn soluble MES in dried whole shoots of navy bean could be used to estimate the nutritional state of plants with respect to this microelement.

In conclusion, this study showed that Zn source and soil characteristics have a great influence on the residual effect of Zn fertilization. The residual effect after 2 years of natural Zn chelates depended on the aging effect and also on Zn losses. The aging effect was greater in calcareous than in acidic soils. In both soils, there were significant decreases from the first to the second crop for the three natural organic Zn chelates in terms of total leached Zn, Zn concentration in the most labile fraction (WSEX) and total Zn uptake by plants. This effect was greater for the Zn-S, S-EDDS chelate than for the Zn-AML and Zn-PHP sources. In the second crop grown in calcareous soil, there was no significant lixiviation of micronutrient. However, the three Zn sources applied to the acidic soil were associated with Zn leaching (although in amounts of less than 3.5% of total Zn applied), with the Zn-S,S-EDDS being the source that produced the lowest level of Zn leaching. In the acidic soil, the Zn-AML treatments produced the highest levels of Zn uptake, and similar levels were taken up by navy bean plants for the three treatments in the calcareous soil. New applications of Zn would be necessary to prevent Zn deficiencies in the navy bean plants of a subsequent crop grown in a calcareous soil. For a crop grown in an acidic soil, it would not be necessary to apply a new Zn treatment. It is necessary to conduct further studies into the residual effect over a longer period. According to the findings of other researches (Boawn 1974; Brennan 2001), inorganic Zn sources such as ZnO or $ZnSO_4$ have a greater residual effect in calcareous soils than the natural sources of organic Zn chelates used in this experiment.

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