Kinetic Behavior of Fe(*o,o*-EDDHA)–Humic Substance Mixtures in Several Soil Components and in Calcareous Soils

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Ferric ethylenediamine-*N,N*′-bis-(*o*-hydroxyphenylacetic)acid chelate (Fe(*o,o*-EDDHA)) is one of the most effective Fe fertilizers in calcareous soils. However, humic substances are occasionally combined with iron chelates in drip irrigation systems in order to lower costs. The reactivity of iron chelate–humic substance mixtures in several soil components and in calcareous soils was investigated through interaction tests, and their behavior was compared to the application of iron chelates and humic substances separately. Two commercial humic substances and two Fe(*o,o*-EDDHA) chelates (one synthesized in the laboratory and one commercial) were used to prepare iron chelate–humic substance mixtures at 50% (w/w). Various soil components (calcium carbonate, gibbsite, amorphous iron oxide, hematite, tenorite, zincite, amorphous Mn oxide, and peat) and three calcareous soils were shaken for 15 days with the mixtures and with iron chelate and humic substance solutions. The kinetic behavior of Fe(*o,o*-EDDHA) and Fe non-(*o,o*-EDDHA) (Fe bonded to (*o,p*-EDDHA) and other polycondensated ligands) and of the different nutrients solubilized after the interaction assay was determined. The results showed that the mixtures did not significantly reduce the retention of Fe(*o,o*-EDDHA) and Fe non-(*o,o*-EDDHA) in the soil components and the calcareous soils compared to the iron chelate solutions, but they did produce changes in the retention rate. Moreover, the competition between humic substances and synthetic chelating agents for complexing metal cations limited the effectiveness of the mixtures to mobilize nutrients from the substrates. The presence of Fe(*o,p*-EDDHA) and other byproducts in the commercial iron chelate had an important effect on the evolution of Fe(*o,o*-EDDHA) and the nutrient solubilization process.

KEYWORDS: Calcareous soils; Fe(*o,o*-EDDHA); iron deficiency; humic substances; soil components

INTRODUCTION

Although iron is usually the most abundant plant nutrient in the mineral phase of soils (*1*), Fe deficiency is a common nutritional problem in plants growing in calcareous soils because of the low solubility of Fe under the soil conditions prevailing in these environments (*2*). Nowadays, soil application of Fe(*o,o*-EDDHA) chelates (iron ethylenediamine-*N,N*′-bis(*o*-hydrox-yphenylacetic) acid) is the most common agricultural practice to avoid and/or relieve lime-induced iron deficiency, since they are able to increase the amount of Fe available for plant absorption in calcareous soils (*3*). Nonetheless, commercial Fe(*o,o*-EDDHA) chelates are costly and show different degrees of effectiveness under field conditions because their Fe(*o,o*-EDDHA) content can vary from 3% to 5.9% (*4*). Furthermore, they have variable extra amounts of soluble Fe chelated by (*o,p*-EDDHA) (authorized chelating agent by EC regulation no. 2003/2003) and other polycondensated ligands formed in the synthesis of (*o,o*-EDDHA) (*5, 6*). Hernández-Apaolaza et al. (*6*), Yunta et al. (*7*), and Schenkeveld et al. (*8*) have found that both Fe(*o,p*-EDDHA) and FeEDDHA byproducts have adequate stability in solution, but they are less effective than (*o,o*-EDDHA) as iron fertilizers in soil application (*6–8*).

The behavior of commercial EDDHA chelates in soils and soils components has been widely studied (*6–17*). In general, a large percentage of Fe(*o,o*-EDDHA) (>90%) remains in solution when commercial chelates are added to different soils (*8–16*) while significant losses of Fe(*o,p*-EDDHA) and FeEDDHA byproducts are observed (*6–8, 16*). According to Schenkeveld et al. (*8*), the percentage of chelate retained after 6 weeks of interaction with different soils ranges from 80% to 100% for Fe(*o,p*-EDDHA) and from 60% to 90% for FeEDDHA byproducts, depending on soil physicochemical properties. The retention of iron chelates in soils is mainly produced by their sorption on solid surfaces and/or iron or chelating agent displacement

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by different soluble ions of soil solution (*11, 16*). Álvarez-Fernández et al. (*11*) and Hernández-Apaolaza and Lucena (*17*) have observed that among several soil components, ferrihydrite and acid peat are the most reactive soil constituents with respect to Fe(*o,o*-EDDHA) sorption whereas the lesser reactive substrates are calcium carbonate and calcium montmorillonite. These authors have also found that the interaction of Fe(*o,o*-EDDHA) with tenorite and zinc hydroxysulfate produces important losses of iron in solution but they are mainly due to iron displacement from chelate by Cu and Zn, respectively (*11*). Hernandez-Apaolaza et al. (*6*) and Lucena et al. (*16*) have proved that Fe(*o,p*-EDDHA) and FeEDDHA byproducts are slightly more retained in the different soil components than Fe(*o,o*-EDDHA) except for ferrihydrite, in which a higher retention is produced. However, Schenkeveld et al. (*8*) have suggested that clay minerals are the main adsorption surface for Fe(*o,p*-EDDHA) and FeEDDHA byproducts.

On the other hand, humic substances, including humic and fulvic acids, have been considered as natural chelators for cationic micronutrients because they, as a result of their high content of oxygen-containing functional groups, can form stable complexes with many metal cations, including Fe (*18*). High molecular weight humic substances (humic acids) could act as a reservoir of easily accessible micronutrients to plants, and low molecular weight humic substances (fulvic acids) could form soluble complexes with micronutrients and move them toward the roots (*19*). Lobartini and Orioli (*20*) have shown that iron-deficient plants could use Fe–humic substance complexes as a source of iron, leading to the disappearance of chlorosis symptoms.

A habitual action in crops with iron chlorosis is the combined application of iron chelates and humic substances in drip irrigation systems, since it is a promising and economically attractive approach for counteracting Fe deficiency and enhancing the efficiency of synthetic iron chelates. Sánchez-Sánchez et al. (*21–23*) and Cerdán et al. (*24*) have found that the application of Fe(*o,o*-EDDHA)–humic substance mixtures at different rates in calcareous soils improves iron nutrition and some quality parameters of fruits in grapevine, citrus, and tomato with respect to Fe(*o,o*-EDDHA) application. However, the cause of this improvement is currently not well-understood. Two hypotheses have been suggested: (1) humic substances could increase nutrient availability in soil because of their ability to solubilize and mobilize iron and other nutrients from the soil surface (*18, 25, 26*); (2) there is a physiologic action of humic substances on plants (*18, 27*).

This research was aimed at assessing whether iron chelate–humic substance mixtures can effectively increase nutrient availability in soil with respect to Fe(*o,o*-EDDHA). We analyzed the reactivity of iron chelate–humic substance mixtures, iron chelate and humic substance solutions in calcareous soils, and several soil components through interaction tests. More specifically, the kinetic behavior of chelated iron and the different nutrients solubilized after the interaction assay was determined. Because Fe(*o,o*-EDDHA) and Fe non-(*o,o*-EDDHA) (*o,p*-EDDHA and FeEDDHA byproducts) fractions are present in commercial iron chelates, this distinction was also included in this study.

MATERIALS AND METHODS

Iron Chelates. A commercial iron chelate that declared 6% of soluble Fe and 4.5% of Fe chelated as Fe(*o,o*-EDDHA) (Qc) and a standard Fe(*o,o*-EDDHA) chelate synthesized in the laboratory (Qs) (*28*) were used in this study.

Table 1. Composition of Commercial Humic Substances

parameters	HS1	HS2
total humic substance (%)	63.6	53.5
humic acid (%)	29.1	1.2
fulvic acid (%)	34.5	52.3
insoluble solid residue (%)	36.4	
Ca (g·kg^{-1})	22.4	20.0
Mg (g·kg^{-1})	6.2	1.4
K (g·kg^{-1})	42.9	90.5
Na (g·kg^{-1})	81.2	133.7
Fe (mg·kg^{-1})	1.7	2.6
Cu (mg·kg^{-1})	1.1	0.4
Mn (mg·kg^{-1})	0.2	0.0043
Zn (mg·kg^{-1})	0.5	0.0045
N (%)	4.91[a]/6.16[b]	3.96[a]
C (%)	41.00[a]/55.10[b]	42.99[a]
H (%)	5.99[a]/5.94[b]	5.60[a]
O (%)	48.10[a]/32.80[b]	47.04[a]
S (%)	0.00[a]/0.00[b]	0.41[a]
C aliphatic (%)	3.2[a]/5.5[b]	7.7[a]
C heteroaliphatic (%)	27.1[a]	17.0[a]
C aromatic (%)	4.6[a]/48.3[b]	3.9[a]
C phenolic (%)		0.9[a]
C carboxylic (%)	6.1[a]/1.3[b]	8.5[a]

[a] Fulvic fraction. [b] Humic fraction.

For preparing the standard Fe(*o,o*-EDDHA), *o,o*-EDDHA (acid form, 99.0% purity) (Sigma, E4135) was dissolved in NaOH (Panreac, analytical grade) (1:3 molar ratio). After complete dissolution, a volume of Fe(NO₃)₃·9H₂O, whose concentration was calculated to be 5% in excess of the molar amount of *o,o*-EDDHA, was added. At the end of Fe addition, the solution pH was adjusted with HCl (Panreac, analytical grade) to 7.0. The solution was left to stand for 24 h in the darkness to allow the excess Fe to precipitate as oxides. The final solution, with an Fe concentration of 1.7×10^{-4} M, was filtered through 0.45 μm nylon filters (Millipore) and made up to volume with distilled water.

To characterize the commercial Fe(*o,o*-EDDHA) chelate, soluble Fe, Cu, Mn, and Zn concentrations were measured by atomic absorption spectrometry (AAS) according to the method described by EC regulation no. 2003/2203 of the European Parliament and of Council of October 13, 2003, relating to fertilizers (*29*). The Fe(*o,o*-EDDHA) quantification was done following the ion-pair chromatography method described by Lucena et al. (*30*). The analysis of this fertilizer showed that it contained 5.6% soluble Fe of which 2.7% was chelated by (*o,o*-EDDHA). It is indicated that the amount of soluble Fe in the commercial iron chelate is larger than the Fe(*o,o*-EDDHA) amount. The difference between both of them is attributed to the soluble iron fraction bonded to *o,p*-EDDHA and other EDDHA byproducts usually present in commercial iron chelates (*6*). This iron fraction was called Fe non-(*o,o*-EDDHA). Amounts of 0.02% of soluble Cu, 0.08% of soluble Mn, and 0.04% of soluble Zn were also found in the commercial iron chelate formulation.

Humic Substances. Two commercially available solid humic substances derived from leonardite (HS1) and plant residues (HS2) were used in the study. The commercial humic substances were analyzed for humic and fulvic acid content as described the International Humic Substance Society (*31*) (**Table 1**). Macro- and micronutrients content in commercial products was also determined by ICP after a dry mineralization followed by acid digestion (**Table 1**).

Solutions of the commercial humic substances were desalted using Spectrapore-3 tubing (MW 3500 cut-off) against distilled water. Afterward, dialyzed solutions were acidified to pH 1.5 with HCl (Panreac, analytical grade) to get precipitation of humic acids, and they were separated from fulvic acids by centrifugation. Precipitated humic acids were resolved with 0.1 N NaOH (Panreac, analytical grade), and dialysis was repeated. The dialyzed humic and fulvic acids solutions was lyophilized. Finally, elemental analysis and distribution of carbon with ¹³C NMR (*32*) of purified humic and fulvic fractions were determined (**Table 1**).

Table 1 shows that HS2 was mainly constituted of fulvic acids whereas HS1 contained a mixture of fulvic and humic acids, although

Table 2. Physicochemical Characteristics of Calcareous soils

parameters	analytical method	LS	PS	GS
texture	hydrometer	clay loam	silty clay	silty clay
pH	saturation extract	7.8	7.9	8.0
EC (dS·m^{-1})	saturation extract	0.38	0.35	0.70
organic matter (g·kg^{-1})	oxidation	9.0	9.0	5.0
carbonates (g·kg^{-1})	gasometry	483	665	616
active lime (g·kg^{-1})	gasometry	220	135	169
Na (g·kg^{-1})	ammonium acetate extract	0.7	0.4	0.5
K (g·kg^{-1})	ammonium acetate extract	0.5	0.6	0.4
Ca (g·kg^{-1})	ammonium acetate extract	8.7	3.5	4.6
Mg (g·kg^{-1})	ammonium acetate extract	9.1	5.0	6.2
P$_2$O$_5$ (mg·kg^{-1})	Burriel–Hernando	140	148	90
N total (g·kg^{-1})	Kjendahl	20	10	10
Fe (mg·kg^{-1})	DTPA extractable	1.9	2.2	2.6
Cu (mg·kg^{-1})	DTPA extractable	4.5	4.4	2.3
Mn (mg·kg^{-1})	DTPA extractable	2.4	4.4	3.6
Zn (mg·kg^{-1})	DTPA extractable	0.4	1.4	1.3

the fulvic fraction was higher than the humic fraction. The ^{13}C NMR spectra indicated that HS2 and the fulvic fraction of HS1 were richer in heteroaliphatic groups than humic fraction, while the humic fraction of HS1 contained more aromatic structures (**Table 1**). Results are in agreement with the O content in the fulvic fractions (*19*). These differences between both humic substances could produce differences in their capacity to bind metal nutrients in soils and soil components.

Calcareous Soils and Soil Components. Three calcareous soils, lemon soil (LS), peach soil (PS), and grapevine soil (GS), from commercial orchards located in the Mediterranean area of Spain were used as substrates. Soil texture (hydrometer method) (*33*), pH (saturation extract) (*34*), and EC (saturation extract) (*34*) are presented in **Table 2**. The soil content of total organic matter (oxidation method) (*35*), total carbonates (gasometry) (*34*), active carbonates (gasometry on the fraction of carbonates extracted with 0.5 ammonium oxalate) (*34*), exchangeable cations (ammonium acetate method) (*36*), total nitrogen content (Kjendahl method) (*37*), available P (Burriel-Hernando method) (*38*), and potentially plant available micronutrients (DTPA extractable fraction) (*39*) are also included in **Table 2**.

Peat (Floraska Substrate), CaCO$_3$ (Panreac, analytical grade), Cu, Zn, Al, and Fe oxides synthesized in the laboratory, and a commercial iron oxide (Panreac, analytical grade) were used. Synthetic iron oxide was prepared according to the procedure described by Sims and Bingham (*40*). Aluminium, copper, and zinc oxides were synthesized by mixing solutions of AlK(SO$_4$)$_2$, CuSO$_4$·5H$_2$O and ZnSO$_4$·H$_2$O (Panreac, analytical grade) with NaOH (Panreac, analytical grade) at room temperature. The precipitates were filtered and washed, first with deionized water until the conductivity of the washes was lower than 0.1 dS/m and afterward with ethanol (80%) until the pH was approximately 7. To obtain Mn oxide, heated ethanol was added to KMnO$_4$ solution (Panreac, analytical grade). All oxides were dried at 65°C for 24 h and then ground. The different oxides were characterized by XRD with a Seifert JSO-DEBYEFLEX 2002 apparatus with Cu radiation Kα ($\lambda = 1.540\,598$ Å). The analysis conditions were 45 kV and 35 mA, time/step = 3 s, step size = 0.1, and raw data were measured from 6° (2θ) to 70° (2θ). The analysis determined that the synthetic iron and manganese oxides were amorphous, copper oxide corresponded with the tenorite phase, zinc oxide corresponded with the synthetic zincite phase, aluminium oxide corresponded with the synthetic gibbsite phase, and commercial iron oxide corresponded with the hematite phase.

Interaction Studies. Samples of 2 g of soil or soil component interacted with 20 mL of iron chelate–humic substance mixtures (Q + HS), humic substance (HS), and iron chelate (Q) solutions. Both standard and commercial iron chelate solutions (Qs and Qc, respec-

tively) had a 1.7×10^{-4} M soluble Fe concentration. Standard iron chelate solution (Qs) was prepared following the experimental procedure described previously (iron chelates section). Commercial iron chelate solution (Qc), containing approximately 0.17 g·L^{-1} product, was prepared by dissolving the formulation in distilled water and filtering it through a 0.45 μm nylon filter (Millipore). HS samples were prepared by dissolving the same quantity of the commercial products as a Qc solution (0.17 g·L^{-1} product) in distilled water. Q + HS samples were mixtures at 50% (w/w) of chelate and humic substance with the same concentrations as Q and HS solutions (0.17 g·L^{-1} each product). As control, an amount of 2 g of soil or soil components was added to 20 mL of deionized water. After 24, 48, 96, 168, and 360 h of continuous stirring at 25 °C in the darkness, the soil suspensions were centrifuged at 4000 rpm, the pellet was discarded, and the supernatant was filtered through a 0.22 μm membrane filter. The pH value, Fe(*o,o*-EDDHA) content by HPLC (*30*), and soluble Fe, Cu, Mn, Zn, Al, and Ca concentration by AAS were determined in the filtrate. The experiment was performed in triplicate.

HPLC Analysis. A Shimadzu chromatographic system was used for HPLC analysis, with an LC-7A pump, an SIL-10A autosampler, an N SPD-M6A photodiode array detector, and Windows 98 chromatographic software CLASS-LC10 V.1.6. For FeEDDHA, the column used was a Lichrospher 100RP-18 (5 μm) (Hp, ref no. 841334), 250 mm × 4 mm, with a flow rate of 1.5 mL/min, an oven temperature 25°C, a detection wavelength of 300 nm, and an injection volume of 50 μL. The mobile phase was constituted of 2% tetrabutylammonium hydroxide (v/v) (Sigma) and 30% acetonitrile (v/v) (HPLC Scharlau FEROSA). Fe(*o,o*-EDDHA) solutions were used as calibration standards.

Kinetic Analysis. The experimental data that described the evolution of Fe and other nutrients in solution after interaction with the soils and the soil components were fitted in different equations. The behavior of Fe(*o,o*-EDDHA) and Fe non-(*o,o*-EDDHA) in the solutions was fitted in eq a, whereas the solubilization process of different nutrients from the soils and the soil components was described by eqs b and c.

$$[\text{Fe}] = [\text{Fe}]_0 - \frac{[\text{Fe}]_{\text{lost}} \cdot t}{\tau_{1/2} + t} \tag{a}$$

$$[\text{nutrient}] = [\text{nutrient}]_0 + \frac{[\text{nutrient}]_{\text{sol}} \cdot t}{\tau_{1/2} + t} \tag{b}$$

$$[\text{nutrient}] = \frac{[\text{nutrient}]_{\text{eq}} \cdot t}{t - \left(\frac{\sigma}{2}\right)} \tag{c}$$

The parameter [Fe] was the Fe concentration that remained in solution (M). [Fe]$_0$ was the initial Fe concentration in solution (M) (time of interaction = 0). [Fe]$_{\text{lost}}$ was the loss of iron in solution after interaction assay due to iron retention in soils or soil components (M). [nutrient] was the soluble nutrient concentration in solution (M). [nutrient]$_0$ was the initial nutrient concentration in solution (M) (time of interaction = 0). [nutrient]$_{\text{sol}}$ was the nutrient concentration solubilized from calcareous soils or soil components after interaction assay (M). [nutrient]$_{\text{eq}}$ was the nutrient concentration in solution at equilibrium. t was the period of interaction (days), and $\tau_{1/2}$ was the time, in days, that the reaction took in reaching half of [Fe]$_{\text{lost}}$ or [nutrient]$_{\text{sol}}$. σ was the time, in days, that [nutrient] was double of the value of [nutrient]$_{\text{eq}}$. The Fe concentration that remained in solution at equilibrium ([Fe]$_{\text{eq}}$) was calculated by subtracting [Fe]$_{\text{lost}}$ from [Fe]$_0$, while the nutrient concentration in solution at equilibrium for eq b ([nutrient]$_{\text{eq}}$) was calculated by adding [nutrient]$_{\text{sol}}$ to [nutrient]$_0$.

Equations a–c were derived with respect to time to establish the iron retention rate in the substrates and the solubilization rate of the different nutrients from the soils and the soil components, so eqs df were obtained:

$$V = \frac{\text{d}([\text{Fe}])}{\text{d}t} = \frac{[\text{Fe}]_{\text{lost}} \cdot \tau_{1/2}}{(\tau_{1/2} + t)^2} \tag{d}$$

$$V = \frac{\text{d}([\text{nutrient}])}{\text{d}t} = \frac{[\text{nutrient}]_{\text{sol}} \cdot \tau_{1/2}}{(\tau_{1/2} + t)^2} \tag{e}$$

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$$V = \frac{d([\text{nutrient}])}{dt} = \frac{[\text{nutrient}]_{eq} \cdot \left(\frac{\sigma}{2}\right)}{\left(t - \frac{\sigma}{2}\right)^2} \qquad (f)$$

All statistical calculations were performed using SPSS, version 12.0, software.

RESULTS AND DISCUSSION

Kinetic Behavior of Fe(o,o-EDDHA) and Fe Non-(o,o-EDDHA) from Iron Chelate–Humic Substance Mixtures in Soil Components and Calcareous Soils. *Behavior of Fe(o,o-EDDHA) over the Interaction Assay.* Fe(o,o-EDDHA) content in Qs (standard iron chelate solution), Qs + HS1, Qs + HS2, Qc (commercial iron chelate solution), Qc + HS1, and Qc + HS2 remained constant for the 15 days of interaction assay with CaCO$_3$, amorphous iron oxide, gibbsite, and the three soils tested (LS, GS, and PS) (data not shown). Moreover, there were no significant differences in the behavior of Fe(o,o-EDDHA) between Q + HS mixtures and the Q solution for the entire interaction period with the substrates mentioned above (data not shown). This suggests that the effectiveness of the o,o-EDDHA chelating agent to maintain soluble iron under these experimental conditions was not affected by humic substances.

In accordance with several studies (*9–17*), Fe(o,o-EDDHA) maintained a larger amount of soluble iron after interaction with calcareous soils, CaCO$_3$, or gibbsite because of its relatively low reactivity with these substrates. Álvarez-Fernández et al. (*11*) and Hernández-Apaolaza and Lucena (*17*) observed that iron (hydr)oxides (ferrihydrite) are the most reactive soil constituents, allowing a low recovery of Fe(o,o-EDDHA) (~60%). According to the results obtained by these authors, it was expected that the Fe(o,o-EDDHA) concentration decreased strongly after the interaction with amorphous iron oxide. However, Fe(o,o-EDDHA) was not retained in this substrate (data not shown). This opposite behavior could be related to the pH values obtained after the interaction assay, since the sorption of iron chelate on iron oxide surfaces is pH-dependent. The pH average value of Q and Q + HS solutions after interaction (8.7) was above the point zero of charge of amorphous iron oxide (*41*). Consequently, the oxide had a net surface negative charge and the sorption of an anion ligand as Fe(o,o-EDDHA) did not occur.

The Fe(o,o-EDDHA) concentration in Qs, Qs + HS, Qc, and Qc + HS solutions suffered an important decrease for interaction with hematite, tenorite, zincite, amorphous Mn oxide, and peat ([Fe]$_{lost}$) (**Tables 3** and **4**). Because Fe(o,o-EDDHA) is stable at the pH values observed in the solution after the interaction with each soil component (5.5 for hematite, 8.0 for tenorite, 7.5 for zincite, 8.0 for amorphous Mn oxide, 5.5 for peat) (*15, 42*), this drop must be fundamentally produced by the sorption of Fe(o,o-EDDHA) and/or the iron displacement from chelate by the cations present in the solid phase (*10, 11, 17*). To determine the kinetic behavior of Fe chelated by (o,o-EDDHA) in these soil components, the experimental data were fitted in eqs a and d.

The retention of Fe(o,o-EDDHA) from Qs, Qs + HS1, and Qs + HS2 in these soil components increased in the following order: peat ≤ hematite < zincite ~ tenorite < amorphous Mn oxide (**Table 3**). However, the sequence for Qc, Qc + HS1, and Qc + HS2 solutions was zincite < peat < hematite ~ tenorite < amorphous Mn oxide (**Table 4**). The most notable difference among the commercial and the standard iron chelate solutions was their reactivity with zincite. Whereas Fe(o,o-EDDHA) of the commercial iron chelate solutions was hardly retained in this substrate (**Table 4**), substantial losses of Fe(o,o-EDDHA) were found in the standard iron chelate solutions after interaction with zincite (**Table 3**). The lowest reactivity of commercial iron chelate with this soil material could be related to the presence of Fe(o,p-EDDHA) and FeEDDHA byproducts (Fe non-(EDDHA)) in its formulation. Various authors (*6, 7, 16*) have proved that this iron fraction is more reactive with different soil components than Fe(o,o-EDDHA). Consequently, Fe non-(o,o-EDDHA) could be more susceptible to the competition effects and/or the sorption process. So it may reduce the displacement of Fe from Fe(o,o-EDDHA) chelate by exchangeable Zn and/or it may saturate adsorption sites of zincite surface, avoiding the sorption of Fe(o,o-EDDHA).

For all substrates, the Fe(o,o-EDDHA) concentration that remained at equilibrium ([Fe]$_{eq}$) in Qs + HS1 and Qs + HS2 was statistically similar to the one in the Qs solution (**Table**

Table 3. Kinetic Parameters of Fe(o,o-EDDHA) in Qs and Qs + HS Solutions After Interaction Assay[a]

substrate	treatment	[Fe]$_0$ (M) (×10^4)	[Fe]$_{lost}$ (M) (×10^4)	[Fe]$_{eq}$ (M) (×10^4)	$\tau_{1/2}$ (h)	$V_{t=\tau_{1/2}}$ (mol of Fe/(h·kg soil)) (×10^5)	R^2
hematite	Qs	1.61 ± 0.03	0.4 ± 0.1	1.2 ± 0.1	300 ± 80	0.040 ± 0.005	0.9803
	Qs + HS1	1.65 ± 0.03	0.35 ± 0.05	1.3 ± 0.1	100 ± 60	0.09 ± 0.03	0.9602
	Qs + HS2	1.64 ± 0.03	0.28 ± 0.04	1.35 ± 0.07	50 ± 20	0.15 ± 0.05	0.9506
tenorite	Qs	1.70 ± 0.05	1.00 ± 0.05	0.7 ± 0.1	11 ± 3	2.3 ± 0.4	0.9876
	Qs + HS1	1.72 ± 0.04	1.05 ± 0.05	0.65 ± 0.1	8 ± 2	3.3 ± 0.6	0.9945
	Qs + HS2	1.70 ± 0.05	1.0 ± 0.1	0.7 ± 0.1	10 ± 4	2.5 ± 0.5	0.9765
zincite	Qs	1.69 ± 0.04	0.95 ± 0.05	0.8 ± 0.1	6 ± 2	4.0 ± 0.6	0.9933
	Qs + HS1	1.69 ± 0.02	0.97 ± 0.02	0.72 ± 0.04	6 ± 2	4.0 ± 0.3	0.9982
	Qs + HS2	1.53 ± 0.04	0.80 ± 0.05	0.73 ± 0.09	8 ± 3	4.5 ± 0.4	0.9897
amorphous Mn oxide	Qs	1.7 ± 0.1	1.30 ± 0.2	0.4 ± 0.3	110 ± 10	0.30 ± 0.05	0.9642
	Qs + HS1	1.64 ± 0.04	1.10 ± 0.05	0.5 ± 0.1	44 ± 8	0.6 ± 0.2	0.9935
	Qs + HS2	1.7 ± 0.2	1.30 ± 0.4	0.4 ± 0.4	40 ± 40	0.8 ± 0.3	0.8292
peat	Qs	1.69 ± 0.03	0.25 ± 0.05	1.45 ± 0.05	10 ± 7	0.6 ± 0.1	0.9409
	Qs + HS1	1.59 ± 0.02	0.2 ± 0.1	1.4 ± 0.1	12 ± 9	0.4 ± 0.3	0.9198
	Qs + HS2	1.70 ± 0.05	0.4 ± 0.1	1.4 ± 0.2	16 ± 15	0.5 ± 0.1	0.8577

[a] Qs: standard Fe(o,o-EDDHA). HS: humic substance.

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Table 4. Kinetic Parameters of Fe(o,o-EDDHA) in Qc and Qc + HS Solutions After Interaction Assay[a]

substrate	treatment	$[Fe]_0$ (M) ($\times 10^4$)	$[Fe]_{lost}$ (M) ($\times 10^4$)	$[Fe]_{eq}$ (M) ($\times 10^4$)	$\tau_{1/2}$ (h)	$V_{t=\tau 1/2}$ (mol of Fe/(h·kg soil)) $\times 10^5$	R^2
hematite	Qc	0.79 ± 0.05	0.45 ± 0.04	0.3 ± 0.1	270 ± 60	0.04 ± 0.02	0.9964
	Qc + HS1	0.78 ± 0.02	0.35 ± 0.1	0.4 ± 0.1	200 ± 100	0.04 ± 0.03	0.9510
	Qc + HS2	0.80 ± 0.02	0.5 ± 0.2	0.3 ± 0.2	190 ± 80	0.07 ± 0.06	0.9799
tenorite	Qc	0.84 ± 0.02	0.47 ± 0.02	0.37 ± 0.04	4 ± 2	3 ± 2	0.9935
	Qc + HS1	0.835 ± 0.005	0.45 ± 0.01	0.39 ± 0.01	3.8 ± 0.6	3.0 ± 0.5	0.9990
	Qc + HS2	0.84 ± 0.01	0.46 ± 0.02	0.38 ± 0.03	2.8 ± 0.7	4.1 ± 0.7	0.9987
zincite	Qc	0.75 ± 0.01	0.16 ± 0.03	0.59 ± 0.04	102 ± 60	0.04 ± 0.01	0.9458
	Qc + HS1	0.77 ± 0.04	0.4 ± 0.1	0.4 ± 0.1	154 ± 141	0.06 ± 0.02	0.9473
	Qc + HS2	0.74 ± 0.02		0.73 ± 0.04			
amorphous Mn oxide	Qc	0.8 ± 0.2	1.0 ± 0.3	0.2 ± 0.5	80 ± 70	0.3 ± 0.2	0.8760
	Qc + HS1	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.2	50 ± 30	0.40 ± 0.08	0.9311
	Qc + HS2	0.8 ± 0.1	1.1 ± 0.3	0.5 ± 0.5	80 ± 70	0.3 ± 0.2	0.8699
peat	Qc	0.71 ± 0.02	0.33 ± 0.03	0.38 ± 0.05	3 ± 2	2.8 ± 0.4	0.9926
	Qc + HS1	0.72 ± 0.02	0.33 ± 0.03	0.39 ± 0.05	5 ± 2	1.7 ± 0.8	0.9917
	Qc + HS2	0.76 ± 0.01	0.39 ± 0.04	0.37 ± 0.05	4 ± 1	2 ± 1	0.9963

[a] Qc: commercial Fe(o,o-EDDHA). HS: humic substance.

3). Hence, HS1 and HS2 did not reduce the Fe(o,o-EDDHA) retention in the different substrates. For tenorite, zincite, and peat, humic substances had no effect on the $\tau_{1/2}$ value or on the Fe(o,o-EDDHA) retention rate ($V_{t=\tau 1/2}$) (**Table 3**). However, the $\tau_{1/2}$ values for Qs + HS1 and Qs + HS2 were lower than the one for the Qs solution for interaction with hematite and amorphous Mn oxide. Therefore, humic substances significantly increased retention rate of Fe(o,o-EDDHA) ($V_{t=\tau 1/2}$) with respect to Qs (**Table 3**). The fact that this behavior was only observed upon interaction with hematite and amorphous Mn oxide suggests that the interaction between humic substances and these substrates might play an important role. So the presence of humic substances could change the adsorption surface characteristics of hematite and amorphous Mn oxide, increasing the rate to which Fe(o,o-EDDHA) is removed from Qs + HS solutions. Nonetheless, further research is needed to explain such behavior. On the other hand, statistical differences between Qs + HS1 and Qs + HS2 mixtures were not observed (**Table 3**) despite the structural differences between HS1 and HS2 (**Table 1**).

According to the values of the kinetic parameters shown in **Table 4**, the presence of HS1 and HS2 in Qc + HS1 and Qc + HS2 did not have any effect on the kinetic behavior of Fe(o,o-EDDHA) in the different substrates except for zincite. HS2 avoided the retention of Fe(o,o-EDDHA) in this soil component. Apparently the humic substance may have a larger ability to bind Zn of the zincite surface, reducing the competence of this cation with Fe for (o,o-EDDHA) (**Table 4**). In contrast, HS1 may act as a competitor of o,o-EDDHA for Fe, since the losses of Fe(o,o-EDDHA) in Qc + HS1 solution ($[Fe]_{lost}$) increased with respect to the commercial iron chelate solution (Qc). Unlike the mixtures prepared with standard iron chelate, humic substances did not change the $\tau_{1/2}$ value or the Fe(o,o-EDDHA) retention rate ($V_{t=\tau 1/2}$) with respect to Qc (**Table 4**). These results indicate that the presence of (o,p-EDDHA) and other EDDHA byproducts in the commercial iron chelate (5, 6) produced some variations in the humic substances effects on the kinetic behavior of Fe(o,o-EDDHA).

Behavior of Fe Non-(o,o-EDDHA) over the Interaction Assay. The Fe non-(o,o-EDDHA) (Fe(o,p-EDDHA) and FeEDDHA byproducts) content of Qc, Qc + HS1, and Qc + HS2

declined quickly after the interaction with $CaCO_3$, hematite, tenorite, zincite, amorphous Mn oxide, gibbsite, peat, and the three calcareous soils tested. Like Fe(o,o-EDDHA), this iron fraction has adequate stability at the pH values observed in Qc and Qc + HS solutions upon the interaction assay (data cited in Behavior of Fe(o,o-EDDHA) over the Interaction Assay) (6, 7, 16). Hence, the losses of Fe non-(o,o-EDDHA) must be also produced by its sorption and/or the iron displacement from chelate by the cations present in the solid phase. To determine the kinetic parameters that described the behavior of Fe non-(o,o-EDDHA), the experimental data were fitted in eqs a and d (**Table 5**). Little or no Fe non-(o,o-EDDHA) was detected in the Qc solution and Qc + HS mixtures after the interaction with tenorite, zincite, and amorphous Mn oxide ($[Fe]_{eq}$) (**Table 5**). For hematite and peat, Fe non-(o,o-EDDHA) concentration declined about 80% (**Table 5**), whereas the lowest retention of Fe non-(o,o-EDDHA) ($[Fe]_{lost}$) was found after interaction with $CaCO_3$ and gibbsite (**Table 5**). These results are in good agreement with those obtained by Hernández-Apaolaza et al. (6), Álvarez-Fernández et al. (11), and Lucena et al. (16). These authors found that iron oxides were the most reactive and calcium carbonate was a less reactive soil constituent with respect to the Fe non-(o,o-EDDHA) fraction. Moreover, Yunta et al. (7) reported that the Fe(o,p-EDDHA) concentration strongly decreased after the interaction between commercial iron chelates and substrates with high Cu availability because the Cu was the principal competitor of Fe(o,p-EDDHA).

Table 5 shows that the $[Fe]_{lost}$ values in Qc + HS1 and Qc + HS2 solutions were statistically similar to the one in Qc solution for the different substrates. So HS1 and HS2 did not reduce Fe non-(o,o-EDDHA) retention by soil components, but they did have different effects on the $\tau_{1/2}$ value and the retention rate ($V_{t=\tau 1/2}$) (**Table 5**). For hematite and amorphous Mn oxide, HS1 decreased $V_{t=\tau 1/2}$ in Qc + HS1 compared to the Qc solution, while HS2 increased the value of this parameter. Humic substances produced the reverse effect after interaction with peat

Table 5. Kinetic Parameters of Fe Non-(*o,o*-EDDHA) in Qc and Qc + HS Solutions After Interaction Assay[a]

substrate	treatment	[Fe]$_0$ (M) ($\times 10^4$)	[Fe]$_{lost}$ (M) ($\times 10^4$)	[Fe]$_{eq}$ (M) ($\times 10^4$)	$\tau_{1/2}$ (h)	$V_{t=\tau 1/2}$ (mol of Fe/(h·kg soil)) ($\times 10^5$)	R^2
CaCO$_3$	Qc	0.94 ± 0.04	0.5 ± 0.1	0.4 ± 0.2	30 ± 10	0.4 ± 0.3	0.9578
	Qc + HS1	0.95 ± 0.05	0.55 ± 0.05	0.4 ± 0.1	40 ± 20	0.34 ± 0.06	0.9563
	Qc + HS2	1.05 ± 0.05	0.60 ± 0.05	0.5 ± 0.1	50 ± 20	0.30 ± 0.06	0.9660
hematite	Qc	0.80 ± 0.02	0.64 ± 0.02	0.16 ± 0.04	2.0 ± 0.2	8.0 ± 0.5	0.9965
	Qc + HS1	0.86 ± 0.02	0.68 ± 0.04	0.2 ± 0.1	5.1 ± 0.2	3.4 ± 0.5	0.9891
	Qc + HS2	0.93 ± 0.01	0.64 ± 0.02	0.29 ± 0.2	0.8 ± 0.1	20 ± 5	0.9984
tenorite	Qc	0.80 ± 0.08	0.78 ± 0.06	0.0 ± 0.2	5.0 ± 0.4	3.9 ± 0.4	0.9953
	Qc + HS1	0.73 ± 0.04	0.73 ± 0.04	0.00 ± 0.05	2.5 ± 0.9	7 ± 1	0.9958
	Qc + HS2	0.91 ± 0.06	0.87 ± 0.06	0.1 ± 0.2	2.1 ± 0.1	11 ± 2	0.9962
zincite	Qc	0.82 ± 0.06	0.80 ± 0.06	0.0 ± 0.1	2 ± 1	10 ± 2	0.9960
	Qc + HS1	0.77 ± 0.02	0.77 ± 0.02	0.00 ± 0.04	4.7 ± 0.5	4 ± 1	0.9995
	Qc + HS2	0.84 ± 0.06	0.82 ± 0.06	0.0 ± 0.1	6 ± 1	3 ± 2	0.9957
amorphous Mn oxide	Qc	0.756 ± 0.001	0.756 ± 0.001	0.000 ± 0.005	$(1.05 ± 0.01) \times 10^{-7}$	$(180 ± 25) \times 10^6$	0.9998
	Qc + HS1	0.75 ± 0.05	0.9 ± 0.2	0.2 ± 0.3	18 ± 6	1.3 ± 0.8	0.9786
	Qc + HS2	0.757 ± 0.001	0.757 ± 0.001	0.000 ± 0.002	$(1 ± 1) \times 10^{-14}$	$(1.96 ± 0.03) \times 10^{15}$	0.9998
gibbsite	Qc	0.90 ± 0.02	0.56 ± 0.02	0.34 ± 0.04	11.7 ± 0.8	1.2 ± 0.6	0.9994
	Qc + HS1	0.79 ± 0.06	0.6 ± 0.1	0.2 ± 0.2	17 ± 6	0.9 ± 0.4	0.9796
	Qc + HS2	0.887 ± 0.002	0.583 ± 0.004	0.304 ± 0.009	16 ± 4	0.9 ± 0.3	0.9972
peat	Qc	0.88 ± 0.02	0.74 ± 0.03	0.14 ± 0.05	2 ± 1	9.3 ± 0.1	0.9961
	Qc + HS1	0.84 ± 0.04	0.72 ± 0.04	0.12 ± 0.05	0.7 ± 0.7	26 ± 1	0.9989
	Qc + HS2	0.98 ± 0.02	0.79 ± 0.03	0.19 ± 0.04	3 ± 2	6.6 ± 0.1	0.9902
GS	Qc	0.94 ± 0.02	0.68 ± 0.04	0.26 ± 0.05	58 ± 13	0.29 ± 0.08	0.9913
	Qc + HS1	0.971 ± 0.009	0.71 ± 0.01	0.26 ± 0.01	44 ± 2	0.4 ± 0.3	0.9998
	Qc + HS2	0.9 ± 0.1	2 ± 1	1 ± 1	285 ± 110	0.2 ± 0.2	0.9677
LS	Qc	0.92 ± 0.04	0.60 ± 0.05	0.3 ± 0.1	12 ± 5	1.3 ± 0.3	0.9786
	Qc + HS1	0.90 ± 0.05	0.7 ± 0.1	0.2 ± 0.2	17 ± 9	1.0 ± 0.3	0.9517
	Qc + HS2	1.05 ± 0.05	0.75 ± 0.05	0.3 ± 0.1	16 ± 6	1.2 ± 0.2	0.9750
PS	Qc	0.97 ± 0.01	0.67 ± 0.02	0.30 ± 0.04	17 ± 1	1.0 ± 0.5	0.9990
	Qc + HS1	1.08 ± 0.02	0.79 ± 0.06	0.3 ± 0.1	17 ± 3	1.1 ± 0.5	0.9955
	Qc + HS2	1.08 ± 0.02	0.81 ± 0.04	0.27 ± 0.04	18 ± 2	1.1 ± 0.5	0.9981

[a] Qc: commercial Fe(*o,o*-EDDHA). HS: humic substance.

(**Table 5**). For tenorite, both humic substances increased the retention rate of Fe non-(*o,o*-EDDHA) ($V_{t=\tau 1/2}$) with respect to the Qc solution (**Table 5**). The irregular trend of Q + HS mixtures for interaction with each soil component complicated the interpretations of the results and made it difficult to establish the mechanisms of humic substances for increasing or decreasing the retention rate of Fe non-(*o,o*-EDDHA) in the different soil components with respect to iron chelate solutions.

Unlike the other soil components tested, a slight increase of soluble Fe concentration was observed when Qc and Qc + HS interacted with amorphous Fe oxide. This implies that Fe was extracted from amorphous Fe oxide. To determine the kinetic parameters that describe the iron solubilization process, the experimental data were fitted in eqs b and e. **Table 6** shows that the [Fe]$_{sol}$ value for Qc + HS was similar to the one for HS and it was 10 times higher than the amount of Fe extracted from amorphous iron oxide with Qc solution. Furthermore, the mixture dropped significantly the solubilization rate of Fe compared to the application of HS and Qc separately. These results suggest that the use of humic substances could have more beneficial effects on Fe availability than the combined application of iron chelate with humic mixtures, since the lower $V_{t=\tau 1/2}$ value of Qc + HS could not be enough to cover the plant needs (**Table 6**). However, additional studies involving plants would be needed to comment on this.

According to the [Fe]$_{lost}$ value shown in **Table 5**, the retention of Fe non-(*o,o*-EDDHA) in the three soils tested was very close to the one in CaCO$_3$. This is in agreement with the fact that these soils had higher contents of CaCO$_3$ compared to other reactive soil components (**Table 2**). For LS and PS soils, the kinetic behavior of Fe non-(*o,o*-EDDHA) in Qc + HS mixtures was statistically similar to the one in Qc. However, HS2 increased the losses of Fe non-(*o,o*-EDDHA) in Qc + HS2 during the interaction with GS soil with respect to Qc (**Table 5**). This effect was not observed for HS1 because there were no significant differences between the kinetic behavior of Fe non-(*o,o*-EDDHA) in Qc and Qc + HS1 solutions after interaction with the grape soil (**Table 5**).

Kinetic Behavior of Nutrients Solubilized with Iron Chelate–Humic Substance Mixtures from Soil Components and Calcareous Soils. The kinetic parameters that described the solubilization process of different nutrients from the soil components are included in **Tables 6** and **7**. The experimental data of Mn mobilization were fitted in eqs c and f, since the Mn concentration reached the maximum value during the first 24 h of the interaction assay, but afterward Mn solubility dropped steadily. However, the solubilization process of the other nutrients was represented by a hyperbolic curve and the experimental data were fitted in eqs b and e.

Kinetics of Fe(*o,o*-EDDHA)–Humic Substance Mixtures

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Table 6. Kinetic Parameters of Different Nutrients Solubilized from Soil Components[a]

substrate	nutrient	treatment	[nutrient]$_0$ (M) ($\times 10^5$)	[nutrient]$_{sol}$ (M) ($\times 10^5$)	[nutrient]$_{eq}$ (M) ($\times 10^5$)	$\tau_{1/2}$ (h)	$V_{t=\tau 1/2}$ (mol/(h·kg soil)) ($\times 10^6$)	R^2
CaCO$_3$	soluble Ca	H$_2$0 (ctrl)	nd[b]	24 ± 1	24 ± 1	21 ± 10	3 ± 2	0.9267
		HS1	9.5 ± 0.2	25 ± 3	34 ± 3	6 ± 4	6 ± 2	0.9655
		HS2	8.5 ± 0.2	24 ± 5	32 ± 5	9 ± 5	8 ± 3	0.9683
		Qs	nd	22 ± 3	22 ± 3	2 ± 1	28 ± 3	0.9952
		Qs + HS1	9.4 ± 0.1	24 ± 3	33 ± 3	10 ± 6	6 ± 1	0.9225
		Qs + HS2	8.6 ± 0.2	27 ± 2	36 ± 2	5 ± 4	14 ± 10	0.9504
		Qc	nd	42 ± 3	42 ± 3	8 ± 4	13 ± 8	0.9458
		Qc + HS1	9.2 ± 0.4	36 ± 5	45 ± 5	4 ± 2	24 ± 15	0.9168
		Qc + HS2	8.9 ± 0.5	35 ± 4	44 ± 5	7 ± 6	12 ± 2	0.9387
amorphous Fe oxide	soluble Fe	H$_2$0 (ctrl)	nd[b]	0.188 ± 0.03	0.188 ± 0.03	0.14 ± 0.07	3 ± 2	0.9999
		HS1	0.0005 ± 0.0001	1.1 ± 0.4	1.1 ± 0.4	7 ± 2	0.4 ± 0.1	0.9875
		HS2	0.0009 ± 0.0001	1.24 ± 0.04	1.24 ± 0.04	13 ± 3	0.25 ± 0.08	0.9899
		Qs	17.00 ± 0.04	0.19 ± 0.06	17.2 ± 0.1	0.13 ± 0.03	4 ± 1	0.9833
		Qs + HS1	17.20 ± 0.02	1.06 ± 0.04	18.26 ± 0.06	5 ± 2	0.5 ± 0.2	0.9811
		Qs + HS2	17.1 ± 0.1	1.3 ± 0.1	18.4 ± 0.2	11 ± 6	0.3 ± 0.2	0.9899
		Qc	17.081 ± 0.007	0.191 ± 0.009	17.27 ± 0.01	0.15 ± 0.03	3.2 ± 0.8	0.9901
		Qc + HS1	17.2 ± 0.2	1.6 ± 0.4	18.8 ± 0.6	260 ± 90	0.015 ± 0.008	0.9323
		Qc + HS2	17.1 ± 0.1	1.5 ± 0.3	18.6 ± 0.4	110 ± 58	0.03 ± 0.02	0.9895
tenorite	soluble Cu	H$_2$0 (ctrl)	nd[b]	0.9 ± 0.1	0.9 ± 0.1	60 ± 20	0.03 ± 0.01	0.9538
		HS1	0.0003 ± 0.0001	0.7 ± 0.3	0.7 ± 0.3	60 ± 20	0.03 ± 0.03	0.9816
		HS2	0.0001 ± 0.0002	0.8 ± 0.3	0.8 ± 0.3	50 ± 10	0.04 ± 0.02	0.9229
		Qs	nd[b]	3.9 ± 0.2	3.9 ± 0.2	60 ± 10	0.18 ± 0.05	0.9845
		Qs + HS1	0.0003 ± 0.0001	3.5 ± 0.4	3.5 ± 0.4	60 ± 20	0.15 ± 0.08	0.9566
		Qs + HS2	0.00012 ± 0.00002	4.0 ± 0.2	4.0 ± 0.2	50 ± 10	0.20 ± 0.05	0.9527
		Qc	0.054 ± 0.003	10.5 ± 0.1	10.6 ± 0.1	3.6 ± 0.7	7 ± 2	0.9982
		Qc + HS1	0.06 ± 0.01	10.7 ± 0.2	10.8 ± 0.2	4 ± 1	7 ± 2	0.9945
		Qc + HS2	0.06 ± 0.01	10.8 ± 0.4	10.9 ± 0.4	5 ± 2	5 ± 5	0.9850
zincite	soluble Zn	H$_2$0 (ctrl)	nd[b]	24.5 ± 0.4	24.5 ± 0.4	1.5 ± 0.8	40 ± 23	0.9973
		HS1	0.00013 ± 0.00005	32 ± 5	32 ± 5	10 ± 4	8 ± 3	0.9777
		HS2	nd[b]	37 ± 2	37 ± 2	10 ± 3	10 ± 1	0.9790
		Qs	nd[b]	29.0 ± 0.6	29.0 ± 0.6	7 ± 1	10 ± 2	0.9966
		Qs + HS1	0.00015 ± 0.00003	37 ± 2	37 ± 2	8 ± 3	13 ± 5	0.9817
		Qs + HS2	nd	37 ± 1	37 ± 1	7 ± 2	13 ± 5	0.9930
		Qc	0.10 ± 0.02					
		Qc + HS1	0.13 ± 0.05					
		Qc + HS2	0.14 ± 0.04					
peat	soluble Zn	H$_2$0 (ctrl)	nd[b]	0.71 ± 0.09	0.71 ± 0.09	22 ± 12	0.08 ± 0.05	0.9720
		HS1	0.00014 ± 0.00004	1.4 ± 0.3	1.4 ± 0.3	76 ± 45	0.02 ± 0.01	0.8706
		HS2	nd[b]	1.0 ± 0.1	1.0 ± 0.1	64 ± 23	0.04 ± 0.02	0.9536
		Qs	nd[b]	1.5 ± 0.2	1.5 ± 0.2	22 ± 13	0.2 ± 0.1	0.8930
		Qs + HS1	0.00014 ± 0.00003	1.2 ± 0.3	1.2 ± 0.3	25 ± 15	0.13 ± 0.06	0.9132
		Qs + HS2	nd[b]	1.2 ± 0.3	1.2 ± 0.3	30 ± 14	0.2 ± 0.1	0.9263
		Qc	0.11 ± 0.02	2.0 ± 0.4	2.1 ± 0.4	160 ± 70	0.03 ± 0.02	0.9435
		Qc + HS1	0.12 ± 0.05	1.6 ± 0.5	1.7 ± 0.5	80 ± 20	0.06 ± 0.02	0.9810
		Qc + HS2	0.14 ± 0.04	2.1 ± 0.4	2.2 ± 0.4	140 ± 60	0.04 ± 0.02	0.9488

[a] Ctrl: control. HS: humic substance. Qs: standard Fe(*o,o*-EDDHA). Qc: commercial Fe(*o,o*-EDDHA). [b] nd: not detectable.

Table 7. Kinetic Parameters of Mn Solubilized from Amorphous Mn Oxide Substrate[a]

treatment	[Mn]$_0$ (M) ($\times 10^5$)	[Mn]$_{eq}$ (M) ($\times 10^5$)	σ (h)	$V_{t=\sigma}$ (mol/(h·kg soil)) ($\times 10^6$)	R^2
H$_2$0 (ctrl)	nd[b]	0.20 ± 0.04	24 ± 6	0.17 ± 0.08	0.8479
HS1	0.00006 ± 0.00001	0.23 ± 0.04	28 ± 4	0.16 ± 0.05	0.8981
HS2	nd[b]	0.23 ± 0.04	28 ± 4	0.16 ± 0.05	0.8981
Qs	nd[b]	0.5 ± 0.1	36 ± 2	0.31 ± 0.06	0.9528
Qs + HS1	0.00006 ± 0.00002	0.5 ± 0.1	34 ± 4	0.4 ± 0.2	0.8945
Qs + HS2	nd[b]	0.31 ± 0.09	42.2 ± 0.8	0.15 ± 0.02	0.9912
Qc	0.25 ± 0.09	0.39 ± 0.07	28 ± 6	0.3 ± 0.1	0.8379
Qc + HS1	0.24 ± 0.01	0.5 ± 0.1	30 ± 6	0.3 ± 0.1	0.7688
Qc + HS2	0.25 ± 0.09	0.39 ± 0.07	28 ± 6	0.3 ± 0.1	0.8379

[a] Ctrl: control. HS: humic substance. Qs: standard Fe(*o,o*-EDDHA). Qc: commercial Fe(*o,o*-EDDHA). [b] nd: not detectable.

Results reported in **Tables 6** and **7** show that the interaction of deionized water (control) with all soil components produced the solubilization of nutrients. Nonetheless, humic substances HS1 and HS2 significantly increased the nutrient concentration

in solution at equilibrium ([nutrient]$_{eq}$) compared to control except for tenorite and amorphous Mn oxide (**Tables 6** and **7**). For these substrates, [nutrient]$_{eq}$ values did not differ from those in the control (**Tables 6** and **7**). This fact seems to indicate that the humic substances were not able to form soluble Cu and Mn complexes from tenorite and amorphous Mn oxide, respectively. For CaCO$_3$, the initial Ca content inherent to humic substance formulations produced an increase of [Ca]$_{eq}$ in HS solutions with respect to the one in the control, even though differences in [Ca]$_{sol}$ values between both treatments after interaction with CaCO$_3$ were not observed (**Table 6**). On the other hand, although the functional group composition of HS1 was different from the one of HS2 (**Table 1**), a similar kinetic behavior was observed for both humic substances for interaction with all soil components (**Tables 6** and **7**).

The concentrations of Ca and Fe solubilized ([nutrient]$_{sol}$) from CaCO$_3$ and amorphous Fe oxide, respectively, with the standard iron chelate solution (Qs) were statistically similar to those with the control (**Table 6**). These results are in agreement with the fact that losses of Fe(*o,o*-EDDHA) in Qs solution were not observed after the interaction with these substrates, and therefore, there was no free (*o,o*-EDDHA) chelating agent to complex Ca and Fe from CaCO$_3$ and amorphous Fe oxide, respectively. A similar Fe solubilization from amorphous Fe oxide was found for commercial iron chelate solution (Qc) (**Table 6**). However, unlike the standard iron chelate solutions, the interaction of Qc with CaCO$_3$ significantly increased Ca concentration with respect to control. This increase could be associated with the displacement of Fe from non-(*o,o*-EDDHA) chelating agents and the affinity of these chelators to form soluble complexes with Ca.

Both standard and commercial iron chelate solutions increased the extraction of Cu, Mn, and Zn from tenorite, amorphous Mn oxide, and peat, respectively, compared to control (**Tables 6** and **7**). For amorphous Mn oxide, the kinetic behavior of Qc was similar to Qs (**Table 7**). However, commercial iron chelate solution decreased the Zn solubilization rate from peat with respect to the standard iron chelate solution, even though the concentration of Zn at equilibrium ([Zn]$_{eq}$) was statistically similar for both chelates (**Table 6**). On the other hand, the Cu concentration in Qc after the interaction with tenorite was 2.6 times higher than the one obtained for Qs. An explanation for this might be sought in the large ability of (*o,p*-EDDHA) to dissolve Cu from the solid phases (*7, 8*). In spite of the fact that higher amounts of Cu were solubilized with the iron chelate solutions from tenorite (**Table 6**), the amount of retained Fe ($2.0 \times 10^{-6} \pm 0.1 \times 10^{-6}$ mol of Fe for Qs and $2.5 \times 10^{-6} \pm 0.1 \times 10^{-6}$ mol of Fe for Qc) was larger than the amount of solubilized Cu ($0.78 \times 10^{-6} \pm 0.09 \times 10^{-6}$ mol of Cu with Qs and $1.98 \times 10^{-6} \pm 0.04 \times 10^{-6}$ Cu mol with Qc). Hence, the retention of chelated Fe was not only due to iron displacement from chelate by Cu (*11*) but also due to the sorption processes on the tenorite surface. In the same way, the important differences between the amount of Fe retained in amorphous Mn oxide and peat ($0.50 \times 10^{-6} \pm 0.02 \times 10^{-6}$ mol of Fe for Qs and $2.1 \times 10^{-6} \pm 0.1 \times 10^{-6}$ mol of Fe for Qc in peat; $2.6 \times 10^{-6} \pm 0.4 \times 10^{-6}$ mol of Fe for Qs and $3.5 \times 10^{-6} \pm 0.6 \times 10^{-6}$ mol of Fe for Qc in amorphous Mn oxide) and the amount of Mn and Zn solubilized from these substrates ($0.16 \times 10^{-6} \pm 0.01 \times 10^{-6}$ mol of Zn with Qs and $0.42 \times 10^{-6} \pm 0.08 \times 10^{-6}$ mol of Zn with Qc from peat; $0.10 \times 10^{-6} \pm 0.05 \times 10^{-6}$ mol of Mn with Qs and $0.078 \times 10^{-6} \pm 0.005 \times 10^{-6}$ mol of Mn with Qc from amorphous Mn oxide) were indicative of adsorption being the dominant factor in regulating iron

concentration in solution. Unlike tenorite, the losses of Fe(*o,o*-EDDHA) in Qs after interaction with zincite were fundamentally due to the displacement of iron from chelate by Zn, since the solubilized Zn ($5.8 \times 10^{-6} \pm 0.5 \times 10^{-6}$ mol of Zn) was statistically larger than the retained Fe ($1.9 \times 10^{-6} \pm 0.1 \times 10^{-6}$ mol of Fe). For the commercial iron chelate solution, the Zn solubilization from zincite was not observed, even though losses of Fe(*o,o*-EDDHA) and Fe non-(*o,o*-EDDHA) were found in Qc after interaction with this soil component. This implies that the sorption of Fe non-(*o,o*-EDDHA) may have inactivated the surface zincite, avoiding the Zn solubilization and therefore the competence between this cation and Fe for (*o,o*-EDDHA). This is in agreement with the fact that Fe(*o,o*-EDDHA) in commercial iron chelate solutions was hardly retained in this substrate (**Table 4**).

In general, the concentration of nutrient solubilized ([nutrient]$_{sol}$) with Q + HS mixtures was less than the sum of the amount of nutrient solubilized with the iron chelate and the humic substance solutions (**Table 6**). This implies that the nutrient availability in the mixtures was partly limited because of the competition between the iron chelates and the humic substances for complexing these nutrients. According to our results, the solubilization process from amorphous Fe oxide and zincite for Qs + HS mixtures was fundamentally controlled by the reaction between humic substances and the soil components, since the kinetic parameters of the mixtures were statistically similar to those of the humic substance solutions (**Table 6**). In contrast, the standard iron chelate controlled the mobilization of Cu and Zn from tenorite and peat, respectively, in Qs + HS (**Table 6**). In the same way, the observed trend in the Qc + HS mixtures after interaction with the different soil materials suggests that the commercial iron chelate controlled the solubilization process from all substrates except for amorphous Fe oxide (**Table 6**). In this case, the [Fe]$_{sol}$ values in Qc + HS were similar to those in humic substance solutions.

Cu was extracted from the three soils tested with the different treatments in the study (Q, Q + HS, and HS) ([Cu]$_{sol}$, **Table 8**), whereas the mobilization of other nutrients such as Zn or Mn was not detected. The kinetic parameters that described Cu solubilization from the calcareous soils are shown in **Table 8**.

The concentration of Cu solubilized ([Cu]$_{sol}$) with the standard iron chelate was similar to the one with deionized water (control) (**Table 8**), as was expected because losses of Fe(*o,o*-EDDHA) were not observed after the interaction with the soils. However, increases of the Cu concentration in Qc solution was found with respect to the control or Qs (**Table 8**). This indicates that Cu in Qc was fundamentally dissolved through complexation with the *o,p*-EDDHA chelating agent present in commercial iron chelate formulations (*7, 8*).

For the three calcareous soils, HS solutions were able to form soluble complexes with Cu, increasing the availability of this nutrient in soil solution (**Table 8**). Unlike tenorite, the concentration of Cu solublized from the soils with HS solutions was higher than the one with the control (**Table 8**). This difference was a function of the crystalline degree of tenorite and the amount of Cu present in the extractable fraction of the soils. The concentration of Cu solubilized from the three soils tested was similar for both humic substances (**Table 8**). However, HS2 mobilized Cu more quickly than HS1 (**Table 8**).

The Cu concentration solubilized ([Cu]$_{sol}$) with the mixtures was again statistically lower than the sum of Cu extracted with the iron chelate and the humic substance separately (**Table 8**). Nonetheless, unlike the kinetic behavior observed for the soil components, Qc + HS2 slightly increased the solubilization rate

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Table 8. Kinetic Parameters of Cu Solubilized from Calcareous Soils[a]

soil	treatment	$[Cu]_0$ (M) ($\times 10^7$)	$[Cu]_{sol}$ (M) ($\times 10^7$)	$[Cu]_{eq}$ (M) ($\times 10^7$)	$\tau_{1/2}$ (h)	$V_{t=\tau 1/2}$ (mol/(h·kg soil)) ($\times 10^8$)	R^2
LS	H₂0 (Ctrl)	nd[b]	0.61 ± 0.09	0.61 ± 0.09	13 ± 6	0.11 ± 0.04	0.9808
	HS1	0.029 ± 0.005	1.8 ± 0.2	1.8 ± 0.2	23 ± 6	0.20 ± 0.03	0.9889
	HS2	0.011 ± 0.002	2.0 ± 0.2	2.0 ± 0.2	12.3 ± 0.7	0.41 ± 0.07	0.9998
	Qs	nd[b]	0.59 ± 0.05	0.59 ± 0.05	14 ± 3	0.10 ± 0.09	0.9885
	Qs + HS1	0.023 ± 0.006	1.66 ± 0.09	1.66 ± 0.09	14 ± 4	0.29 ± 0.06	0.9801
	Qs + HS2	0.012 ± 0.002	2.02 ± 0.04	2.02 ± 0.04	14 ± 2	0.36 ± 0.05	0.9954
	Qc	5.4 ± 0.1	3.2 ± 0.4	8.6 ± 0.5	22 ± 3	0.36 ± 0.04	0.9961
	Qc + HS1	5.9 ± 0.4	3.6 ± 0.4	9.5 ± 0.8	34 ± 7	0.26 ± 0.03	0.9856
	Qc + HS2	5.8 ± 0.5	3.8 ± 0.6	9 ± 1	13 ± 3	0.73 ± 0.08	0.9898
PS	H₂0 (Ctrl)	nd[b]	0.58 ± 0.05	0.58 ± 0.05	14 ± 5	0.10 ± 0.02	0.9684
	HS1	0.029 ± 0.005	2.0 ± 0.1	2.0 ± 0.1	18 ± 7	0.28 ± 0.04	0.9554
	HS2	0.011 ± 0.002	2.20 ± 0.09	2.20 ± 0.09	13 ± 2	0.42 ± 0.08	0.9948
	Qs	nd[b]	0.52 ± 0.06	0.52 ± 0.06	15 ± 6	0.08 ± 0.02	0.9524
	Qs + HS1	0.023 ± 0.006	2.2 ± 0.2	2.2 ± 0.2	31 ± 12	0.2 ± 0.4	0.9391
	Qs + HS2	0.012 ± 0.002	2.20 ± 0.05	2.20 ± 0.05	13 ± 2	0.42 ± 0.06	0.9953
	Qc	5.4 ± 0.1	3.3 ± 0.1	8.7 ± 0.2	24 ± 4	0.34 ± 0.06	0.9915
	Qc + HS1	5.9 ± 0.4	3.8 ± 0.2	9.7 ± 0.6	19 ± 4	0.50 ± 0.09	0.9863
	Qc + HS2	5.8 ± 0.5	4 ± 1	10 ± 2	17 ± 4	0.69 ± 0.02	0.9857
GS	H₂0 (Ctrl)	nd[b]	0.34 ± 0.08	0.34 ± 0.08	19 ± 5	0.04 ± 0.04	0.9758
	HS1	0.029 ± 0.005	1.32 ± 0.04	1.32 ± 0.04	39 ± 4	0.08 ± 0.03	0.9967
	HS2	0.011 ± 0.002	1.30 ± 0.09	1.30 ± 0.09	19 ± 7	0.17 ± 0.03	0.9689
	Qs	nd[b]	0.36 ± 0.02	0.36 ± 0.02	20 ± 5	0.04 ± 0.03	0.9684
	Qs + HS1	0.023 ± 0.006	1.32 ± 0.05	1.32 ± 0.05	49 ± 7	0.07 ± 0.01	0.9957
	Qs + HS2	0.012 ± 0.002	1.28 ± 0.08	1.28 ± 0.08	20 ± 6	0.16 ± 0.03	0.9699
	Qc	5.4 ± 0.1	2.6 ± 0.2	8.0 ± 0.2	26 ± 7	0.25 ± 0.07	0.9798
	Qc + HS1	5.9 ± 0.4	4.5 ± 0.2	10.4 ± 0.6	25 ± 6	0.46 ± 0.08	0.9813
	Qc + HS2	5.8 ± 0.5	3.3 ± 0.4	9 ± 1	9 ± 4	0.92 ± 0.04	0.9984

[a] Ctrl: control. HS: humic substance. Qs: standard Fe(*o,o*-EDDHA). Qc: commercial Fe(*o,o*-EDDHA). [b] nd: not detectable.
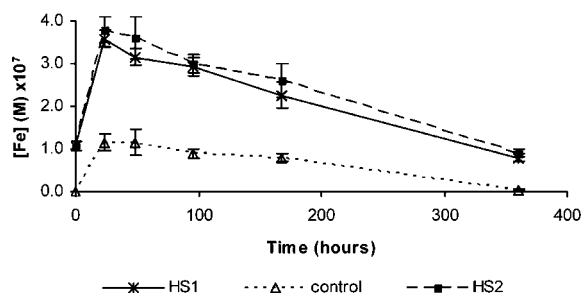

Figure 1. Evolution of Fe solubilized from LS soil by deionized water (control) and HS1 and HS2 solutions.

with respect to Qc and HS2 (**Table 8**). This increase of Cu solubilization rate could be beneficial for plant nutrition, since it may be enough to cover the plant needs and, therefore, it may relieve Cu deficiencies in plants.

Finally, the humic substances also solubilized Fe from the calcareous soils compared to the control. The evolution of Fe solubilized from the lemon soil (LS) is shown as an example in **Figure 1**; there were no differences among the three soils tested. Statistically significant differences were not found in the iron solubilization process between HS1 and HS2. There is no doubt that the commercial humic substances were able to form soluble iron complexes, but they had low stability under these experimental conditions (**Figure 1**). However, the ability of humic substances to maintain iron in solution does not determine their effectiveness as an iron fertilizer. It is well-known that humic substances can also retain Fe in a form available for mobilization and uptake by plant (*19*) and stimulate the iron uptake mechanisms on plant (*27*). Consequently, further research involving plants is needed to establish the agronomic value of humic substances.

Conclusion. The results from this study show that the combined application of humic substance and iron chelate did

not decrease Fe retention in different soil components and calcareous soils, although these mixtures did produce some variation in Fe retention rate with respect to the application of iron chelate. The mechanisms of humic substances for producing such changes are poorly understood because of the complexity of the system, and additional studies would be needed for their full understanding. Nonetheless, there can be no doubt that the presence of *o,p*-EDDHA and other polycondensed ligands in commercial iron chelates (*6*) played a dominant role in the effects of humic substances on the kinetic behavior of Fe(*o,o*-EDDHA). On the other hand, it should be emphasized that the competition between humic substances and synthetic chelating agents for complexing metal cations limited the effectiveness of the mixtures to mobilize nutrients from substrates compared to the application of iron chelate or humic substance solutions separately.

In spite of the observation that humic substances were able to extract only small amounts of iron from calcareous soils, a combination of these humic substances with Fe(*o,o*-EDDHA) did not increase iron solubility in calcareous soils with respect to Fe(*o,o*-EDDHA). However, iron availability for plant in soil is not only represented by its forms in solution. Several studies (*18, 19*) have shown that humic substances could act as a reservoir of easily accessible iron to plants. This property is mainly associated with high molecular weight humic substances (humic acids), while low-weight humic substances (fulvic acids) could solubilize and mobilize iron toward the roots. The fact that humic substances used in this study were richer in fulvic fraction questions that this iron retained by humic substances in a form available for plants can be the cause of the improvement of iron nutrition observed in plants treated with iron chelate–humic substance mixtures at 50% (w/w) (*21–24*). Hence, the physiological action of humic substances on plants (*18, 27*) must be considered as the main cause of this improvement on plant iron nutrition.

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ABBREVIATIONS USED

o,o-EDDHA, ethylenediamine-*N,N*′-bis-(*o*-hydroxyphenylace-tic)acid; Fe(*o,o*-EDDHA), ferric ethylenediamine-*N,N*′-bis-(*o*-hydroxyphenylacetic)acid chelate; *o,p*-EDDHA, ethylenediamine-*N*-(*o*-hydroxyphenylacetic) -*N*′- (*p*-hydroxyphenylacetic) acid; Fe non-(*o,o*-EDDHA), soluble iron nonchelated by ethylene-diamine-*N,N*′-bis-(*o*-hydroxyphenylacetic)acid chelating agent; Qc, commercial iron chelate solution; Qs, standard iron chelate solution; HS, humic substance solution, Qs + HS, standard iron chelate–humic substance mixture; Qc + HS, commercial iron chelate–humic substance mixture; AAS, atomic absorption spec-trometry; HPLC, high-performance liquid chromatograhy; ^{13}C NMR, ^{13}C nuclear magnetic resonance; XRD, X-ray diffraction.

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