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Influence of the Soil/Solution Ratio, Interaction Time, and Extractant on the Evaluation of Iron Chelate Sorption/Desorption by Soils

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ABSTRACT: Synthetic Fe chelates are the most efficient agricultural practice to control Fe deficiency in crops, EDTA/Fe^{3+} and *o,o*-EDDHA/Fe^{3+} being the most commonly used. Their efficacy as Fe sources and carriers in soils can be severely limited by their retention on it. The aim of this work is to evaluate the possible bias introduced in the studies of the iron chelate retention by soils. For that purpose, results obtained for EDTA and EDDHA iron chelates from two batch studies with different soil/solution ratios were compared with data obtained for a leaching column experiment. Moreover, different extractants were tested to study the *o,o*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} desorption from a calcareous soil, and also the effect of the interaction time in their retention process has been evaluated. In summary, the mobility through a calcareous soil of the studied iron chelates differs greatly depending on the type of iron chelate and also on the procedure used to evaluate the retention and the soil/solution ratio used. In general, the leaching column method is preferred because the achieved conclusions are more representative of the natural conditions, but batch methods are very useful as a preliminary experiment, especially one with a high soil/solution ratio. The iron chelate desorption could be quantified by using a sequential extraction with water, sodium sulfate, and DTPA as extractants. Under the experimental conditions used in this study, *o,o*-EDDHA/Fe^{3+} retention increased with interaction time.

KEYWORDS: iron chelates, soil, retention, desorption, batch, column

INTRODUCTION

Iron chlorosis is a plant nutritional disorder mainly occurring in alkaline and/or calcareous soils. Intervenial leaf yellowing, due to the reduction of leaf photosynthetic pigments concentration, is the most characteristic visual symptom in Fe-deficient plants, which display severely reduced fruit quality, size, and yield.[1] Fe chlorosis is a limiting factor for agricultural production in many areas in the world. In the Mediterranean region, for example, it was estimated that from 20 to 50% of fruit crops are affected by Fe deficiency symptoms.[2] Currently, the use of synthetic Fe chelates derived from polyamine−carboxylic acids is the most common and efficient agricultural practice to control Fe chlorosis,[3] the iron chelates of ethylenediaminetetraacetic acid (EDTA) and ethylenediaminedi-(*o*-hydroxyphenylacetic) acid (*o,o*-EDDHA) being the most used.

The synthesis pathway applied for manufacturing commercial *o,o*-EDDHA/Fe^{3+} products is a Mannich-like reaction[4,5] that produces a mixture of three regioisomeric compounds, *o,o*-EDDHA, *o,p*-EDDHA, and *p,p*-EDDHA, in variable amounts. After the addition of the inorganic Fe salts to the chelating agent to form the chelate, the *o,o*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} complexes are formed, but the *p,p*-EDDHA does not form the Fe^{3+} complex because the two *p*-hydroxyphenyl groups are sterically impeded to bind Fe^{3+}.[6] The aromatic ligand *o,o*-EDDHA contains two chiral carbons. Hence, four possible isomers are (*R,R*), (*R,S*), (*S,R*), and (*S,S*), but owing to the internal symmetry of the molecules, the (*R,S*) and (*S,R*) enantiomers are the same molecule denoted as the meso isomer. The (*R,R*) and (*S,S*) enantiomers are mirror images that cannot be separated by chemical processes, and generally they are called the racemic mixture.[6]

The retention of a compound in the soil profile controls the mobility of many substances in the environment. The sorption of chelates in soils has been recognized in early investigations of the agricultural uses of synthetic chelating agents; it had been concluded that the effectiveness of Fe chelates as Fe sources and carriers in soils can be severely limited by the retention of Fe chelates or chelating agents on the solid phase.[7−9] The factors affecting sorption include the type of chelating agent, the metal ion, time, pH, salt concentrations, and soil texture. The iron chelate retention on soils has been quantified by several methodologies; it is generally accepted that the measurement method has a great influence. Particularly, the background ionic medium, the solid/solution ratio, and the use of flow-through or closed reactor used to quantify the soil retention are of major importance.[10]

In many cases, the ratio of solid mass versus solution volume should theoretically not influence the proportion of adsorbed compound. However, numerous authors observed a significant and nonlinear dependence of the solid/solution ratio on the amount of adsorption.[11] Adsorption is often observed to decrease with the solid "concentration". This phenomenon is called the "solid effect". The solid/solution ratio of soils is too high to be used in batch experiments, but can be achieved in column experiments. For batch experiments with these natural porous media, the range from 1 g of solid for 2 mL to 1 g of solid for 4 mL is advised.[11] However, for strongly adsorbed compounds, this ideal solid/solution ratio is sometimes too high to detect the compound remaining in solution.[10] Many authors[10] considered that the best experimental choice is a solid/solution ratio that is representative of the natural conditions.

On the other hand, the repacked column method, in which the column is packed with the solid particles and the solution goes

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through, has the advantage of being an open-flow method. Therefore, the chemical kinetics and the release stage can be studied more easily than with the batch method.[12] Moreover, the solid/solution ratio is representative of natural porous media. The disadvantages of this method are, for example, that the duration of the experiment can be long, especially for strongly adsorbed solutes; the system is not perfectly mixed; and hydrodynamic dispersion, preferential pathways, immobile water, etc., may occur.

To improve the knowledge on the interaction of FeEDDHA isomers with soil and soil constituents, several studies have been done.[8,9,13,14] Organic matter and iron (hydr)oxides have been identified as the most reactive and calcium carbonate and clay (calcium montmorillonite) as less reactive soil constituents with respect to EDDHA/Fe^{3+} sorption.[15] Most of these studies used a batch experiment design with different soil/solution rates. Moreover, Lucena et al.[16] studied the interaction of different iron chelates with several soils and soil constituents by three different approaches: a theoretical modeling using the speciation program MINTEQA2, a batch experiment, and a leaching column test. They concluded that modeling helped to detect ineffective chelates, but it will not distinguish among the more stable ones.

The determination of soluble chelate in batch, column, and incubation experiments is a useful tool to compare the reactivities of different chelates and to determine the long-lasting effect of the chelate at the soil solution. However, these methods do not provide information about the fate of the retained chelate. Processes involved in Fe chelate diminution from the solution can be related to degradation, displacement, or sorption mechanisms.[3] A reversible sorption mechanism of the chelate onto the soil surfaces will increase the long-lasting efficacy of the chelate in agronomic conditions. For that reason, in this paper we studied the chelate desorption by extracting it with different extractants.

To evaluate the efficiency of the chelates as correctors of Fe deficiency and to study the potential mobility of the chelates in the soil profile, it is important to have a reliable methodology to assess the soluble and retained fractions of the chelates after the interaction with soils. This methodology should be obtained, keeping in mind the real soil conditions such as the solid/solution ratio.

The aim of this work is to evaluate the possible bias introduced in the studies of the iron chelate retention by soils due to the soil/solution rate, interaction time, and methodology used (batch or repacked columns) and to study different extractants for the desorption of the retained chelate on the soil.

EXPERIMENTAL PROCEDURES

Iron Chelate Solutions. The chelating agents used were *o,o*-EDDHA (Promochem), *o,p*-EDDHA provided by Syngenta Crop Protection, and Na_2EDTA (Merck). For the preparation of the Fe *o,o*-EDDHA and Fe *o,p*-EDDHA solutions, ligands were dissolved in sufficient NaOH (1:3 molar ratio). For the FeEDTA solution, the chelating agent was dissolved in deionized water. Then an amount of $FeCl_3 \cdot 6H_2O$ (Merck), calculated to be 5% in excess of the molar amount of ligand, was slowly added. During chelation, the pH was maintained between 6.0 and 8.0 and adjusted to 7.0 at the end. Solutions were left to stand overnight to allow the excess Fe to precipitate as oxides. Final solutions were filtered through Whatman no. 2 filters and made up to volume with deionized water. Light exposure of all chelate solutions was avoided during their preparation and storage because of their potential photodecomposition.

Table 1. Physicochemical Characteristics of the Soil Used

texture	sandy loam
sand	65
silt	12
clay	23
pH (H_2O)	7.69
pH (KCl)	7.15
EC extract 1:5 (dS m^{-1})	0.235
oxidized OM (g kg^{1-})	8.0
$N_{Kjeldahl}$ (g kg^{-1})	0.79
C/N	10.5
$CaCO_3$ total (g kg^{-1})	150
active lime (g kg^{-1})	40
macronutrients (Soltampour, $cmol_c$ kg^{-1})	
Ca	1.76
Mg	1.72
K	1.26
Na	0.15
micronutrients (Soltampour, mg kg^{-1})	
Fe	14.3
Mn	13.0
Cu	1.5
Zn	1.1

Soil Characterization. The soil used was from Carlet (Valencia, Spain) and supports a peach orchard with obvious chlorotic symptoms. Its physicochemical characteristics are described in Table 1.

Batch Experiments. Two types of batch experiments were done. For the first set, 5.0 g of the soil was introduced into a 60 mL plastic stoppered bottle, and then 25.0 mL of a 25 mg Fe/L solution of each iron chelate, 0.01 M HEPES, and 0.01 M $CaCl_2$ 0.01 M solution were added. Additional bottles with the soil and with no chelate addition and with the chelate solution without the solid material were prepared. Bottles were covered with aluminum foil to avoid light exposure. They were stoppered and shaken for 1 h and left to stand for 3 days at 25 °C.[17] Three replicates were done for each chelate and blank. The interaction pH was 7.4 ± 0.2.

In the second set of experiments, 5.0 g of the soil thoroughly mixed with 5.0 g of quartz sand was introduced into 10 mL plastic tubes. Chelate solutions were applied to each tube following the sequence 0.5 mL of deionized water, 0.5 mL of the chelate solution (60 mg Fe/L), and 0.5 mL of deionized water. The amount of liquid was previously estimated to maintain the soil around its water-holding capacity. Tubes were left open to the air. The interaction was evaluated over time at 0, 1, 2, 4, 8, 16, and 30 h and at 1, 2, 4, 8, 16, and 32 days after the application of the chelate. Three replicates were done for each chelate and time. Tubes with soil and without chelate addition were also included in the assay. Samples were left to stand in the dark at 25 °C. The water-holding capacity was maintained in the samples during the experiment by weighing the tubes and adding water when necessary. After the interaction time, samples were introduced in 50 mL centrifugation tubes. To each tube was added 5.0 mL of deionized water, and they were shaken for 15 min and then centrifuged for 5 min at 313*g*, filtered with Whatman no. 42 filters, and introduced into 25 mL volumetric flasks. This process was repeated three times, and the final solutions were made up to volume with deionized water. Total Fe (AAs) and Fe-chelate fraction (HPLC) were determined.

Soil Columns. A 50:50 soil/quartz sand (HCl acid washed and then with deionized water, diameter = 1−3 mm) mixture (w/w) was placed in 50 × 6 cm plastic columns, until 20 cm height (approximately 150 g of soil per column). At the bottom, 10 cm of sand was placed to

Table 2. Extractants Tested To Desorb the Iron Chelate Fe-*o,o*-EDDHA from the Soil

extractant	composition
H_2O	H_2O type I water (Milli-Q)
Na_2SO_4	Na_2SO_4 0.1 M
Na_2SO_4 (pH 4.0)	Na_2SO_4 0.1 M buffered at pH 4 (AcH/Ac^-)
$NaHCO_3$	$NaHCO_3$ 0.5 M
DTPA	DTPA 0.005 M in $CaCl_2$ 0.01 M and TEA at pH 7.8
sequential extraction	
$H_2O + Na_2SO_4$	
$H_2O + NaHCO_3$	
$H_2O + Na_2SO_4$ + DTPA	
$H_2O + NaHCO_3$ + DTPA	

benefit the leaching process, as well as 2 cm at the top to prevent the soil from evaporating. The columns were situated on the top of Büchner funnels. Between each funnel and column a cellulose filter (Whatman no.42) and a plastic grid were interposed to avoid soil losses. The sand was acid washed, and a mixture between soil and sand was used to aid the leaching process, due to the soil texture, which may make leachate collection difficult.

The soil was brought around to its water-holding capacity by adding deionized water and was left to stand for 24 h. A single 2 mL dose of 500 mg Fe L^{-1} solutions of FeEDTA, Fe-*o,o*-EDDHA, and Fe-*o,p*-EDDHA was added to the top of each column. Then, each column was irrigated with 50 mL of deionized water. The experiment was conducted for 27 days, and every 2 days columns were irrigated with 50 mL of deionized water, leachates were collected in plastic containers, and then total and chelated Fe amounts were analyzed. Five columns per treatment were done. Each column was covered with aluminum foil to avoid photodecomposition of the iron chelate.

No significant differences in leachate pH have been found between the different treatments during the experiment. The average leached volume for each column and sampling time was 37 mL.

Desorption Experiments. Ten grams of the soil/sand mixture (50:50 w/w) was placed into polycarbonate test tubes and then 0.5 mL of water, 0.5 mL of the standard solution of *o,o*-EDDHA/Fe^{3+} or *o,p*-EDDHA/Fe^{3+} (60 mg L^{-1}), and 0.5 mL of water were consecutively added. The total liquid amount of 1.5 mL corresponded approximately to the field capacity of the soil. After 24 h of interaction at 25 °C in open tubes, several extractants were assayed (Table 2).

Three consecutive extractions with 5 mL of the corresponding extractant were done for each sample. Each extraction was done for 15 min in a rotary shaker, followed by 5 min centrifugation at 704*g* and filtration through 0.45 μm Millipore membranes. The final volume was made up to 25 mL. Three replicates were done for each soil sample, and three control tubes per extractant were also carried out containing soil and water (1.5 mL) but without chelate. For the sequential extractions, after an extraction process as described above had been completed, another extraction with the corresponding extractant was done to the same soil sample. For all of the extractions, total and chelated Fe amounts were assessed, except for the DTPA extraction, in which only total Fe (AAs) was determined.

Effect of the Interaction Time on the Chelate Desorption. Once the conditions of the extraction procedure and the extractants were established, a second experiment was developed using several interaction times between the iron chelate and the soil.

The procedure was similar to that presented in the first experiment. The chelates tested were *o,o*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Fe^{3+} (20 mg L^{-1} of Fe). Two grams of the soil/sand mixture was placed in polycarbonate tubes, and 0.5 mL of the chelate solution was added to the tubes. Incubation times were 1, 3, 6, and 13 h and 1, 2, 4, 6, 8, 15, 30, and 50 days, and the extraction sequence was water, water + sulfate and water + sulfate + DTPA. Each extraction was made three times with 3 mL of extractant, and then the total volume was raised to 10 mL.

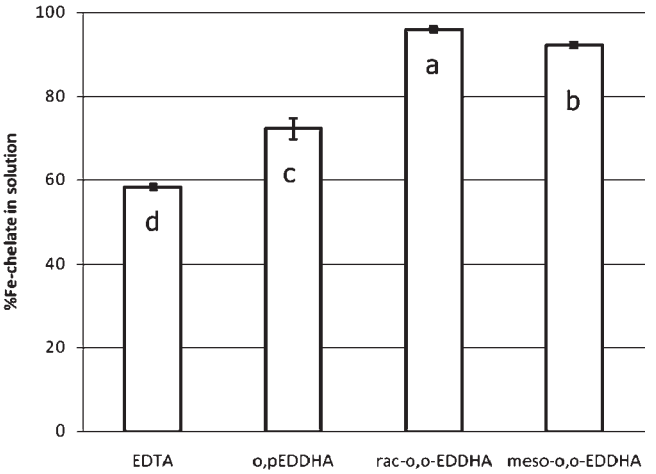

Figure 1. Percentage of Fe chelate that remained in solution after 3 days of interaction between the soil (5 g) and the chelates (25 mL). Different letters indicate significant differences among chelates according to Duncan's multiple-range test ($\alpha > 0.05$). $n = 3$.

Incubation was made in darkness at 25 °C and with the tubes open to the atmosphere. To correct evaporation in long incubation periods, sample weight was controlled and water was added to reach the initial weight every 3−4 days. Average evaporation was <0.08 g per day. Five replicates per sample were prepared. Also, three controls of soil (soil with water instead of chelate) and three controls of chelate (chelate without soil) were used for each period. For all of the extractions, total and chelated Fe were assessed, except for the DTPA extraction, in which only total Fe (AAs) was determined.

Analytical Determinations. The pH was measured with an Orion Research ion analyzer EA 920. Total Fe and Cu concentrations were assessed with atomic absorption spectrophotometry (AAS), using a Perkin-Elmer 4000 spectrophotometer. The Fe chelate content was determined using an ion pair HPLC method.[18] All samples and iron chelate solutions for analysis were filtered through Millipore 0.45 μm filters. The column was a Waters Symmetry C_{18} (150 × 3.9 mm i.d., d_p = 5 μm) connected to HPLC equipment with a Waters 2690 separation module (Alliance), a Waters 996 photodiode array detector, and Empower chromatography data system. The mobile phase consisted of 30% acetonitrile (HPLC grade, Riedel-Häen) and 0.03 M tetrabutylammonium hydroxide (40% Sigma, TBAOH), pH 6.0. The solution was filtered through 0.45 μm Millipore filters.

Data were statistically evaluated by one-way analysis of variance (ANOVA) by using the SPSS program (Statistical Package for the Social Sciences) v. 15.0. Means were also compared using Duncan's test at $P < 0.05$ to find significant differences.

RESULTS AND DISCUSSION

Batch Experiments. The first set of batch experiments (5 g of soil, 25 mL of chelate solution, 3 day interaction time) showed significant differences between the chelate retention on this soil (Figure 1). The highest retention rate was observed for the FeEDTA chelate (approximately 40% of it was not recovered). This fact could not be completely attributed to surface sorption processes, because it also can be explained in terms of the iron chelate stability. As the pH rises above 6.5, the ability of EDTA to chelate iron(III) drastically decreased because the chelate was

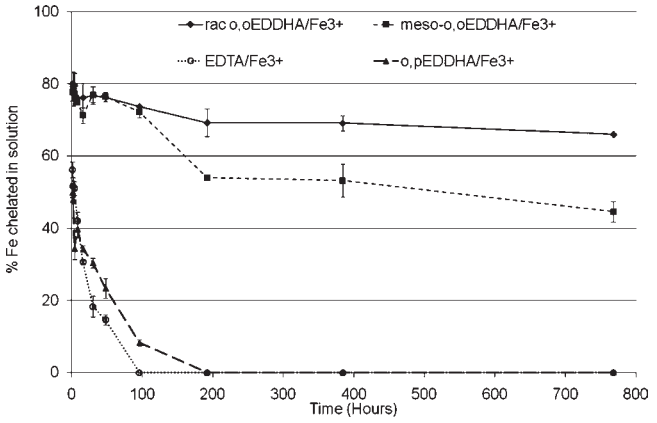

Figure 2. Percentage of Fe chelate that remained in solution over time after the interaction between the soil (10 g of soil/sand mixture 50:50 w/w) and the chelates (1.5 mL). $n = 3$.

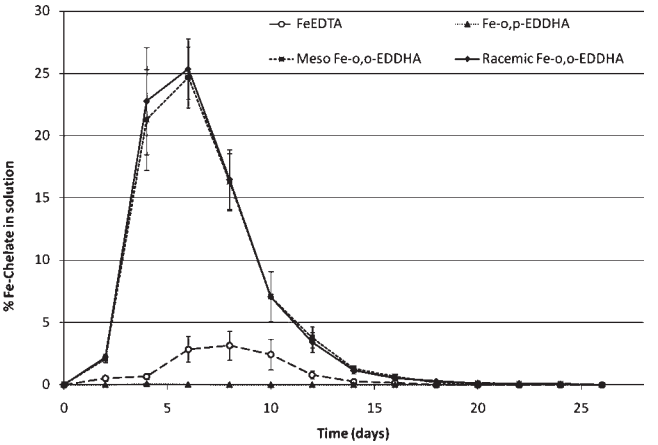

Figure 3. Percentages of FeEDTA, racemic Fe-*o*,*o*-EDDHA, meso Fe-*o*,*o*-EDDHA, and *o*,*p*-EDDHA leached over time from the soil columns. $n = 5$.

broken due to the Zn, Mn, or Ca substitution and caused iron precipitation.[16] At this assay, the interaction pH was 7.4 ± 0.2, so it is supposed that part of the iron was not in a chelated form and was probably precipitated as iron oxides. The AAS measurements assessed only the soluble iron, and the HPLC method used was unable to determine the chelating agent with other cations, so this could induce an overestimation of the chelating agent sorption. However, the *o*,*o*-EDDHA/Fe^{3+} chelate is stable at the typical calcareous soil pH range, so data obtained for this chelate would more precisely reflect its sorption on soils. Moreover, it was determined, by using AAS and colorimetric measurements, that the relationship between total (AAS) and chelated iron (absorbance at 480 nm) that remains in solution after interaction with a calcareous standard soil was 1:1.[17] This means that the whole chelate was retained, neither the chelating agent nor the iron alone.

If the racemic and meso isomers of the *o*,*o*-EDDHA/Fe^{3+} behavior were considered, the meso isomer was slightly more sorbed than the racemic isomer. This fact has previously been observed by several authors.[7,15] The racemic isomer may chelate Fe more strongly than the meso complex, thereby imparting a stronger anionic nature to the racemic complex. Then it was found that the stability constant for FeEDDHA was 2.26 log units greater for the racemic complex than for the meso complex, indicating a 500-fold difference in iron chelating ability.[19]

The *o*,*p*-EDDHA/Fe^{3+} retention was significantly higher in comparison to the ortho−ortho isomer (Figure 1), but lower than the one observed for the FeEDTA. Although *o*,*p*-EDDHA has only five functional groups able to complex the Fe^{3+} ion, its stability is much higher than the stability of EDTA/Fe^{3+} but lower than the one of *o*,*o*-EDDHA/Fe^{3+}.[20] The behavior of *o*,*p*-EDDHA/Fe^{3+} to maintain soluble Fe in different theoretical models is close to that of *o*,*o*-EDDHA/Fe^{3+}, because Ca^{2+} and Mg^{2+} are not important competitors; only the Cu^{2+} ion is a likely competitor with Fe^{3+} for the *o*,*p*-EDDHA ligand.[20] With limited Cu^{2+} availability, *o*,*p*-EDDHA/Fe^{3+} is stable, but when the soil presents a large Cu^{2+} availability, it can displace the Fe^{3+} from the chelate, reducing its availability to plants. The copper concentration of this soil was not so high, so the substitution of iron by copper was not expected.

A second set of batch experiments (5 g of soil, 5 g of sand, 1.5 mL of chelate solution, several interaction times) were done to compare the retention of the same chelates at the same soil at different interaction periods. The data obtained (Figure 2) indicated a trend similar to the one observed for the previous batch experiment. The EDTA/Fe^{3+} chelate remained in solution for approximately 5 days after the beginning of the experiment, but at low concentration. As previously mentioned, this was related to the chelate stability at the interaction pH and the competing cations of the soil. In a similar way, after 1 week, *o*,*p*-EDDHA/Fe^{3+} had disappeared from solution almost completely. Both *o*,*o*-EDDHA/Fe^{3+} isomers remained in solution to a much larger extent. In accordance with other adsorption and soil interaction studies, the racemic isomer concentration was almost constant, displaying hardly any decrease.[8,9,15] The meso isomer that remained in solution showed some initial decrease in concentration until the first week and after that it was almost constant. This behavior was previously found for different soils with a soil/solution ratio of 1:1 (w/v) and for interaction periods between 1 and 6 weeks.[7]

Column Experiment. The chelated Fe leaching curves (Figure 3) followed nearly the same shape for all of the products tested, with the exception of *o*,*p*-EDDHA/Fe^{3+}, which was not leached from the columns at all. There are 2−5 days until the chelates were detected in leachates; then their concentration increased until a maximum and decreased until it disappeared, being under the detection limit for the last 10 days of the experiment. The EDTA/Fe^{3+} did not appear in leachates until the sixth day from the beginning of the experiment due to the great amount of solid used and the length of the column; then a small amount was found in leachates until day 12, and after this day no EDTA/Fe^{3+} was detected. This fact could also be related to kinetic aspects of the substitution of Fe from the chelate by other cations (pH was around 8.3 ± 0.4) or by the sometimes strongly kinetically controlled retention/release phenomena.[10] However, no *o*,*p*-EDDHA/Fe^{3+} was detected at the highest soil/solution rate. For the *o*,*o*-EDDHA/Fe^{3+} enantiomers, a maximum was reached the sixth day, with a percentage of iron chelate found in leachates of 25.4 and 24.7% for the racemic and meso isomer, respectively (Figure 3). After day 16, the amount of both isomers in leachates was negligible. The total isomer concentration calculated by adding the leachates chelate content (Figure 4) showed that >80% of the chelate was collected after 28 days (90.9% for the racemic and 83.6% for the meso isomer), but no significant differences between enantiomer retention by the soil

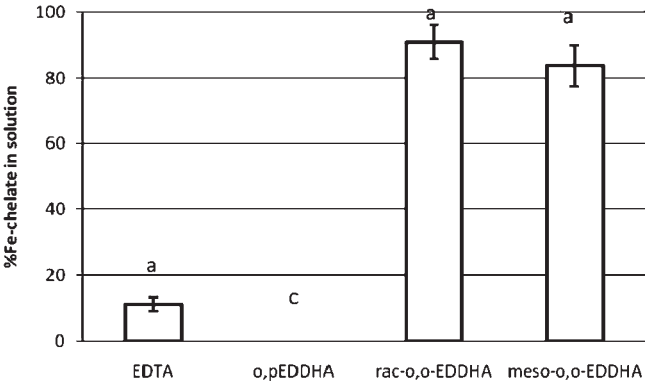

Figure 4. Total percentage of iron chelate leached in 28 days of the column experiment. Different letters indicate significant differences among chelates according to Duncan's multiple-range test ($\alpha > 0.05$). $n = 5$.

were observed. For the same chelate, but for another calcareous soil in a column experiment,[16] it was observed that only 70% of the *o,o*-EDDHA/Fe^{3+} added percolated from the column.

In this experiment a single dose with a great amount of *o,o*-EDDHA/Fe^{3+} has been applied, and most of this chelate has been collected. This could mean that the addition of EDDHA/Fe^{3+} to crops once or twice per year in light soils could favor the leaching of the chelate and the possible contamination of the superficial and subterranean waters, more than if they are applied in a continuous way. That is why a frequent application at low dose with the fertigation solution could be recommended due to the obtained column results.

A comparison between data obtained with a soil/solution ratio of 5 g/25 mL (Figure 1), 10 g of soil mixture/1.5 mL (Figure 2), and 300 g of soil mixture/50 mL (columns, Figures 3 and 4) (w/v) reached different conclusions. Although the trends in both batch experiments were similar, the percentage of chelate that remains in solution showed a great dependence on the soil/solution rate used. If both experimental batch sets were compared at the same interaction time (3 days), it can be seen, in general, that the iron chelate percentage that remained in solution in the 10 g soil/sand mixture + 1.5 mL solution set was much lower than the one observed when 5 g of soil interacted with 25 mL of solution. For the soil/solution ratio 5:25 (w/v) the values of the EDTA/Fe^{3+}, *o,p*-EDDHA/Fe^{3+}, racemic and meso isomer of *o,o*-EDDHA/Fe^{3+} found in solution were 58.2, 72.3, 96.0, and 92.2%, respectively. For the 10:1.5 ratio (w/v), the percentages of chelate in solution at the third day of interaction were 17.1, 25.0, 78.8, and 72.8%, respectively. From the column experiments, a completely different conclusion was reached. At the third day of interaction, a negligible amount of either EDTA/Fe^{3+} or *o,p*-EDDHA/Fe^{3+} was detected in leachates, so we can conclude that both chelates were retained by the soil, and <2% of racemic and meso isomers of *o,o*-EDDHA/Fe^{3+} was recovered. Nevertheless, the batch experiment with a high soil amount seemed to provide more accurate results than the batch methodology traditionally used to evaluate iron chelate retention by soils.

Previous data obtained with 5 g of soil, 10 mL of chelate solution 10^{-4} M (pH 8.0 HEPES, 0.1 M $CaCl_2$), and 3 days as interaction time[16] gave again a similar trend but different numerical data for the same soil and chelate product. For this soil/solution ratio the percentages of the EDTA/Fe^{3+}, *o,p*-EDDHA/Fe^{3+}, and *o,o*-EDDHA/Fe^{3+} found in solution were 45, 60, and 100%, respectively. Given an intermediate behavior between both batch experiments tested in this work, this was expected for its soil/solution ratio.

These data challenge the viability of the traditional batch assays as a tool to determine the persistence of iron chelates in calcareous soils. One reason could be that the sorption mechanisms are driven by various kinetically controlled reactions or physical phenomena, which have the largest variability of reaction times: from a few seconds to many years.[21] Short-term experiments are shown to provide higher values of chelate that remain in solution than long-term assays, and also the solid/solution ratio plays an important role in the amount of retention obtained. The best experimental choice is a solid/solution ratio that is representative of the natural conditions;[10] the solid/solution ratio of soils is too high to be used in batch experiments, but it can be achieved in column experiments. Moreover, maintaining or not a constant composition of the solution can influence the quantity of adsorbed compounds. In batch experiments the displaced competitive substances remain in solution and, thus, may interact with the solid. In contrast, the open-flow methods supply the system with a constant solution and the displaced substances were flushed out and thus did not compete for adsorption. Therefore, adsorption should be higher for the open-flow methods such as leaching columns.[11,22] On the contrary, other studies showed that the adsorption was higher in batch experiments with high solution doses than in an open-flow column.[23] The reasons for this paradoxical behavior were mainly (i) the presence of immobile water in the column, which acts as a kinetic barrier,[24] and (ii) the failure to achieve chemical equilibrium in the column if the mean residence time is significantly lower than the mean reaction time.[25] Because many systems of interest are open and dynamic in nature (solute diffusion in clays, solute transfers in soils and aquifers, particle transport in lakes or rivers, etc.), it can be thought that open-flow methods are closer to these natural conditions and thus should be preferred as often as possible.[26]

However, the batch experiment is by far easier to use than any other method, despite its numerous disadvantages. The solid/solution ratio is often either too high compared with the natural conditions in rivers, lakes, or seas or too low compared with the natural conditions of porous media. Moreover, the hydrodynamic conditions of natural porous media are not met. A long-term experiment with continuous shaking can lead to side reactions[27] such as the destruction of particles, which prevents the study of very slow reactions. As a consequence, the batch method is very useful as a preliminary experiment, but extrapolation to porous media requires other investigations. The repacked column method has the advantage of being an open-flow method, so the chemical kinetics and the release stage can be studied more easily than with the batch method.[8] Moreover, the solid/solution ratio is representative of natural porous media. Furthermore, the experiments can be run either in water-saturated or unsaturated conditions.[11,24] However, this flow-through method has some disadvantages. For example, the duration of the experiment can be long, especially for strongly adsorbed solutes or in the case of compacted unsaturated clay materials with low hydraulic conductivity.

Desorption Experiments. Table 3 presents the percentages of *o,o*-EDDHA/Fe^{3+} extracted from the soil by using different extractants. In general, the chelate extraction gave lower values than the ones obtained for the water extraction done at the batch experiments with 5 g of soil and 25 mL of chelate solution. This is due to the larger interaction produced in this experimental

Table 3. Percentage of Extracted Chelate from the Soil Treated with *o,o*-EDDHA/Fe^{3+} Using Different Extractants

	% Fe-*o,o*-EDDHA extracted			
extractant	racemic	meso	total	% accumulated total
H_2O	75.1	69.9	72.5	72.5
Na_2SO_4	60.9	60.7	60.8	
Na_2SO_4 (pH 4.0)	66.8	52.8	59.8	
$NaHCO_3$	52.1	40.2	46.2	
DTPA	67.2	58.0	61.6	
$H_2O + Na_2SO_4$	9.4	12.5	11.0	83.5
$H_2O + Na_2SO_4$ + DTPA	3.1	1.4	2.3	85.8
$H_2O + NaHCO_3$	2.4	2.1	2.3	74.8
$H_2O + NaHCO_3$ + DTPA	3.5	2.4	3.0	77.8

model, more similar to the natural soil conditions, where the soil/solution ratio allowed a stronger interaction between the compound and the soil components. In fact, only 72.5% of the *o,o*-EDDHA/Fe^{3+} was extracted after 1 day of interaction when normally >90% was recovered from this batch assay after 3 days of intercation. Similar results were obtained between this desorption assay and the second type of batch experiments tested, with 10 g of solid and 1.5 mL of solution when water was used as extractant, due to the similar soil/solution rate used.

With respect to the extractants, several combinations were tested to desorb the *o,o*-EDDHA/Fe^{3+} from the soil, consisting of a single extraction with one extractant or a sequential extraction with two or three different extractants (Table 2). The percentage of *o,o*-EDDHA/Fe^{3+} desorbed is shown in Table 3. Surprisingly, water was the most efficient extractant among the single extractants tested. Elements or compounds solubilized by water are normally referred to as the soluble fractions, whereas sulfate or bicarbonate were used to displace anions from the soil surfaces. Their use would extract the soluble + electrostatic retained fraction (exchangeable chelate). Because the amount of *o,o*-EDDHA/Fe^{3+} recovered was lower by using these extractants than when using water as extractant, a side reaction should occur that reduces the amount of extracted chelate. Some anions such as phosphate or molybdate can reduce metal extraction from soils because they can occupy the exchangeable layer, but this effect is not well described for sulfate or bicarbonate. When added in high amounts, these anions may precipitate, mainly forming calcium sulfates or carbonates. The reduction in the *o,o*-EDDHA/Fe^{3+} recovery may then be explained by the occlusion of the chelate into the new minerals formed. Sulfate seems to be a better extractant of the chelate than the bicarbonate, but the pH of the extractant seems to be irrelevant, surely due to the high pH buffer of the soil.

The DTPA extractant is mainly used to extract available metals (not chelates). The lower amount of *o,o*-EDDHA/Fe^{3+} extracted by using this extractant than with water could be related to a slight displacement of Fe from the *o,o*-EDDHA/Fe^{3+}. The use of sequential extractions (Table 3) showed that most of the chelate can be released from the soils by using first water and then a sulfate extraction (83.5%), with only a slight increase after an additional DTPA extraction (85.8%). The sequential extraction improved by 10% the recovery of the chelate, so the retained *o,o*-EDDHA/Fe^{3+} was in such way sorbed at the soil colloids surface, unless the mechanism of the interaction is still unknown. To explain this extra desorption of the chelate, at least two possibilities could exist; the first is that the chelate was sorbed onto the surface without suffering the splitting of the molecule, and the other is that the chelate molecule splits and either the chelating agent or the iron interacts with the soil surfaces; sequential extraction then removes the sorbate from the sorbent, and the molecule will be reconstructed at the soil solution. To elucidate this fact, further research is needed, for example, via Mössbauer spectrometry, which allows the study of the Fe surroundings. In Table 4 the percentage of *o,p*-EDDHA/Fe^{3+} extracted is presented. As for *o,o*-EDDHA/Fe^{3+}, the best extractant was water, whereas the sequence H_2O + sulfate + DTPA was the best sequential extraction procedure. The desorption obtained by this method, similar to the one obtained at the second batch experiment of the previous section, was much lower than the one obtained for the ortho−ortho isomer, due to the lower stability constant of the molecule. This fact could allow the precipitation of the iron at the soil surfaces or an irreversible sorption of the molecule, the Fe, or the chelating agent. It is necessary to remember that the HPLC method used is only capable of determining the iron chelate, not the chelating agent or chelates of other metals. The increase in desorption with the sequential extraction may suggest some sorption phenomena of the chelate itself or of the chelating agent. As for the *o,o*-EDDHA/Fe^{3+}, further research is required.

Table 4. Percentage of Extracted Chelate from the Soil Treated with *o,p*-EDDHA/Fe^{3+} Using Different Extractants

extractant	% Fe-*o,p*-EDDHA extracted	% accumulated
H_2O	28.8	28.8
Na_2SO_4	6.3	
Na_2SO_4 (pH 4.0)		
$NaHCO_3$	15.1	
DTPA	8.7	
$H_2O + Na_2SO_4$	2.3	31.1
$H_2O + Na_2SO_4$ + DTPA	10.2	41.3
$H_2O + NaHCO_3$		28.8
$H_2O + NaHCO_3$ + DTPA		28.8

Effect of the Interaction Time on the Chelate Desorption. Once the sequential extraction procedure was selected, another

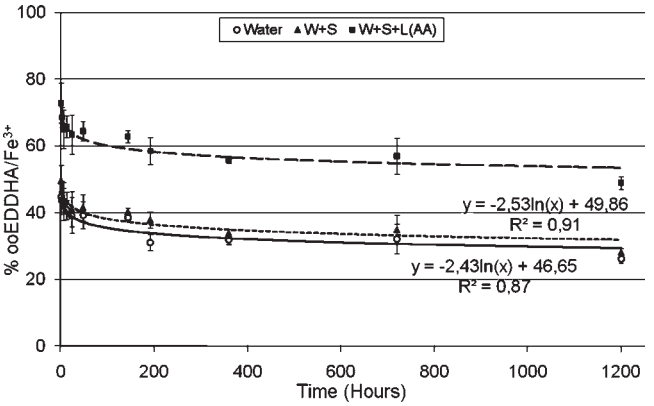

Figure 5. Chelated Fe extracted from the soil treated with *o,o*-EDDHA/Fe^{3+} over the 50 days of the experiment. Extractants: W (water), W+S (water + sulfate), and W+S+L (ligand, DTPA). Data corresponding to the W+S+L extraction are total iron measurements (AAS) instead of iron chelated.

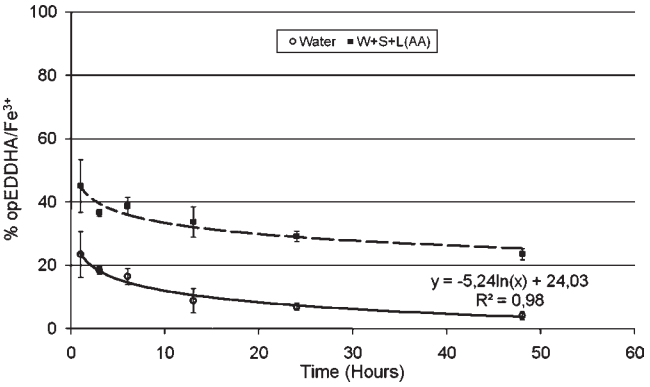

Figure 6. Chelated Fe extracted from the soil treated with *o,p*-EDDHA/Fe^{3+} during the first 48 h of the experiment. Extractants: water, W+S (water + sulfate), and W+S+L (ligand, DTPA). Data corresponding to the W+S+L extraction are total iron measurements (AAS) instead of iron chelated.

question was studied: were the sorption phenomena changed over time or the sorption kinetics? In Figure 5 the percentage of *o,o*-EDDHA/Fe^{3+} obtained by the water and water + sulfate extraction is presented for each period considered, but the desorption increase due to the DTPA extractant was estimated by AAS, because the HPLC data were below the detection limit. Due to the high variability of the data from the DTPA extraction, average data for all of the periods have been considered (1.12 μg Fe g^{-1} of soil), so the W+S+L line is an estimation and not a real data representation.

Water extracted almost 50% of the added chelate at the beginning of the test, with a moderate increase by the sulfate addition. It seemed that the binding of the chelate to the soil surface was quite strong, and the weak sulfate anion could not displace the chelate from the surface. The presence of the chelate (HPLC) in the DTPA extracts was only detectable at the first hour. At the end of the assay only around of 30% of the *o,o*-EDDHA/Fe^{3+} added was desorbed, so 20% of the chelate was more strongly sorbed by the colloids surface after 50 days of interaction, or a cation exchange has occurred in the chelate molecule. Another possibility was the partial degradation of the chelate after the 50 days of experiment. It is well-known that iron chelate solutions are photosensitive, which is why the experiment has been done in the dark, so this fact could not explain the lower chelate recovery; chemical or microbial degradation of this chelate is not well documented. For the column experiment reported in a previous section of this paper, several columns without chelate addition were used as control, but low amounts of *o,o*-EDDHA/Fe^{3+} were detected in leachates (data not shown). We attribute this presence to the chelate added by the grower a year before, so the degradation of the chelate in soils could also be discarded.

In Figure 6 the percentage of *o,p*-EDDHA/Fe^{3+} extracted after the first 48 h of the experiment is presented. In this case the amount extracted with sulfate was always below the detection limit, so water + sulfate extraction obtained the same results as the water extraction. As in the case of *o,o*-EDDHA/Fe^{3+}, the amount extracted by DTPA is an average of the values obtained by AAS and was quite similar to those obtained for *o,o*-EDDHA/Fe^{3+}. Soluble *o,p*-EDDHA/Fe^{3+} was detectable only during the first 48 h. The hypothesis that the retention of the *o,p*-EDDHA/Fe^{3+} in soils could imply a long-lasting effect is not supported by our data.

In summary, the mobility through a calcareous soil of the studied iron chelates differs greatly depending on the type of iron chelate and also the procedure used to evaluate the retention and the soil/solution ratio used. In general, the leaching column method is preferred, mainly when leaching is studied, because the achieved conclusions are more representative of the natural conditions than the ones obtained with the batch assays, but batch methods are very useful as preliminary experiments to evaluate the efficiency of the chelates as correctors of Fe deficiency. A low solution/soil ratio is preferred because it permits observation of the retention procedures (e.g., meso *o,o*EDDHA/Fe^{3+} in comparison with rac *o,o*-EDDHA/Fe^{3+}), which are difficult to study when high solution/soil ratios are used.

In general, with the three methods used to evaluate iron chelate retention by a soil, it is observed that *o,o*-EDDHA/Fe^{3+} had great mobility in this soil, greater for the racemic than for the meso isomer, so care must be taken with ground and superficial waters when this type of chelate is applied to soils. On the other hand, the *o,p*-EDDHA/Fe^{3+} mobility was very low.

The iron chelate desorption, which should correspond to the available metal fraction to plants, could be quantified by using a sequential extraction with water, sodium sulfate, and DTPA as extractants. For our experimental conditions, we can conclude that the *o,o*-EDDHA/Fe^{3+} is retained by this soil, and this retention increased with interaction time. Soluble *o,p*-EDDHA/Fe^{3+} was detectable only during the first 48 h.

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